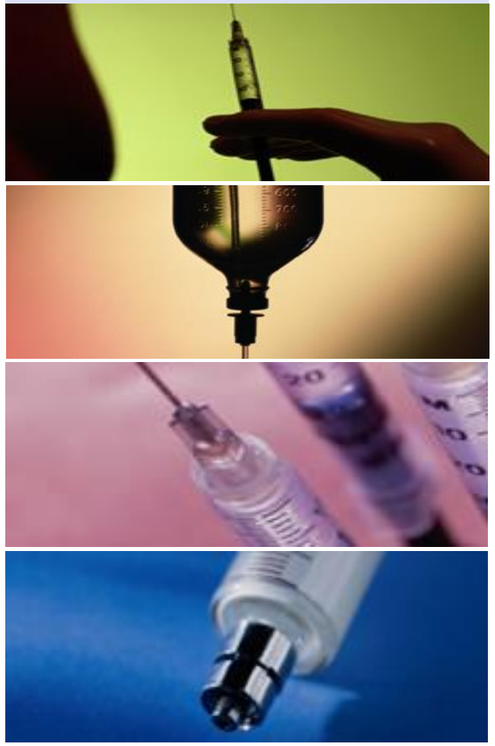

Research of the Month (March 2015)

Preclinical Research

Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

March 2015

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Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

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Abstract

Hypogonadism in men is associated with an increased incidence of type 2 diabetes. Supplementation with testosterone has been shown to protect pancreatic β-cell against apoptosis due to toxic substances including streptozotocin and high glucose. One of the pathological mechanisms of glucose-induced pancreatic β-cell apoptosis is the induction of the local rennin–angiotensin–aldosterone system (RAAS). The role of testosterone in regulation of the pancreatic RAAS is still unknown. This study aims to investigate the protective action of testosterone against glucotoxicity-induced pancreatic β-cell apoptosis via alteration of the pancreatic RAAS pathway. Rat insulinoma cell line (INS-1) cells or isolated male mouse islets were cultured in basal and high-glucose media in the presence or absence of testosterone, losartan, and angiotensin II (Ang II), then cell apoptosis, cleaved caspase 3 expression, oxidative stress, and expression of angiotensin II type 1 receptor (AGTR1) and $p47^{phox}$ mRNA and protein were measured. Testosterone and losartan showed similar effects in reducing pancreatic β-cell apoptosis. Testosterone significantly reduced expression of AGTR1 protein in INS-1 cells cultured in high-glucose medium or high-glucose medium with Ang II. Testosterone decreased the expression of AGTR1 and $p47^{phox}$ mRNA and protein in comparison with levels in cells cultured in high-glucose medium alone. Furthermore, testosterone attenuated superoxide production when co-cultured with high-glucose medium. In contrast, when cultured in basal glucose, supplementation of testosterone did not have any effect on cell apoptosis, oxidative stress, and expression of *AGT1R* and $p47^{phox}$. In addition, high-glucose medium did not increase cleaved caspase 3 in *AGTR1* knockdown experiments. Thus, our results indicated that testosterone prevents pancreatic β-cell apoptosis due to glucotoxicity through reduction of the expression of *ATGR1* and its signaling pathway.

Key Words
- glucotoxicity
- testosterone
- pancreatic β-cell
- apoptosis
- angiotensin II type 1 receptor (*AGTR1*)
- signaling pathway

Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

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Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

- **Testosterone decreased apoptosis of pancreatic β-cells cultured in high-glucose (40mM) medium**

