

The Effect of Sucralose on Levels of Nitric Oxide Synthase in Endothelial Cells

Olivia Rogers
Dr. Yinan Hua
Dr. Sreejayan Nair
School of Pharmacy

Introduction

- Nitric oxide in endothelial cells regulates vascular tone, local cell growth, angiogenesis, and is critical for cardiovascular homeostasis.
- Oxidative stress decreases nitric oxide synthase (eNOS) levels and can cause atherosclerosis, hypertension, cardiovascular disease, asthma, COPD, cancer, and diabetes.
- Sucralose is a commonly used sweetener which is a chlorinated form of sucrose (table sugar). It is 600 times sweeter than sucrose and has no calories.

Aim

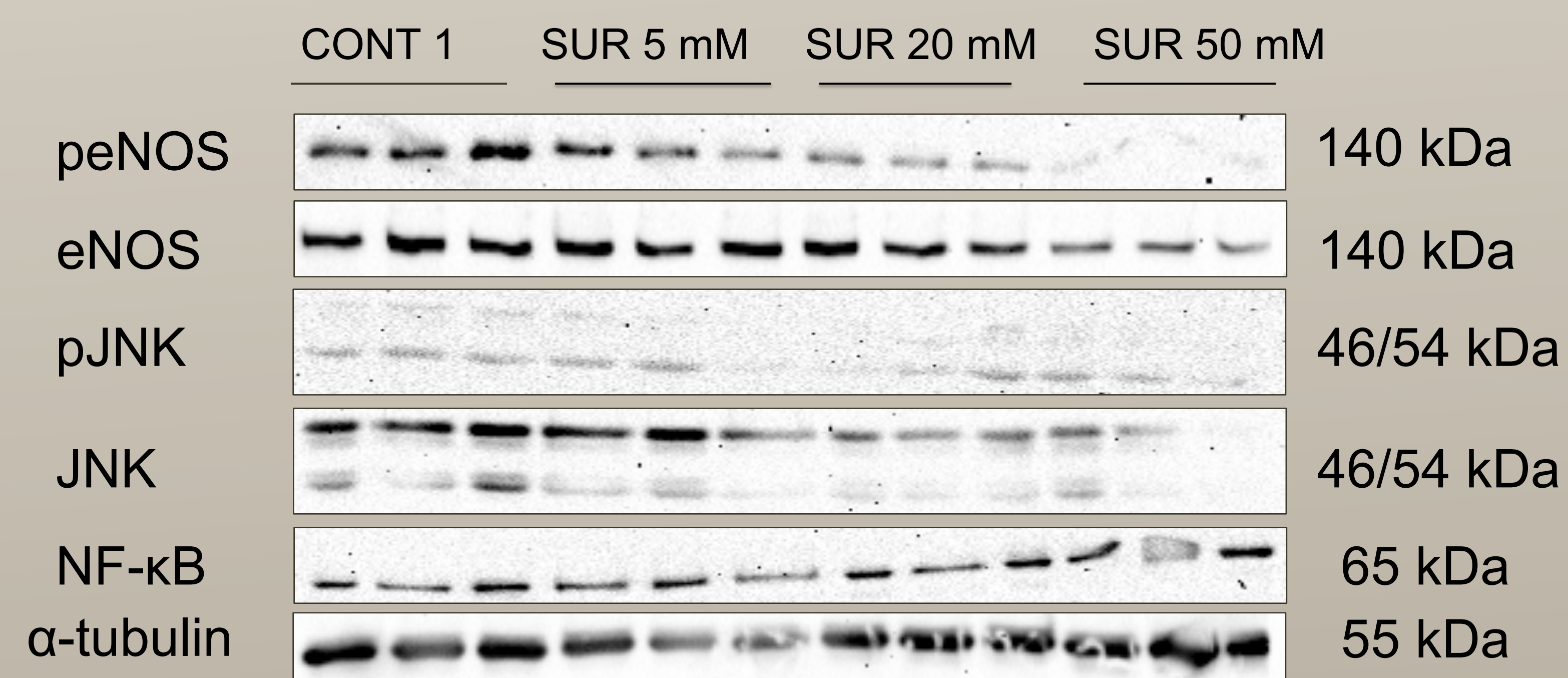
The objective of this study is to test the hypothesis that sucralose triggers oxidative stress and endothelial cell dysfunction in human umbilical vein endothelial cells.

Materials and Method

Cell Culture: Human umbilical vein endothelial cells (HUVECs) were cultured in an incubator at 37°C with 5% CO₂. The medium used was DMEM plus FBS. The cells were treated with concentrations of 5 mM, 20 mM, 50 mM sucralose, as well as a control. DCF Staining: A fluorescent dye detects levels of oxidative stress on endothelial cells. Cells were incubated with a concentration of 20 mM DCF stain for one hour. After this, cells were washed with PBS buffer and observed under an Olympus fluorescence microscope. The darker the green color, the higher oxidative stress the cells contain as shown in Fig. 2.

