# The potential of protection of diabetic hearts and hearts subjected to hyperglycemia from ischemia/reperfusion injury by a novel pathway

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## Background

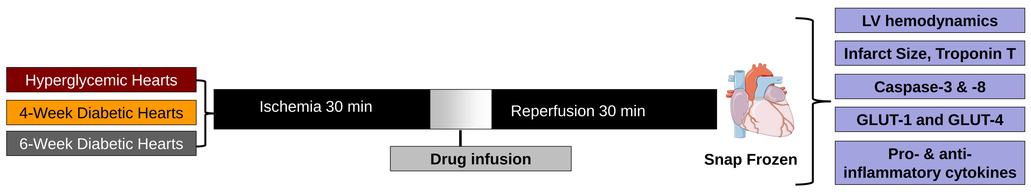
The comorbidities of ischemic heart disease (IHD) and diabetes mellitus (DM) compromise the protection of the diabetic heart from ischemia/reperfusion (I/R) injury.

## Specific aims

The current study aimed to investigate new avenues for potential protection of the diabetic heart.

## Methods

Isolated hearts (n=8 per group) were perfused with a modified Langendorff preparation. All hearts were subjected to I/R and treated with cyclosporine-A, mitochondria permeability transition pore (mPTP) blocker; SNAP, nitric oxide donor; DOG, protein kinase C (PKC) activator; or CRK, mitochondrial ATP-sensitive potassium channel opener at the beginning of reperfusion. Hemodynamic data were collected using suitable software. Infarct size was evaluated using 2,3,5-Triphenyltetrazolium chloride (TTC) staining and troponin T levels. Protein levels were assessed by Western blotting or enzyme-linked immunosorbent assay (ELISA).




## Results

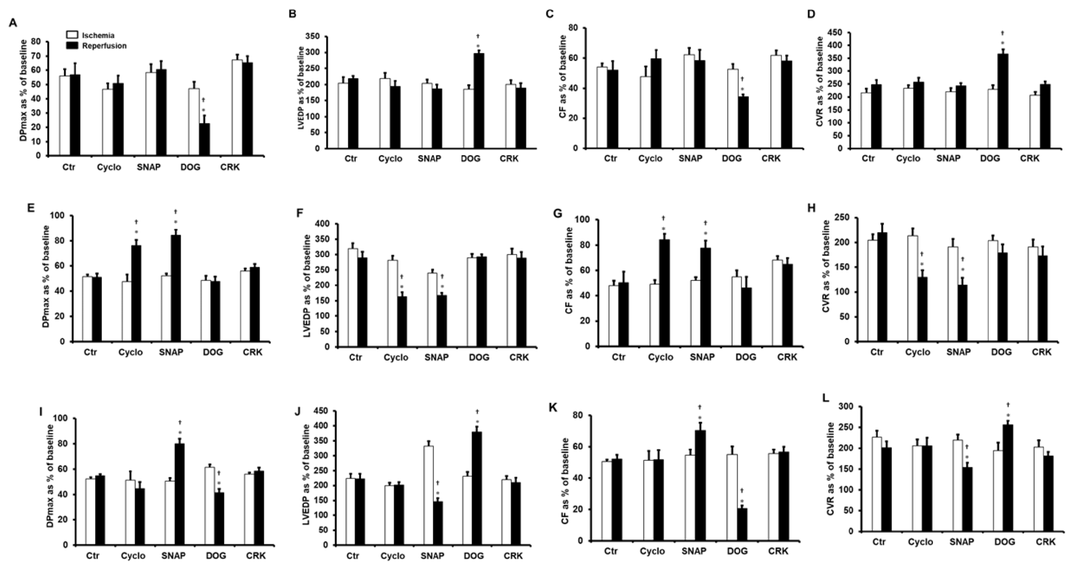



***Figure* 1**: Left ventricle (DPmax and LVEDP) and coronary vascular dynamics (CF and CVR) during post-ischemic recovery after the treatment protocols. A, B, C, and D: acute hyperglycemia, E, F, G, and H: four-week diabetes, I, J, K, and L: six-week diabetes (n=8 per group). The data were computed after 30 min reperfusion and expressed as mean±SEM. DPmax; maximum developed pressure, LVEDP; left ventricular end-diastolic pressure, CF; coronary flow, CVR; coronary vascular resistance, Ctr; control, Cyclo; cyclosporine-A; mitochondria permeability transition pore blocker, SNAP; nitric oxide donor; S-nitroso-N-acetylpenicillamine, DOG; protein kinase C activator; 1,2 octanoyl-sn-glycerol and CRK; mitochondrial ATP-sensitive potassium channel opener; cromakalim. *P<0.05 compared to respective controls and †P<0.05 compared to the respective ischemic period.