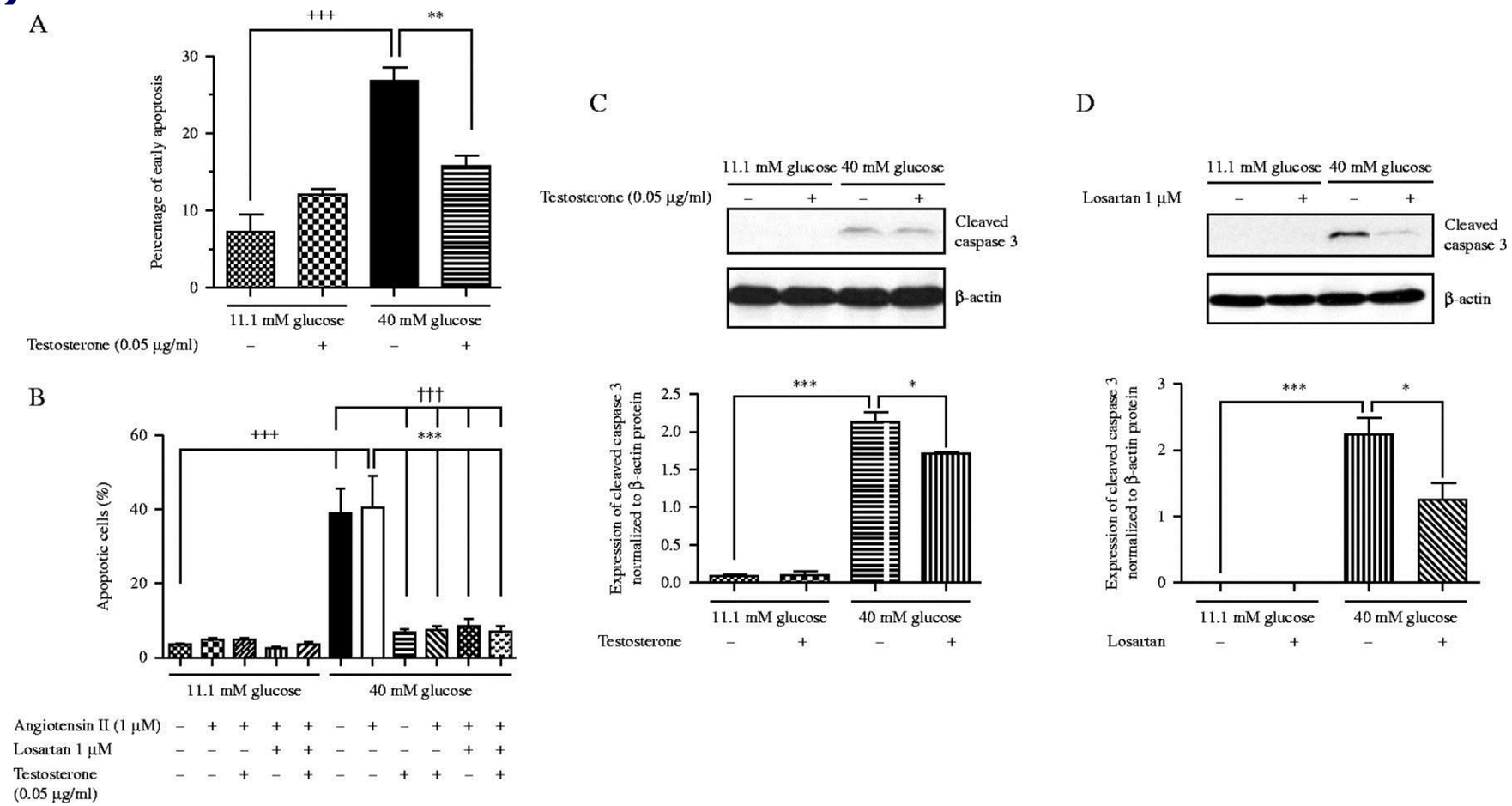

Figure 1 Effect of testosterone, losartan, and Ang II on apoptosis of pancreatic β-cells cultured in basal- and high-glucose media. INS-1 cells were cultured in basal-glucose (11.1mM) or high-glucose (40mM) medium in the presence or absence of testosterone (0.05μg/ml) for 72h. Apoptotic INS-1 cells were detected using Annexin V–FITC/PI staining.

Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

- **Testosterone decreased AGTR1 expression in pancreatic b-cells activated by high-glucose medium and Ang II**

