mRNA EXPRESSION OF GLUCOSE TRANSPORTERS SGLT2 AND GLUT2 IN HUMAN PROXIMAL TUBULAR CELLS: EFFECTS OF TGF-β1 AND IL-1β

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Introduction

Diabetes mellitus (DM) is a major and growing health problem worldwide. Normalizing hyperglycemia is not only crucial for slowing progression of the disease process but also for preventing secondary consequences such as diabetic nephropathy. Sodium glucose cotransporters SGLT2 and SGLT1 in the apical membrane of the proximal tubule have been established as the primary mechanisms of glucose reabsorption in the kidney. Inhibitors of SGLT2 have recently been approved as new antihyperglycemic drugs in type 2 DM. Although studies in mice have shown that pharmacological SGLT2 inhibition itself increases renal SGLT2 protein expression, little is known about the regulation of proximal tubular glucose transporter expression. In the present study we analysed mRNA expression of apical SGLT2 and basolateral GLUT2 in two independent human proximal tubular cell (PTC) lines as well as its regulation by TGF-β1 and IL-1β.

Methods

RPTEC/TERT1 and HK-2 cell culture, real-time PCR.

Results

In order to define cell culture conditions, we first studied SGLT2 mRNA expression in two human PTC lines cultured for 24 h in the presence or absence of growth supplements (e.g. EGF, ITS, Hydrocortisone). In both cell lines, RPTEC/TERT1 and HK-2, 24 h supplement starvation (SS) led to a 1.6-fold and 2.5-fold induction of SGLT2 mRNA expression, respectively ($P<0.01$ and $P<0.001$; n=7) (Fig. 1). After 24 h of stimulation, 10 ng/ml TGF-β1 induced SGLT2 mRNA expression in RPTEC/TERT1 cells 4.4-fold when compared with quiescent controls ($P<0.05$; n=6) and 8.8-fold when compared with cells grown in the presence of supplements ($P<0.001$; n=6) (Fig. 2A). In HK-2 cells, on the other hand, addition of growth supplements, TGF-β1 (10 ng/ml) or IL-1β (10 ng/ml) inhibited SGLT2 mRNA expression when compared with quiescent controls (Fig. 2B). Similar results were obtained when cells were made quiescent for 48 h including a stimulation with TGF-β1 or IL-1β without medium change after 24 h (Fig. 3). Utilizing both experimental protocols, TGF-β1 and IL-1β exerted a strong inhibitory effect on GLUT2 mRNA expression in HK-2 cells after 24 h (Fig. 4).