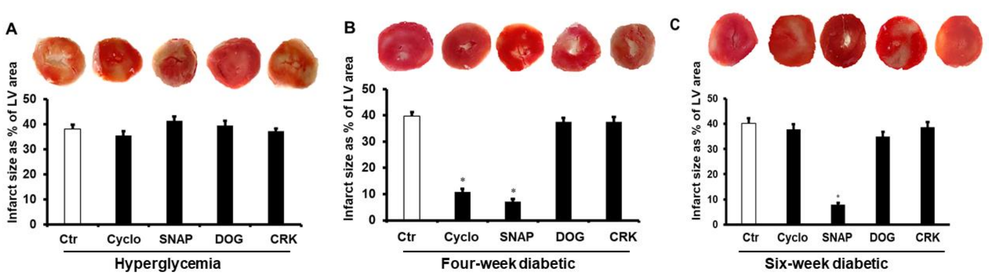



***Figure* 2**: Histological assessment of ischemic injury. Infarct size is presented as an infarcted area as a percentage of the left ventricle area in the experimental models of hyperglycemia and DM presented as mean±SEM (n=4 per group). A: acute hyperglycemia, B: four-week diabetic hearts, and C: six-week diabetic hearts. Ctr; control, Cyclo; cyclosporine-A; mitochondria permeability transition pore blocker, SNAP; nitric oxide donor; S-nitroso-N-acetylpenicillamine, DOG; protein kinase C activator; 1,2 octanoyl-sn-glycerol and CRK; mitochondrial ATP-sensitive potassium channel opener; cromakalim. *P < 0.001 compared to the respective controls.

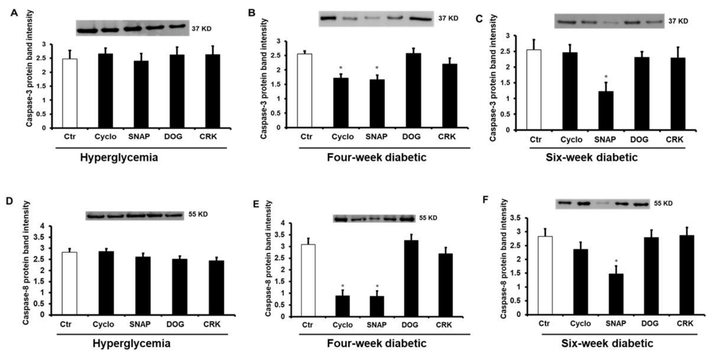



***Figure* 3**: Treatment effects on caspase-3 and caspase-8 protein levels in the supernatants of homogenized left ventricles by immunoblotting presented as mean±SEM (n=4 per group). Western blot shows the protein levels of caspase-3 (A, B, and C), and caspase-8 (D, E, and F). Values are mean±SEM for 4 individual experiments. Ctr; control, Cyclo; cyclosporine-A; mitochondria permeability transition pore blocker, SNAP; nitric oxide donor; S-nitroso-N-acetylpenicillamine, DOG; protein kinase C activator; 1,2 octanoyl-sn-glycerol and CRK; mitochondrial ATP-sensitive potassium channel opener; cromakalim. *P<0.05 compared to respective controls.

