# Effect of SGLT2 inhibitors on fibroblast activation

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## Summary


- Fibroblasts are a dynamic cell type that contribute to cardiac function. TGFβ activates quiescent fibroblasts and increases expression of smooth muscle actin (*Acta2*) and decreases expression of peroxisome proliferator-activated receptor-gamma coactivator (*Pgc1a*).
- SGLT2 inhibitors (SGLT2i) provide cardiovascular benefits independent of glucose control – we hypothesize SGLT2 inhibitors may partially blunt oxidative stress and fibroblast activation.
- Treatment with high glucose activates fibroblasts and increases *Acta2* but does not suppress *Pgc1a*.
- Co-treatment of 3T3s with SGLT2i, TGFβ, and glucose is not sufficient to blunt fibroblast activation. Timing of in-vitro SGLT2i administration likely impacts the transcriptional response of fibroblasts. Future experiments will be performed to pre-treat 3T3s with SGLT2i prior to glucose and TGFβ treatment.
- NRF2 is a key regulator of oxidative stress and changes in nuclear localization can be quantified in 3T3s to evaluate potential effects of SGLT2i on modulating oxidative stress in fibroblasts.


## Introduction

Cardiac remodeling is the process by which the heart responds to increasing workload and is frequently driven by humoral and/or mechanical activation of cardiac fibroblasts (CFs). Heart failure is characterized by sustained activation of CFs and increased workload which eventually contribute to irreversible cardiomyocyte loss and thinning of the ventricular walls.

Recent work demonstrated that fibroblasts isolated from pressure overload models become activated and decrease oxidative stress pathway activity, yet when fibroblasts were isolated from control or exercised animals, these oxidative stress pathways were maintained. This suggests that a robust oxidative stress mechanism promotes cardiac function and is lost or depleted in disease. Indeed, models of diabetic cardiomyopathy demonstrate parallels in the loss of detoxification mechanisms, increased fibrosis, and cardiac dysfunction, suggesting that insufficient glucose control contributes to pathological remodeling.

Sodium glucose linked co-transporter 2 inhibitors (SGLT2i) such as empagliflozin or ertugliflozin were initially developed initially to manage high-glucose conditions for diabetes mellitus but quickly showed cardiovascular benefits in patients without diabetes mellitus. We hypothesize that fibroblasts exposed to glucose experience oxidative stress and are sensitized to pathological stimuli such as TGFβ due to depletion of a protective antioxidant pathway.

## Methods

Mouse embryonic fibroblasts (3T3s) were treated with a combination of glucose, transforming growth factor beta (TGFβ), and either empagliflozin or ertugliflozin. Transcriptional activity was assessed by quantitative PCR (qPCR) against genes known to change in response to oxidative and pathological stimuli such as smooth muscle actin (*Acta2*) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc1a*). Protein expression was assessed by Western blot to evaluate changes in the nuclear localization of the antioxidative response transcription factor nuclear factor erythroid 2 (NFE2)-related factor 2 (NRF2).