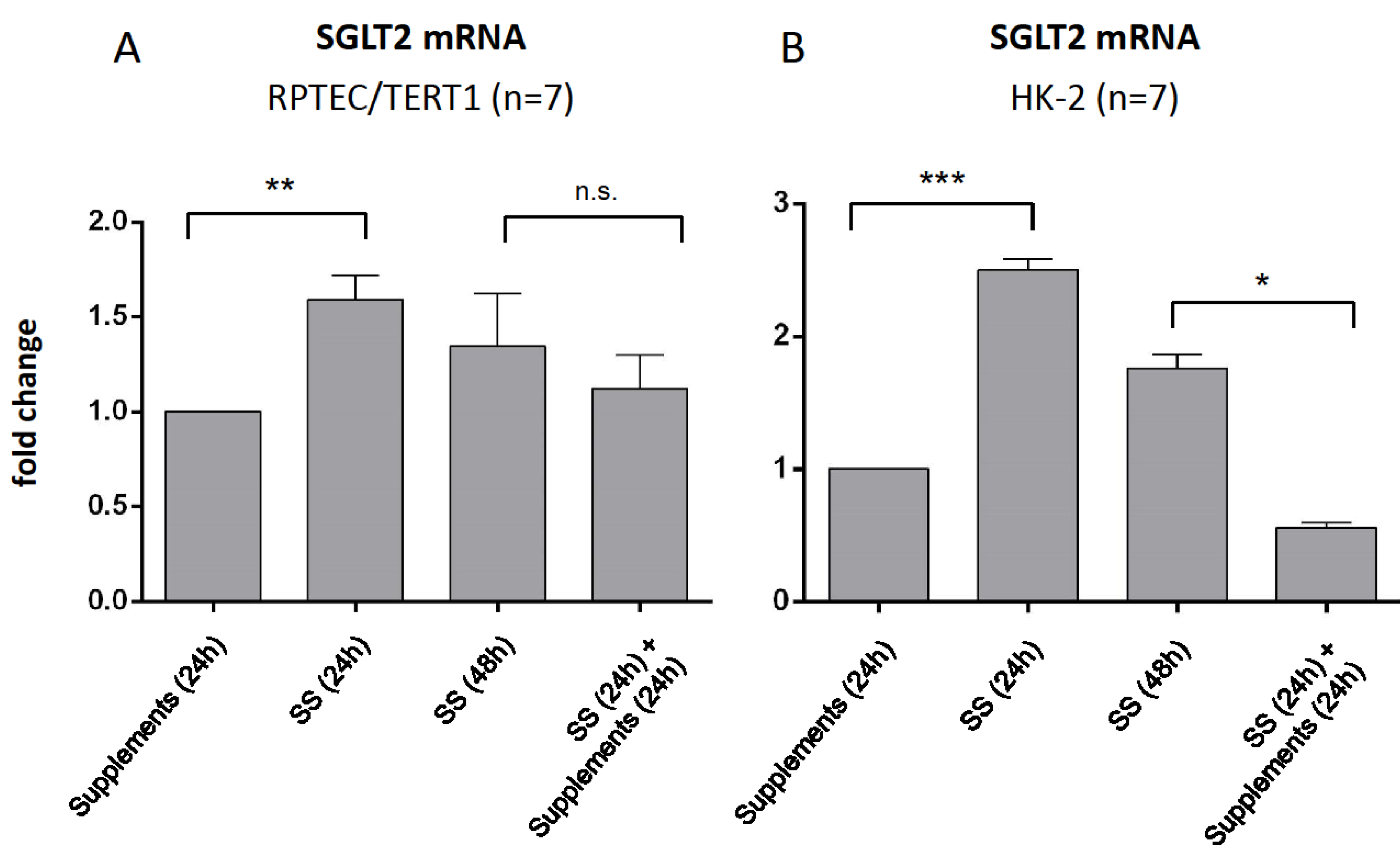

Fig. 1: Effect of cell culture conditions on SGLT2 mRNA expression in RPTEC/TERT1 cells (A) and HK-2 cells (B). SS, supplement starvation.

