GLUCOSAMINE-STIMULATED ENDOPLASMIC RETICULUM STRESS AND AUTOPHAGY ARE ASSOCIATED WITH INSULIN RESISTANCE OF RAT CULTURED PODOCYTES

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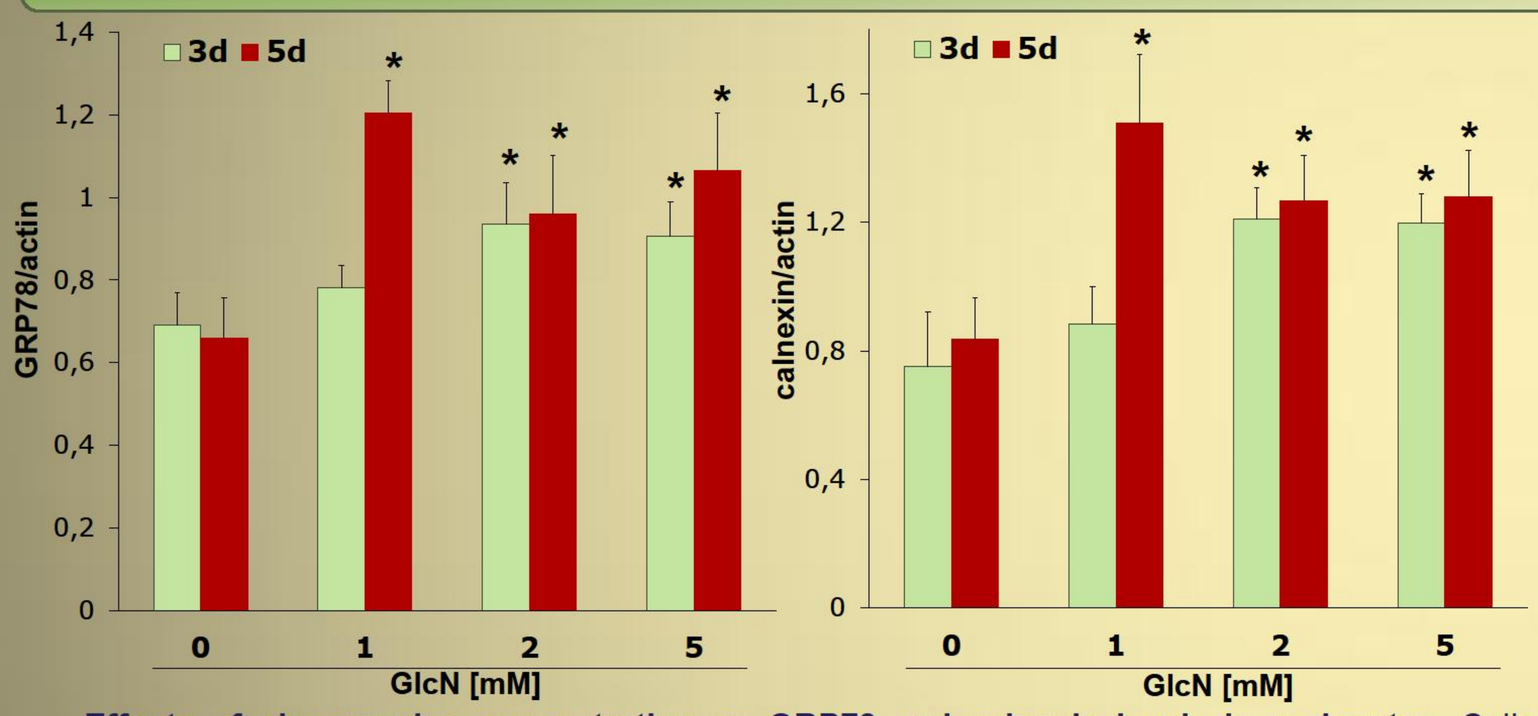
INTRODUCTION AND OBJECTIVES

Diabetic nephropathy (DN) is a chronic progressive disease that affects up to 40% of patients with diabetes mellitus. The early clinical manifestation of DN is microalbuminuria, which can progress to evident proteinuria and renal dysfunction. Glomerular visceral epithelial cells (podocytes), as part of the filtration barrier, play an important role in the development of DN, and their numbers are significantly reduced in both type 1 and type 2 diabetic patients. In addition to the decreased number of podocytes, structural damage to podocytes (podocytopathy) is accompanied by foot process effacement, and it is associated with the progression of proteinuria. Podocytes are direct targets for insulin [1,2], and insulin signaling is essential for normal glomerular function [3]. Disturbances in insulin signaling accompanied by insulin resistance can lead to various intracellular events. Excessive flux through the hexosamine biosynthetic pathway (HBP) and endoplasmic reticulum (ER) stress were related to insulin resistance. The metabolic effects of increased flux through HBP are mediated by increasing *O*-glycosylation of cellular proteins. Our previous reults have demonstrated that in rat podocytes cultured in the presence of glucosamine (GlcN), the amount of *O*-glycosylated proteins is significantly increased [4]. The endoplasmic reticulum (ER) plays a key role in the synthesis and modification of secretory and membrane proteins in all eukaryotic cells. Under normal conditions, these proteins are correctly folded and assembled in the ER. Stress in the endoplasmic reticulum (ER) results from insufficient protein folding capacity or altered ER homeostasis by activating the unfolded protein response (UPR). Autophagy serves as a protective response during ER stress through the degradation of unfolded proteins and damaged organelles. We hypothesized that glucosamine-induced insulin resistance in podocytes is associated with ER stress stimulation accompanied by induction of autophagy.

