Effect of Hyperglycemia on eNOS Function in EPCs

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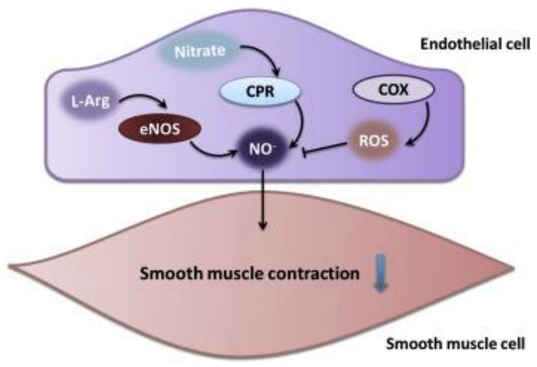
Introduction

Diabetes is one of the critical health issues around the world. Specifically, Type 2 diabetes mullites (T2DM) results in different cardiovascular complications. The main cause of these complications is endothelial dysfunction, which affects the endothelium physiologically and pathologically. The chronic hyperglycemia introduced by T2DM impacts the pivotal enzyme endothelial nitric oxide synthase (eNOS), which is responsible of the production of the vasodilator nitric oxide (NO). Hyperglycemia affects eNOS in terms of phosphorylation and dimerization which results in initiation of oxidative stress. To overcome endothelial dysfunction, endothelial progenitor cells (EPCs) are introduced as a therapeutic tool due to their regenerative characteristics, especially blood outgrowth endothelial cells (BOECs) due to their significant similarity to ECs functionally and morphologically. The effects of hyperglycemia on these cells is not yet established. Thus, this study aims to investigate the effects of hyperglycemia on the eNOS/Akt signaling pathway and its effect on reactive oxygen species (ROS) formation and oxidative stress.



Illustrative image of eNOS signaling pathway in endothelial cells.

Hypothesis

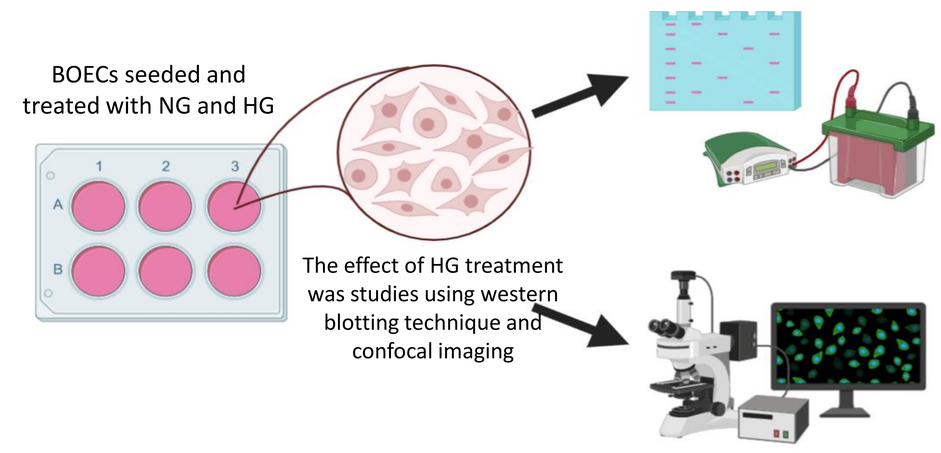
EPCs/BOECs expression of eNOS is impaired in response to hyperglycemia, which reduces NO bioavailability and induces oxidative stress

Aims

1. Treat BOECs with normal and hyperglycemic media in culture and study the effect of hyperglycemic conditions on eNOS phosphorylation and dimerization
2. Study the effect of hyperglycemic conditions on Akt/ protein kinase B which is an essential kinase to regulates eNOS phosphorylation
3. Investigate the effect of hyperglycemia on the generation of reactive oxygen species in BOECs, and

Methodology

- **Cell culture:** BOECs were seeded at 100,000 cells in a 6 well-plate and treated with normal glucose (NG) (5.5mM) and high glucose (HG) (25 mM) for 24, 72 or 144 hrs.
- **Western Blot:** Expression of eNOS, p-eNOS, Akt, and p-AKT was detected using Western blot, total and phosphorylated proteins were corrected by B-actin. Quantification of band density was done using image lab.
- **ROS staining:** CellROX was used to test ROS formation , images were taken by confocal fluorescent microscope (Zeiss) and analyzed using imageJ.