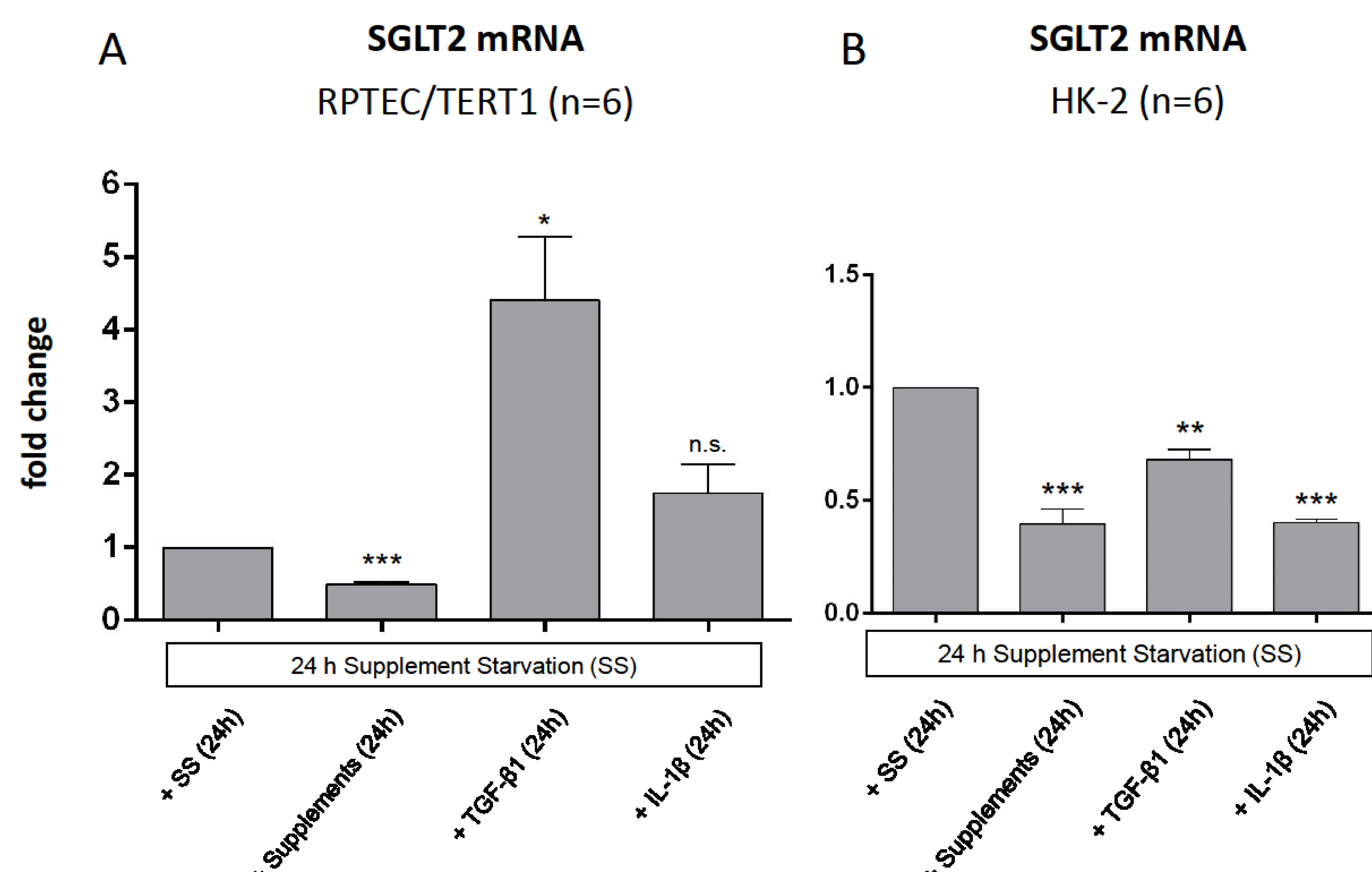

Fig. 2: SGLT2 mRNA expression in RPTEC/TERT1 cells (A) and HK-2 cells (B) after 24h of stimulation with Supplements (EGF, ITS, hydrocorstisone for RPTEC/TERT1; EGF, BPE, 10%FCS for HK-2), TGF-β1 (10 ng/ml) or IL-1β (10 ng/ml).