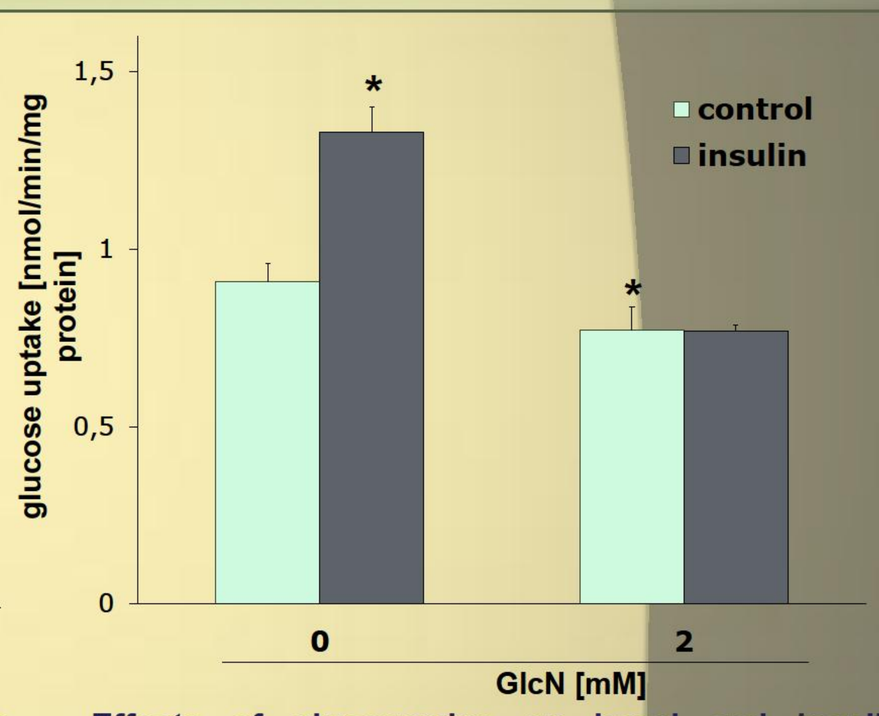
METHODS

Experiments were performed in primary rat podocytes cultured in the presence of 1-5 mM glucosamine (GlcN) for 3 and 5 days. Immunodetection methods were used to examine protein expression of ER chaperones (calnexin and Grp78) and LC3II protein, which is a commonly used marker of autophagy. Insulin-stimulated changes in glucose uptake were used to detect insulin resistance. Glucose uptake was measured by the addition of 1 µCi/well of (1,2-^{3}H)-deoxy-D-glucose diluted in nonradioactive glucose (50 µM final concentration) with or without 300 nM insulin for 3 min to detect insulin resistance. To determine cell viability the colorimetric MTT metabolic activity assay was used.