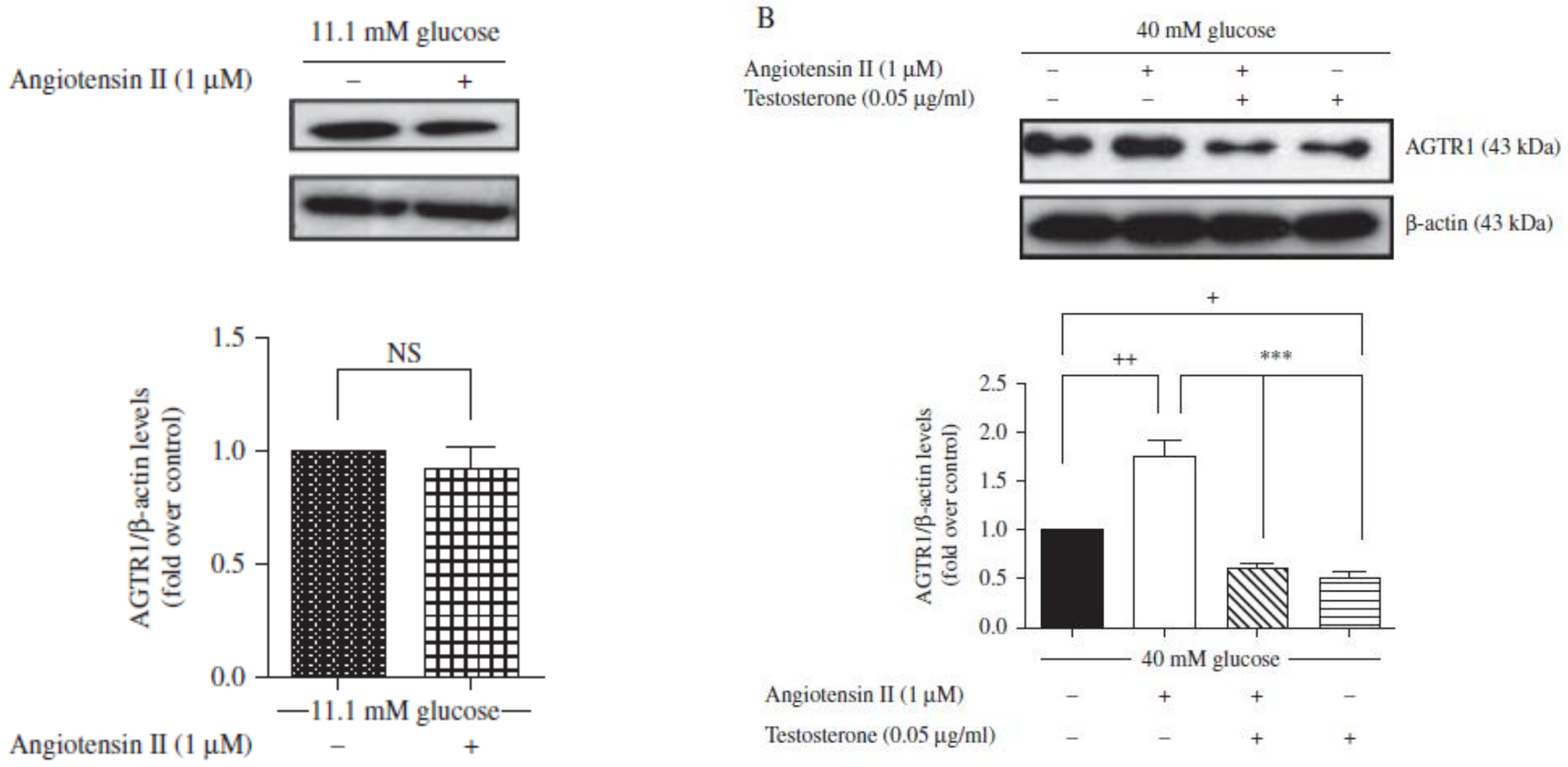

Figure 2 Effect of basal- and high-glucose media, Ang II, and testosterone on expression of AGTR1 protein in INS-1 cells. INS-1 cells were cultured with basal-glucose medium with or without Ang II for 72 h.

Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

- **Testosterone attenuated the expression of AGTR1 mRNA and protein in pancreatic b-cells cultured in high-glucose medium**