Results

A- Exposing BOECs to hyperglycemic media resulted in a reduced eNOS phosphorylation only after 6 days of treatment (Fig 1). In addition, AKT phosphorylation was reduced after 3 and 6 days of treatment (Fig 2).

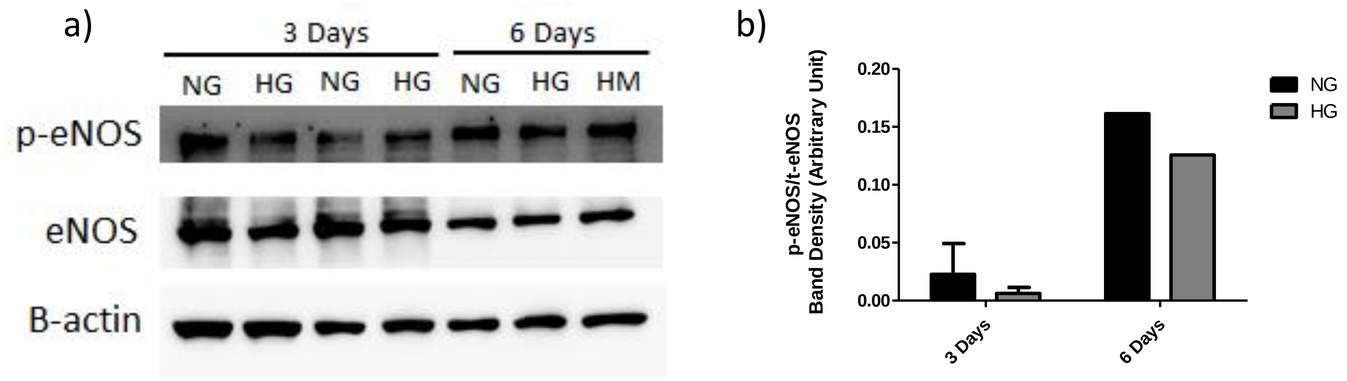


Figure 1. *Protein expression of phosphorylated and total eNOS (a). Protein expression was normalized to B- actin expression, and the ratio of phosphorylated/total protein expression is represented in the bar chart (b).*