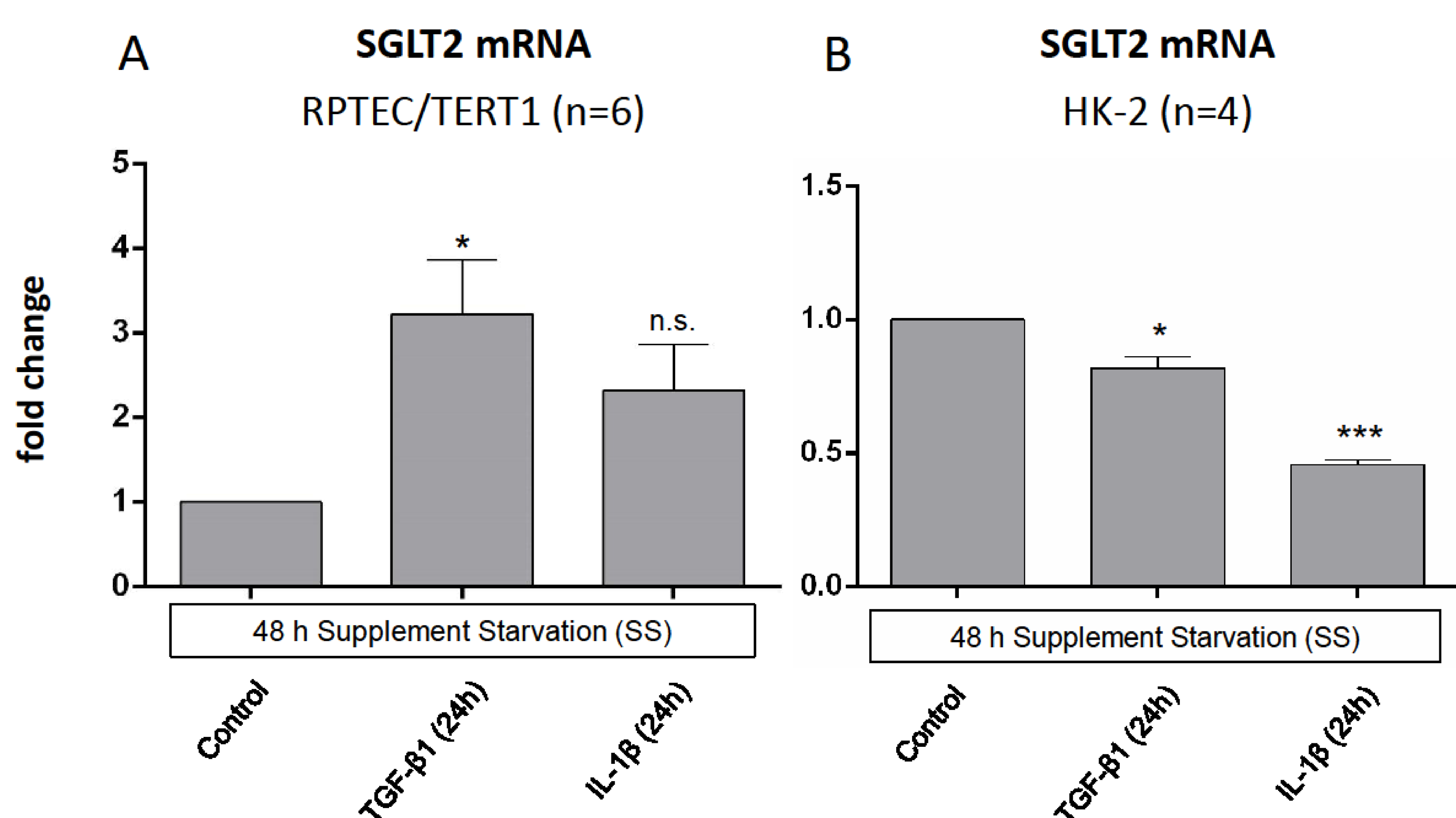

Fig. 3: SGLT2 mRNA expression in RPTEC/TERT1 cells (A) and HK-2 cells (B) after 24h of stimulation with TGF-β1 (10 ng/ml) or IL-1β (10 ng/ml).