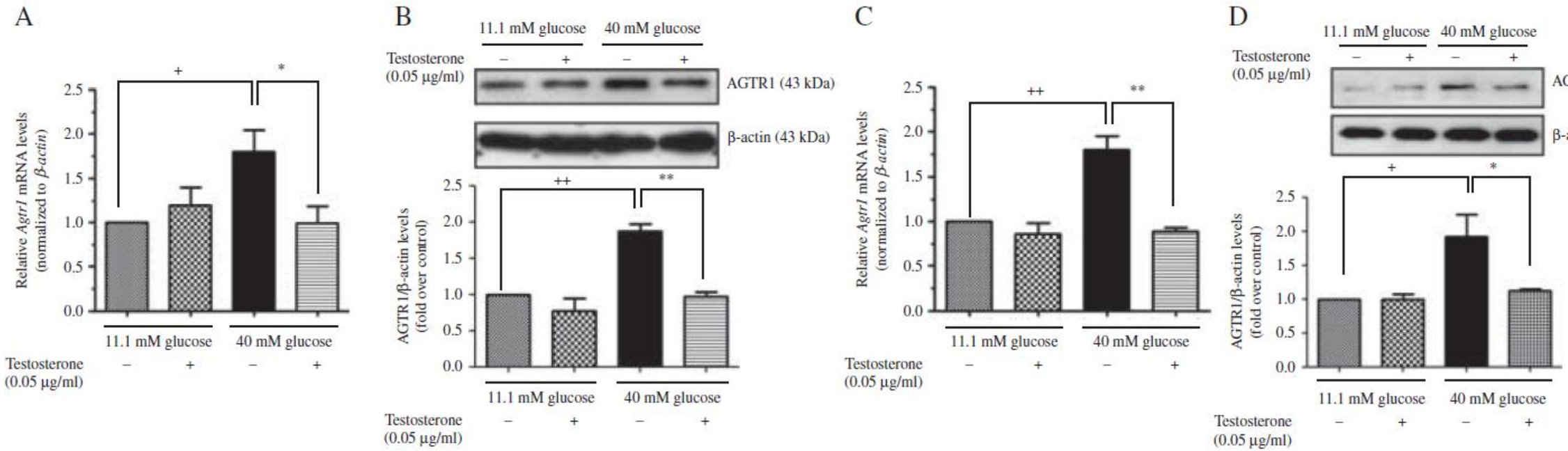

Figure 3 Effect of testosterone on the expression of Agtr1 mRNA and protein in INS-1 cells (A and B) and isolated male mouse pancreatic islets (C and D) cultured in high-glucose medium

Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

- **Testosterone decreased p47phox mRNA and protein expression in pancreatic b-cells cultured in high-glucose medium**

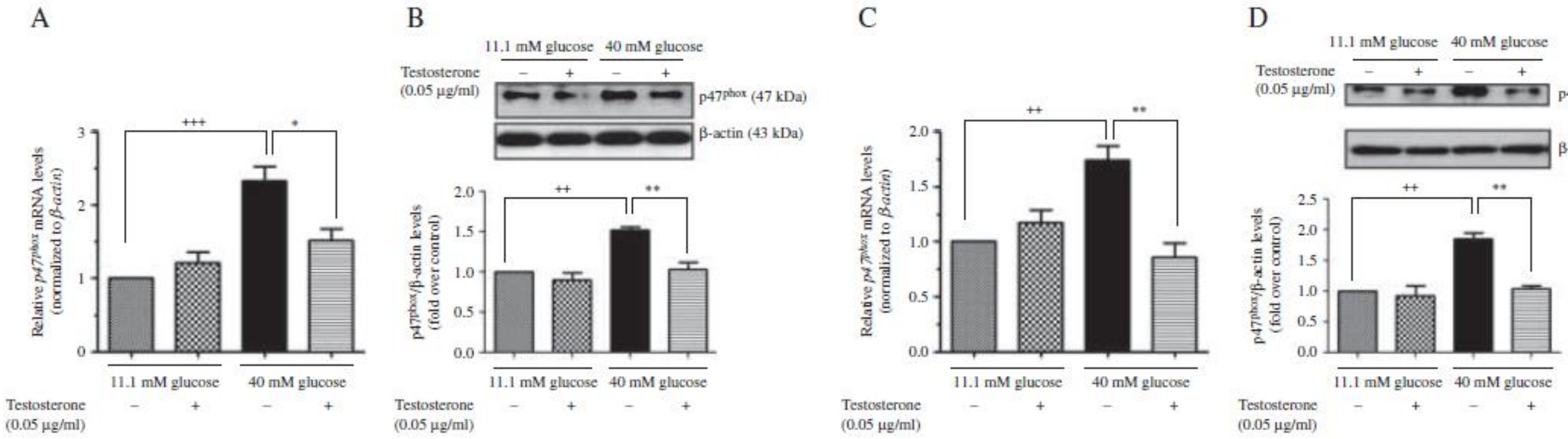

Figure 4 Effect of testosterone on the expression of p47phox mRNA and protein in INS-1 cells (A and B) and isolated male mouse pancreatic islet cells (C and D) cultured in high-glucose medium.