## Results

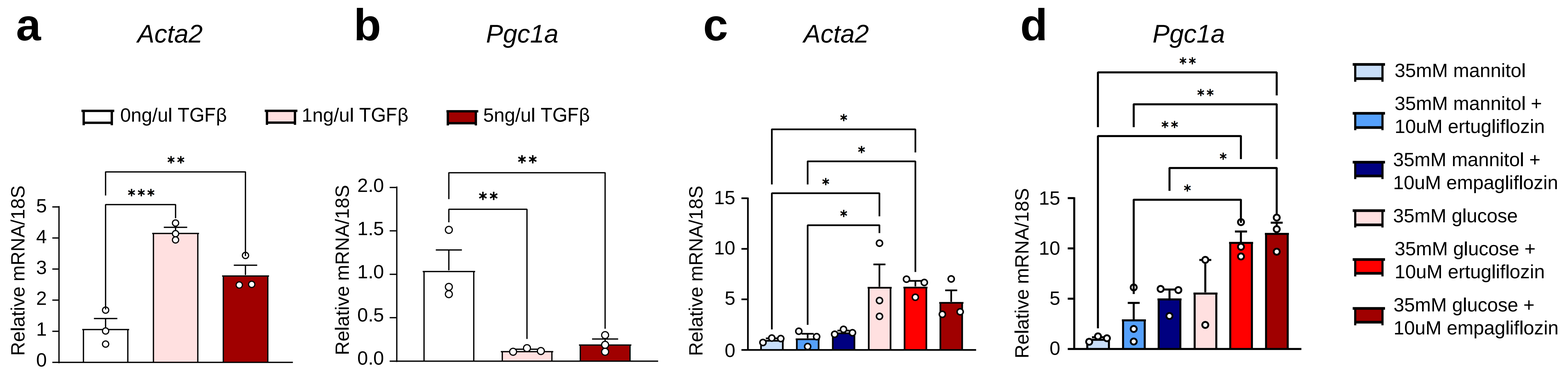


**Figure 1.** TGFβ treatment increases 3T3s treated with 1ng/ul TGFβ **(a,b)** reliably induced *Acta2* expression two- to five-fold over baseline and decreased *Pgc1a* expression to less than 10% of baseline as expected. Treatment with 35mM glucose **(c,d)** was sufficient to activate *Acta2* without decreasing *Pgc1a* expression, suggesting the oxidative response pathway is not depleted with short term glucose treatment. Addition of 10uM empagliflozin or ertugliflozin alone had no significant impact on *Acta2* or *Pgc1a* expression but appear to promote maintenance of *Pgc1a* expression. Addition of 35mM mannitol served as an osmotic control. * p<0.05

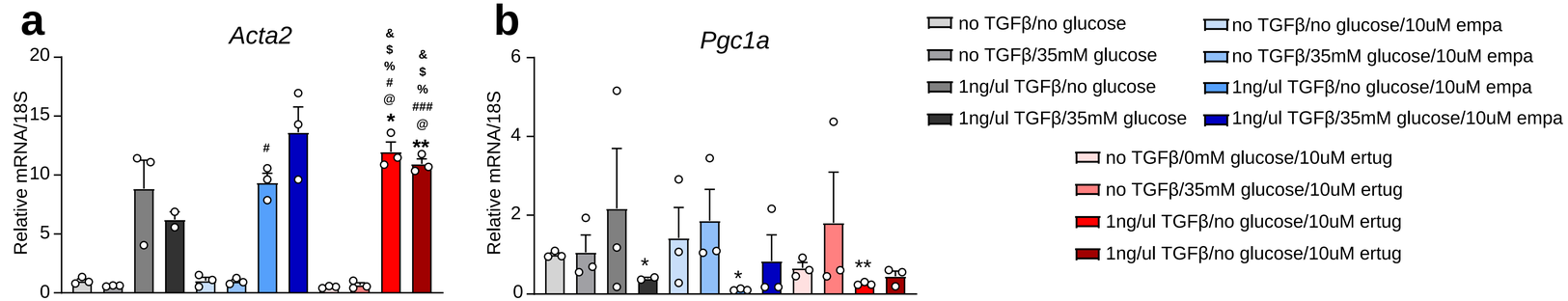


**Figure 2.** Simultaneous treatment of 3T3s with TGFβ, glucose, and either empagliflozin (empa) or ertuglifozin (ertug) did not blunt *Acta2* induction as expected or significantly maintain antioxidant response pathways as assessed by increased expression of *Pgc1a*. * indicates significantly different from no TGF 0mM glucose; @no TGF 35mM glucose; #no TGF 0mM glucose empagliflozin; %no TGF 35mM glucose empagliflozin; $no TGF 0mM glucose ertugliflozin; &no TGF 35mM glucose ertugliflozin; *p<0.05 **p<0.01.

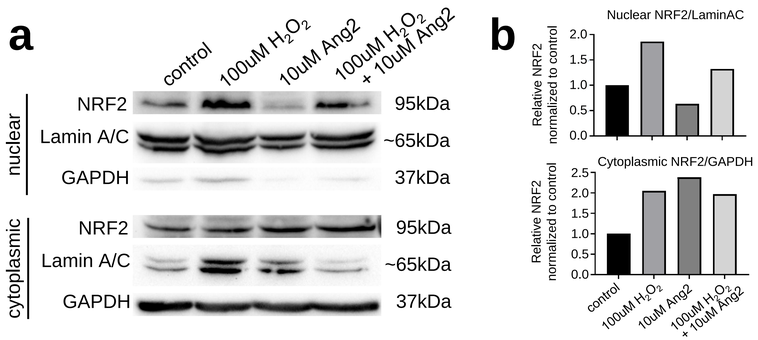


**Figure 3.** Protein expression **(a)** and quantification **(b)** of NRF2 suggests 10uM Ang2 treatment is sufficient to prevent nuclear localization NRF2. Retention of NRF2 in the cytoplasm is consistent with decreased activity of oxidative stress pathways.

## References


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