Abstract: P375

**Effects of simulated hyperglycemia in vitro on insulin signaling in endothelial cells**

**Authors:**
R Madonna¹, P Confalone¹, V Doria¹, R De Caterina¹, ¹Institute of Cardiology - "G. d'Annunzio" University - Italy,

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Emerging evidence in myocytes, hepatocytes and adipocytes indicates that hyperglycemia, a major feature of type 1 diabetes (T1DM), also plays a critical role in the development of insulin resistance and progression of type 2 DM (T2DM). Insulin regulates vascular homeostasis and endothelial function but the role of hyperglycemia in the development and progression of insulin resistance in endothelial cells remains incompletely understood.

We aimed at investigating the impact of high glucose on insulin signaling in human aortic endothelial cells (HAECs). We tested the hypothesis that high glucose per se and/or through its hyperosmolar component may lead to insulin resistance by lowering the metabolic, anti-inflammatory and anti-atherogenic insulin signaling through a down-regulation of the PI3K/AKT pathway.

Serum-starved HAECs were preincubated with 5.5 mmol/L glucose (normoglycemia, NG), high glucose (HG, at 17.5, 30.5 and 50.5 mmol/L), or equimolar concentrations of the hyperosmolar control mannitol (HM) for short- (3 hours) and long-term exposures (24 hours), followed by insulin treatment (1-10-100 nmol/L) for 45 minutes. Expression of insulin receptor-a subunit (IRa), insulin receptor substrate type 1 (IRS-1), eNOS and phosphorylated isoforms of AKT, ERK1/2, and p38 were evaluated.

HG, and to a lesser extent HM, increased the expression of eNOS, while decreasing the expression of AKT and its active phosphorylated isoform pAKT in a concentration-depending manner (p<0.01 versus NG by ANOVA, n=3 independent experiments). In long-term exposure HG, and to a lesser extent HM, increased the expression of ERK1/2 (p<0.01 versus NG by ANOVA, n=3), while at any time point they did not modify the expression of p38 and its active phosphorylated isoforms pERK1/2 and p-P38.

In NG, IRa, pAKT, pERK1/2, p-P38 were increased in insulin treated cells. In HG or HM (17.5 and 30.5 mmol/L), insulin was not able to activate the PI3K/AKT/ eNOS pathway, as compared to the control. Insulin was able to induce the up-regulation of IRS-1, pERK1/2 and p-P38, although no changes of IRa were found (p<0.01 versus NG by ANOVA, n=3).

By decreasing the anti-inflammatory and anti-atherogenic AKT, hyperglycemia and its hyperosmolar component negatively impact insulin signaling in human macrovascular endothelial cells, even when physiological and pathophysiological insulin concentrations are added. The impairment of the PI3K/AKT/eNOS pathway after physiological insulin treatment could contribute to detrimental effects on cardiovascular homeostasis under HG conditions, and might shift toward the activation of certain mitogenic effectors, such as ERK1/2 and p38, the only ones that respond to physiological insulin treatment in HG. Such effects may be relevant for the vascular complications of diabetes and indicate a biochemical basis explaining the progression of insulin resistance as a result of endothelial glucotoxicity in diabetes.