Testosterone reduces AGTR1 expression to prevent β-cell and islet apoptosis from glucotoxicity

Testosterone reduced superoxide production in pancreatic b-cells cultured in high-glucose medium

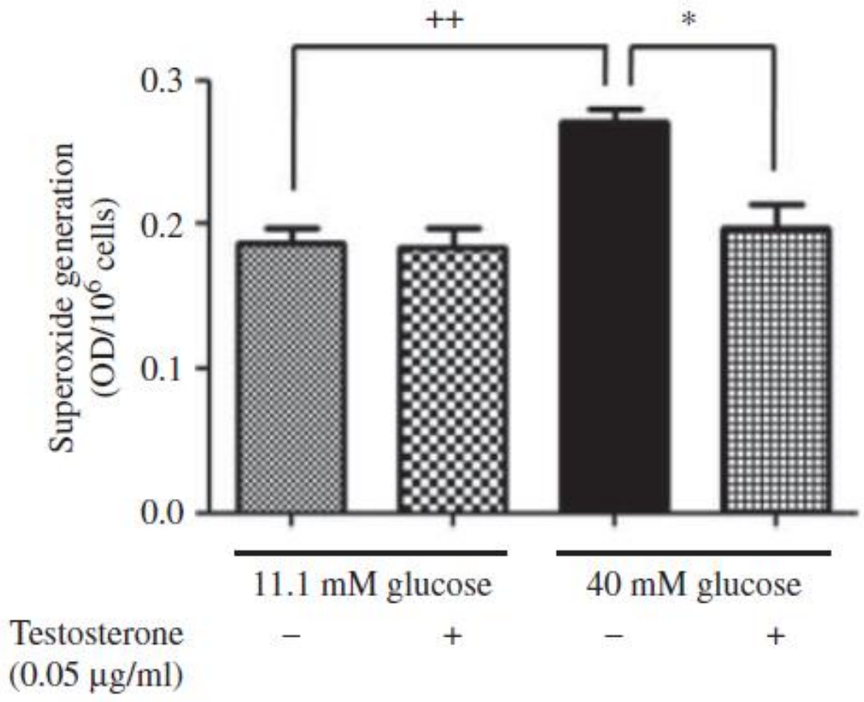

Figure 5 Superoxide production in INS-1 cells cultured in high-glucose medium.

AGTR1 knockdown rescued pancreatic b-cells apoptosis from high-glucose medium

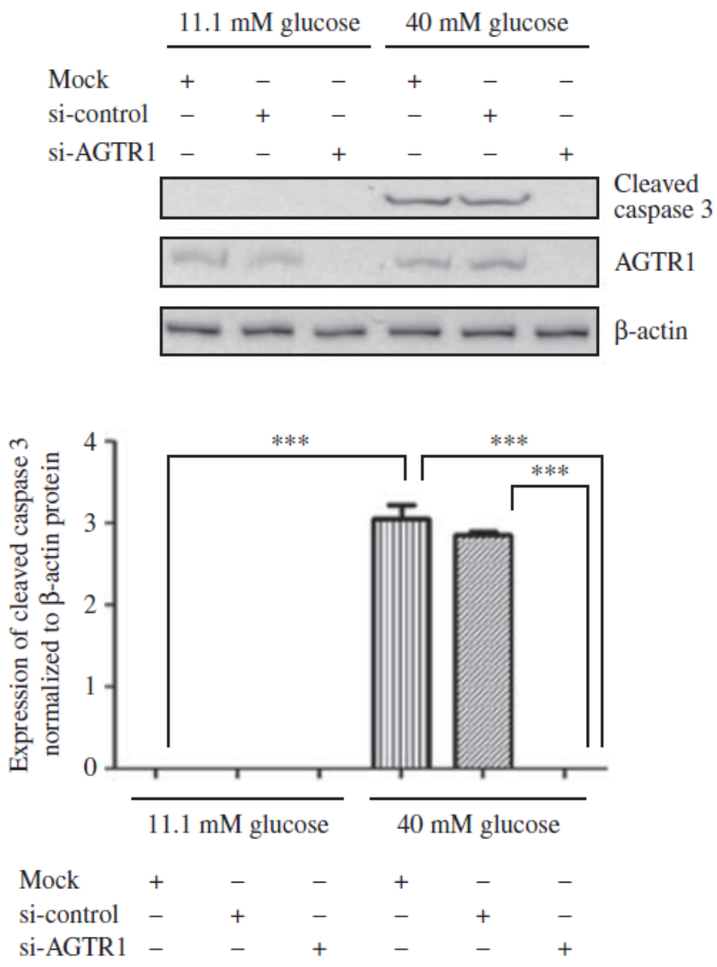

Figure 6 Effect of AGTR1 knockdown on levels of cleaved caspase 3.