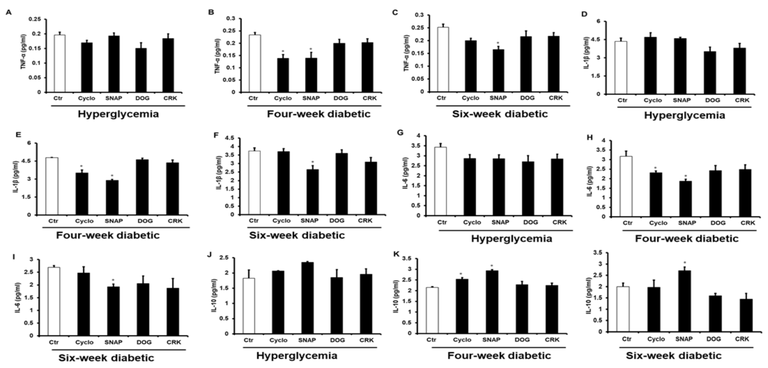



***Figure* 4:** Pro-inflammatory and anti-inflammatory cytokine levels in the cardiac muscle samples after treatment of hearts isolated from nondiabetic rats presented as mean±SEM (n=4 per group). TNF-α protein levels (A, B, and C), IL-1β protein levels (D. E and F), IL-6 protein levels (G, H, and I), and the anti-inflammatory cytokine IL-10 (J, K, and L) protein levels. *P<0.05 compared to the respective controls.

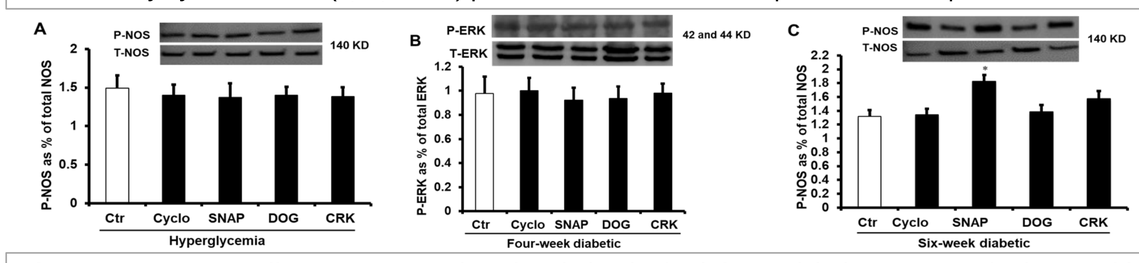



***Figure* 5**: Treatment effects on eNOS phosphorylation in the left ventricle homogenate by immunoblotting presented as mean±SEM (n=4). Western blot showing the protein levels of eNOS, and Phosphorylated eNOS. Ctr; control, Cyclo; cyclosporine-A; mitochondria permeability transition pore blocker, SNAP; nitric oxide donor; S-nitroso-N-acetylpenicillamine, DOG; protein kinase C activator; 1,2 octanoyl-sn-glycerol and CRK; mitochondrial ATP-sensitive potassium channel opener; cromakalim. *P<0.05 compared to respective controls.

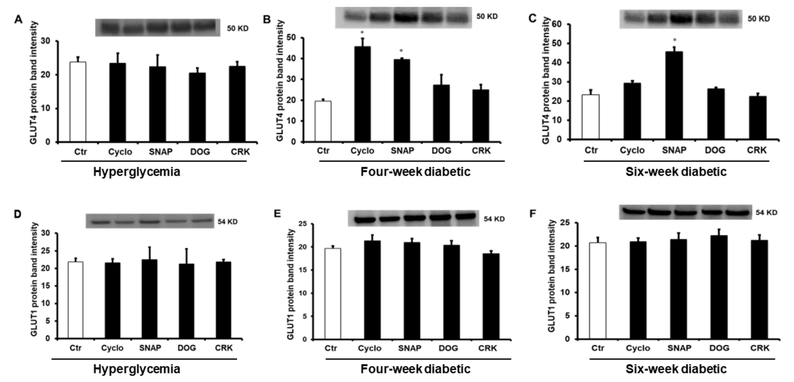



***Figure* 6**: Treatment effects on GLUT1 and GLUT4 protein levels in the left ventricle homogenate by immunoblotting presented as mean±SEM (n=4). Western blot showing GLUT-4 (A, B, and C) and GLUT-1 (D, E, and F) protein levels. Ctr; control, Cyclo; cyclosporine-A; mitochondria permeability transition pore blocker, SNAP; nitric oxide donor; S-nitroso-N-acetylpenicillamine, DOG, protein kinase C activator; 1,2 octanoyl-sn-glycerol and CRK; mitochondrial ATP-sensitive potassium channel opener; cromakalim. *P<0.05 compared to the respective controls.

## Conclusion

Treatment of diabetic hearts with SNAP protected four- and six-week diabetic hearts; however, cyclosporine-A protected four-week diabetic hearts only. This protection followed a pathway involving the eNOS/GLUT-4 axis and showed a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokine levels.

## Acknowledgement

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