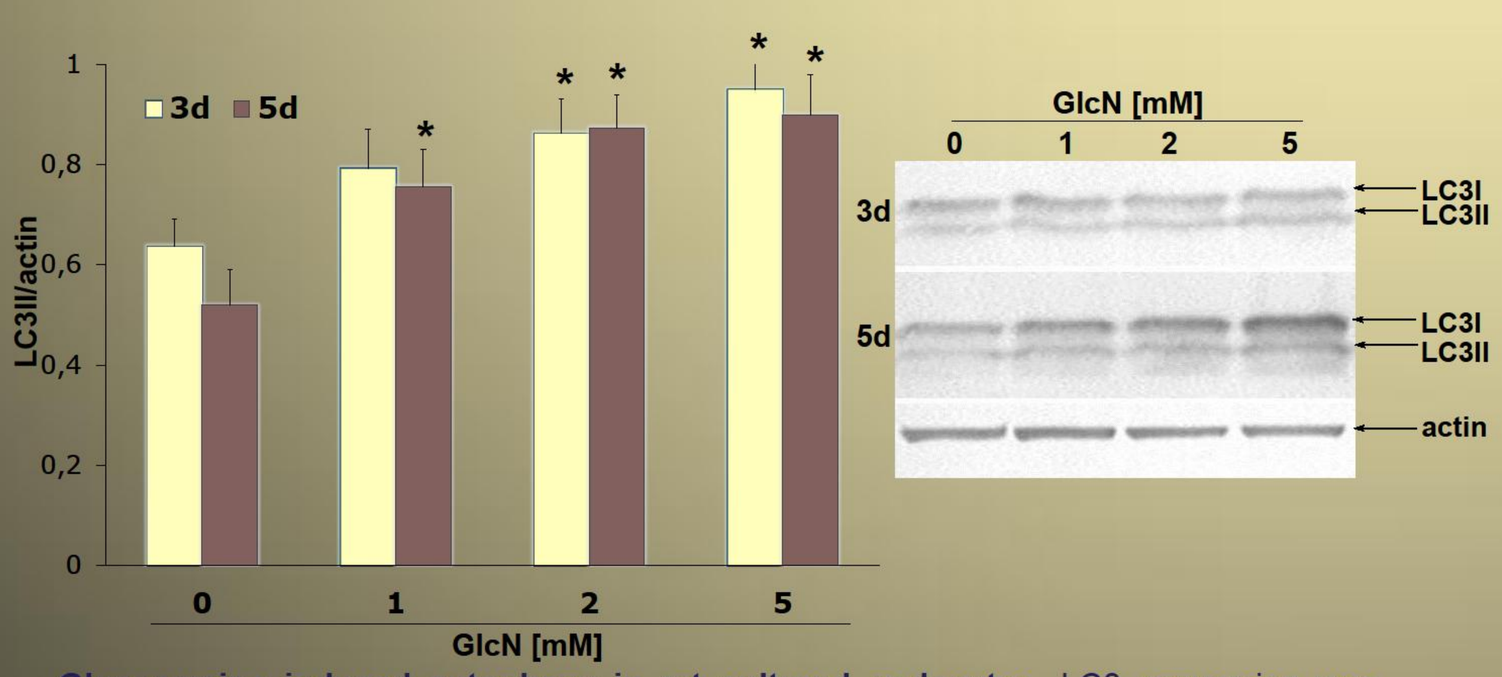
RESULTS



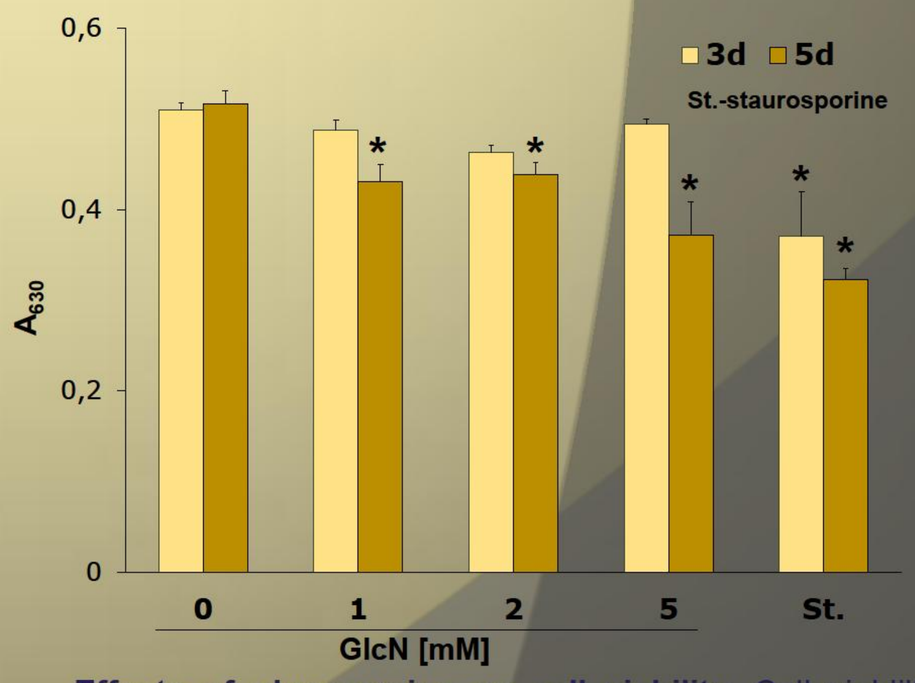
Effects of glucosamine concentration on GRP78 and calnexin levels in podocytes. Cells were preincubated with GlcN (1-5 mM) for 3 or 5 days. Quantitative densitometric analysis was used to determine the ratios of GRP78 or calnexin to actin. Obtained results suggest that GlcN induces ER stress in rat cultured podocytes.



Effects of glucosamine on basal and insulin-stimulated glucose uptake in rat podocytes. Before insulin stimulation cells were preincubated with serum-free medium for 2 hours. Obtained results suggest that GlcN induces insulin resistance in podocytes.



Glucosamine induced autophagy in rat cultured podocytes. LC3 conversion was assessed by immunoblotting. Quantitative densitometric analysis was used to determine the ratios of LC3II to actin. The blots from a representative of three experiments are presented with the densitometry data.



Effects of glucosamine on cell viability. Cell viablilty was determined using the MTT assay. Staurosporine was used as a positive control.

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CONCLUSIONS

We found that glucosamine-induced insulin resistance manifested by impairment of insulin-dependent glucose uptake into podocytes is associated with ER stress and autophagy activation.

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