

# EXPRESSION AND ACTIVITY OF FOXO1/FOXO3A TRANSCRIPTION FACTORS AND ENOS IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS

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## Abstract

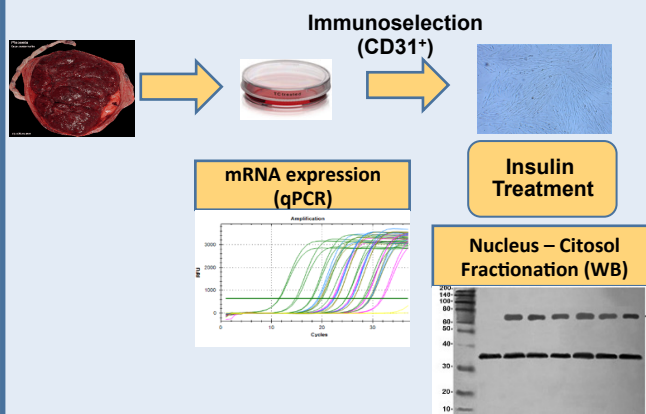
The placenta is a highly vascularized organ that mediates the complex functional interaction between mother and fetus, being the endothelium, the main tissue involved in this interaction. Dysregulation of the endothelial function leads to pregnancy disorders such as gestational diabetes mellitus (GDM). It has been described that, human placental microvascular endothelial cells (hPMECs) from pregnancies with GDM have high basal levels of nitric oxide (NO) and respond to insulin decreasing this synthesis to levels observed in untreated non-pathological cells. In contrast, in cells from normal pregnancies insulin increases NO synthesis. NO is synthesized by endothelial nitric oxide synthase (eNOS), whose expression is regulated by the transcription factors FoxO1/FoxO3a, among others. However, the role of insulin in this pathway is unknown. We propose that FoxO1/FoxO3a control the expression of eNOS in insulin response in these cells through of differential regulation of their receptors, Insulin Receptor IR-A and IR-B. We used cell line of skin microvasculature (HMEC1) in conjunction with hPMEC of healthy and DMG placentas. In HMEC1 (exposed to high or normal glucose levels) and hPMEC (from normal and DMG pregnancies), treated and untreated with insulin, analyzed the expression of FoxO1/FoxO3a by qPCR, and nuclear/cytoplasmic localization by western blotting and immunofluorescence. Also, we evaluated eNOS expression in HMEC1 by these same methods. And we analyze intracellular NO levels by a fluorimetric assay. Preliminary results suggests that FoxO1 cytoplasmic levels decrease in microvascular endothelial cells after insulin treatment. This could be related with FoxO1/FoxO3a nuclear activity increase and therefore eNOS transcription reduction.

## Aim of study

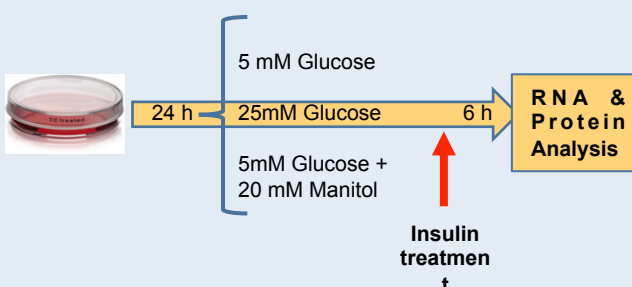
To characterize a cellular model for the study of eNOS activity regulation by Insulin in human microvascular endothelial cells, in the context of Gestational Diabetes Mellitus.