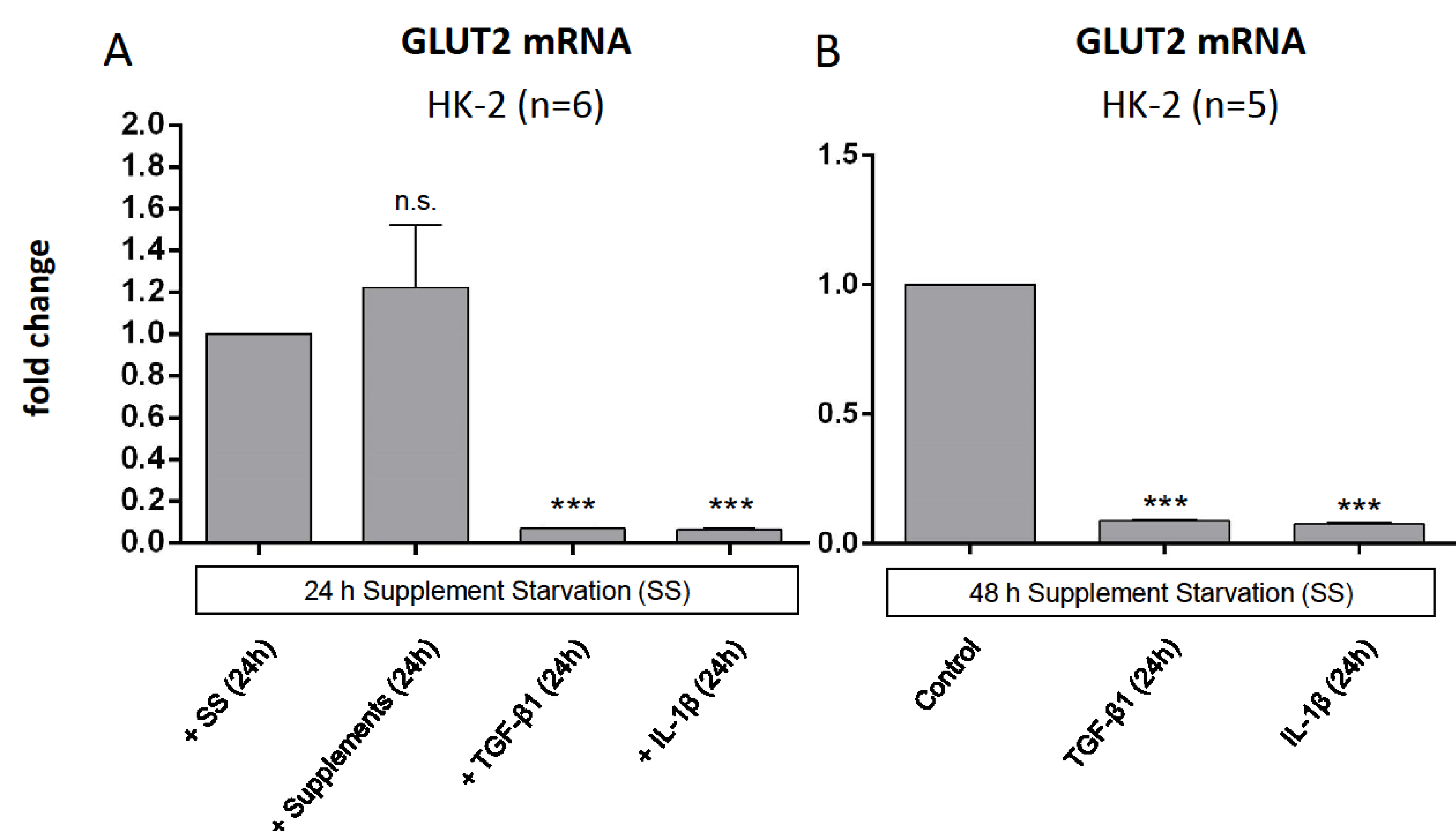

Fig. 4: GLUT2 mRNA expression in HK-2 cells supplement starved for 24h (A) or 48h (B) and stimulated with TGF-β1 (10 ng/ml) or IL-1β (10 ng/ml) for 24h.

Conclusion

Human PTC lines RPTEC/TERT1 and HK-2 represent valuable cell models to study expression of proximal tubular glucose transporters and its regulation. In PTCs stressed by growth supplement starvation, increased expression of SGLT2 may protect cells from intracellular glucose depletion. While TGF-β1 represents a stimulator of SGLT2 mRNA expression in RPTEC/TERT1 cells, both ligands, TGF-β1 and IL-1β, are strong inhibitors of GLUT2 mRNA expression in HK-2 cells. It is tempting to speculate that, during early inflammatory events in DM, mRNA expression of proximal tubular glucose transporters may be differentially affected by proinflammatory and/or profibrotic cytokines.

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