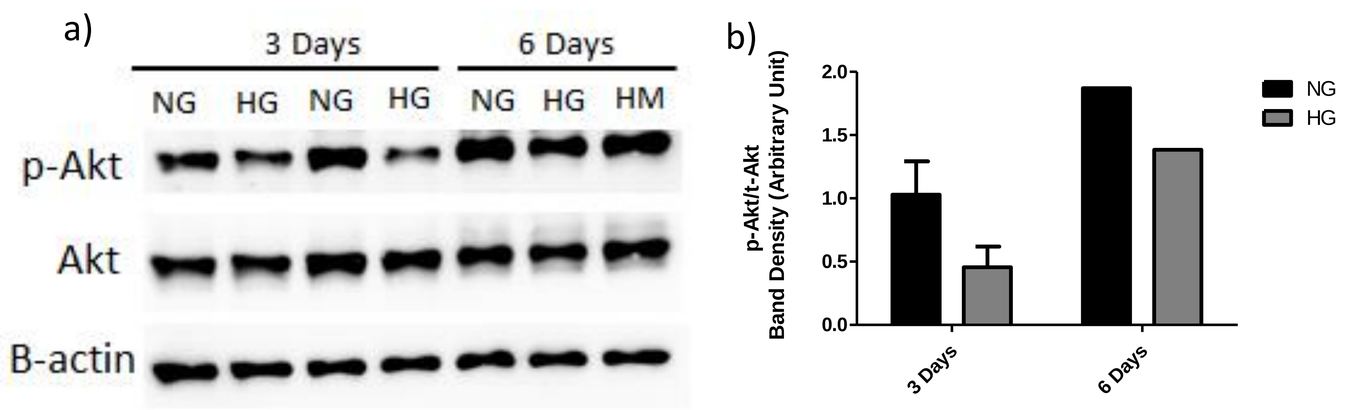


Figure 2. *Protein expression of phosphorylated and total AKT (a). Protein expression was normalized to B- actin expression, and the ratio of phosphorylated/total protein expression is represented in the bar chart (b).*

B- Exposing BOECs to hyperglycemic media for 24hr resulted in an increase in ROS release (Fig 3.A), however, these effects were absent after 72hr (Fig 3.B).

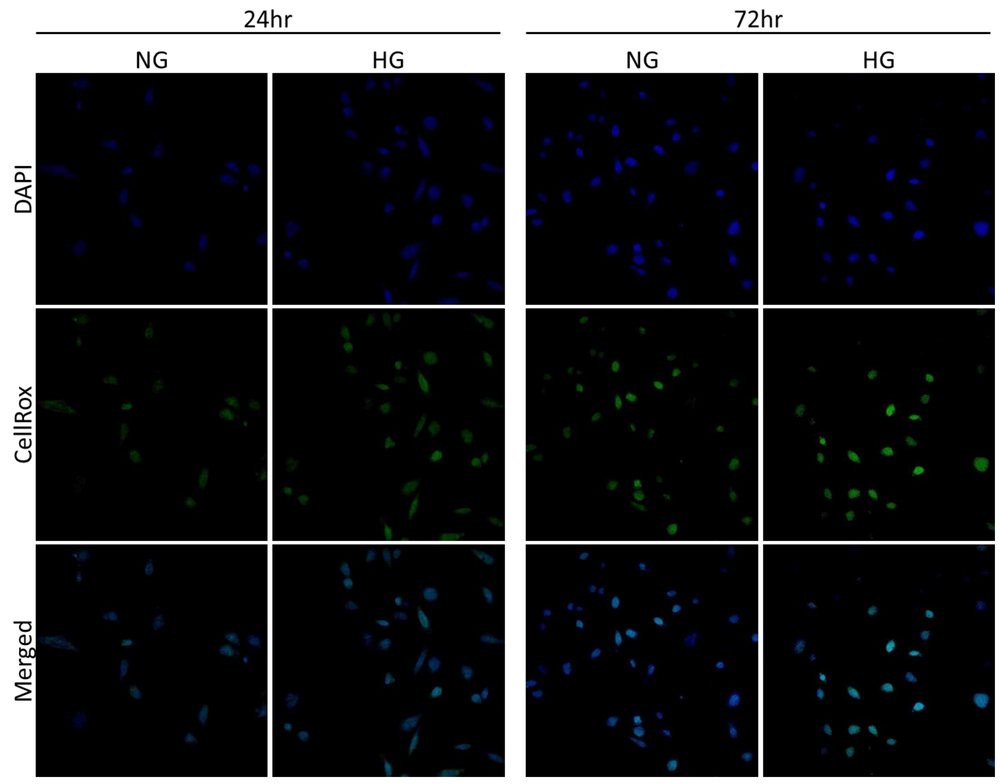


Figure 3. *Effect of hyperglycemia on ROS formation following treatment for 24h (A), and 72hr (B). Bar chart comparison of ROS production in NG and HG treated BOECs represented as corrected total cell fluorescence (CTCF) (C).*

Conclusion

- Hyperglycemia promoted by T2DM reduced eNOS & Akt phosphorylation, which reflects on NO production.
- Acute hyperglycemia induced ROS formation which is known to affect the coupling of eNOS enzyme and to lower NO bioavailability.
- EPCs/BOECs may be involved in future treatments of endothelial dysfunction, due on their similarity to endothelial cells and their regenerative potential.

References

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