## Materials and Methods

### (A) hPMEC primary cultures



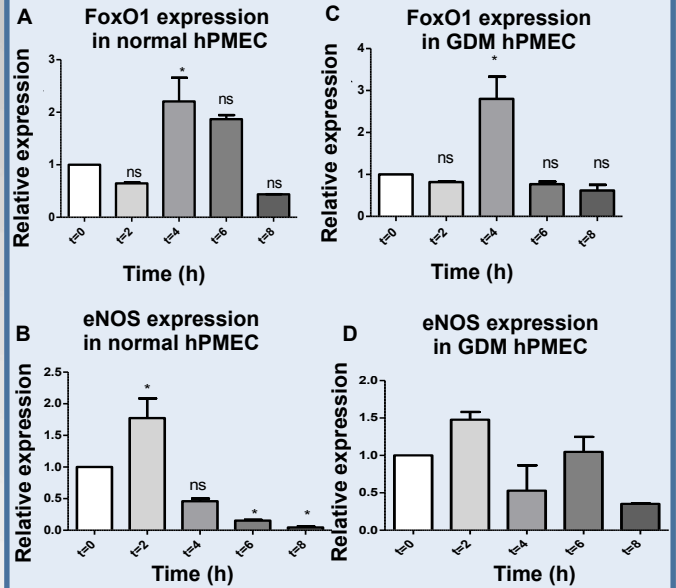
### (B) HMEC1 cell line cultures



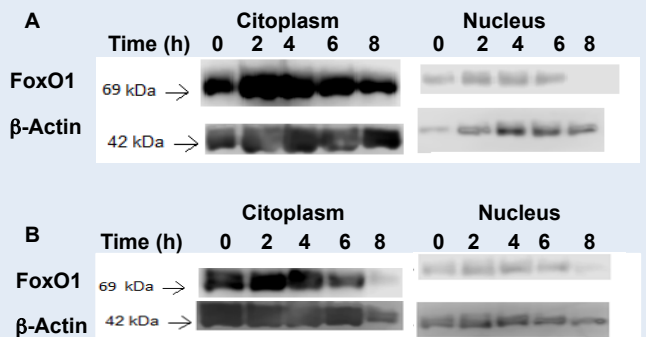
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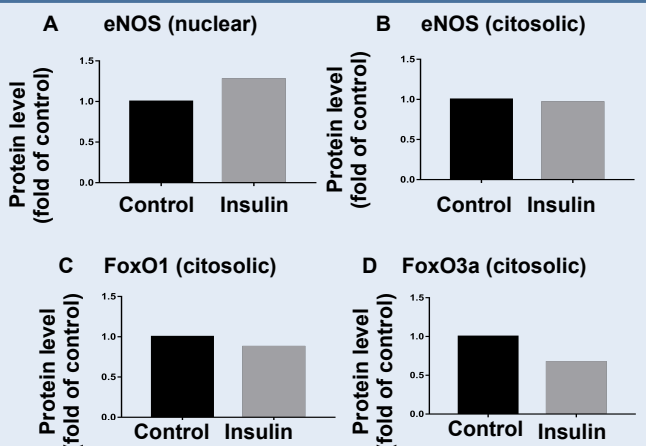
## Results



**Figure 1: mRNA expression of FoxO1 and eNOS in hPMEC primary cultures.** qPCR assay for FoxO1 and eNOS in hPMEC primary cultures from normal (n=1; A-B) and DGM (n=2; C-D) pregnancies, after Insulin treatment (1 nM; 0,2,4,6, 8 hours).eNOS mRNA expression shows a tendency to decrease after insulin treatment. Relative expression, normalized against  $\beta$ -actin.



**Figure 2: Cytoplasmic and nuclear levels of FoxO1 in hPMEC primary cultures.** Western Blot analysis of cytosolic and nuclear fractions of hPMEC primary cultures from normal (n=1; A) and GDM (n=2; B) pregnancies, after Insulin treatment (1 nM; 0,2,4,6,8 hours). FoxO1 cytosolic levels show a tendency to decrease after insulin treatment.



**Figure 3: Protein levels of eNOS, FoxO1 and FoxO3a in HMEC1 culture cells.** Densitometric analysis of Western blot assays against eNOS (A,B), FoxO1 (C) and FoxO3a (D) in HMEC1 cultures after insulin treatment (1nM, 6 hours). FoxO1 and FoxO3a were detected only in cytosolic fractions and shows a tendency to decrease. eNOS shows a tendency to increase in nuclear fraction (A).