Gel-Electrophoresis and Western Blot: Samples were lysed in RIPA buffer and separated onto an SDS gel. After, the proteins were transferred to a nitrocellulose membrane. The membranes were then incubated in P-eNOS, eNOS, P-JNK, JNK, and NF-κB antibodies overnight before being incubated with an anti-rabbit IgG HRP-linked antibody for one hour at room temperature.

Results



A. Fig. 1 Western Blot Analysis

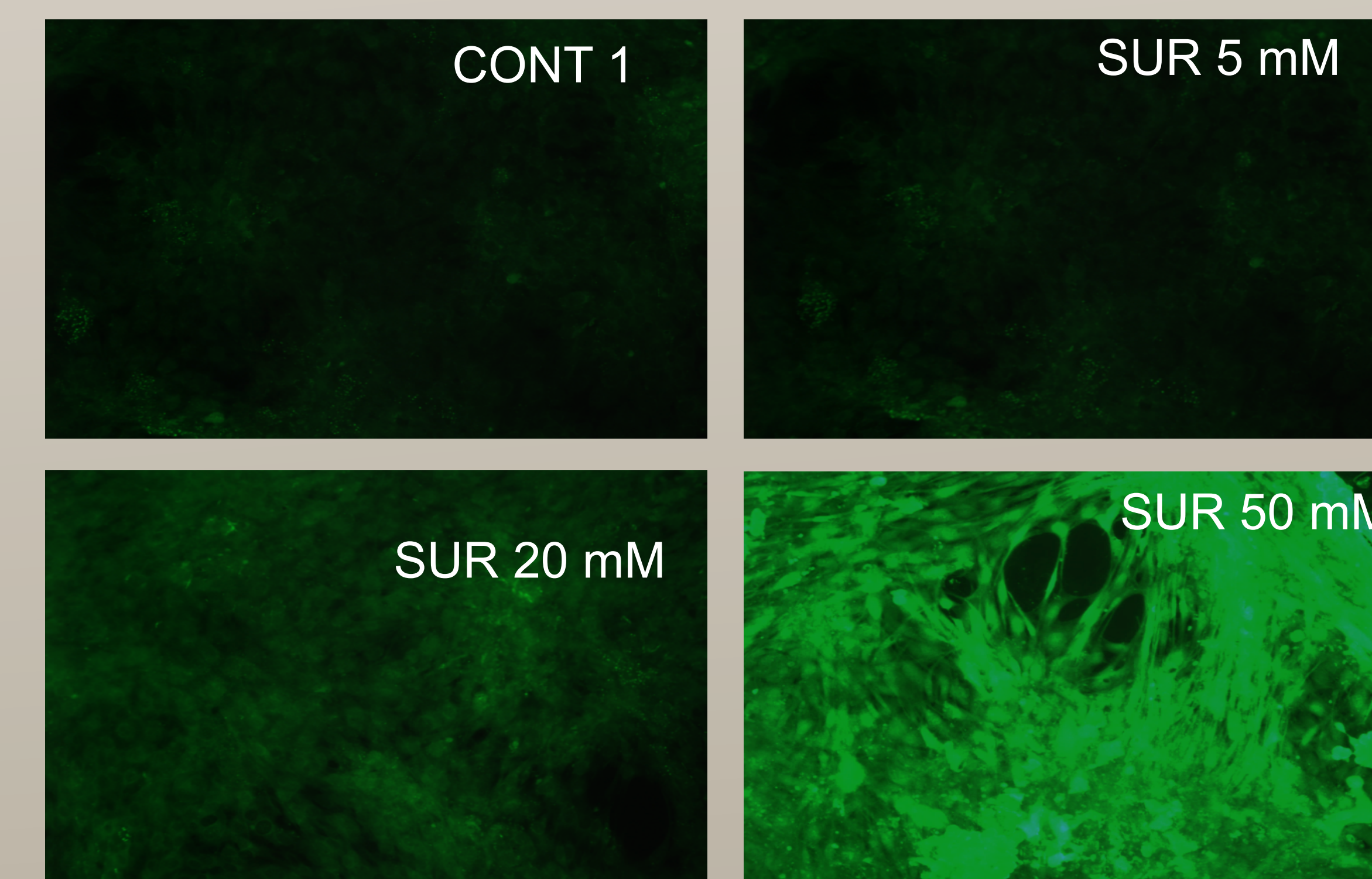
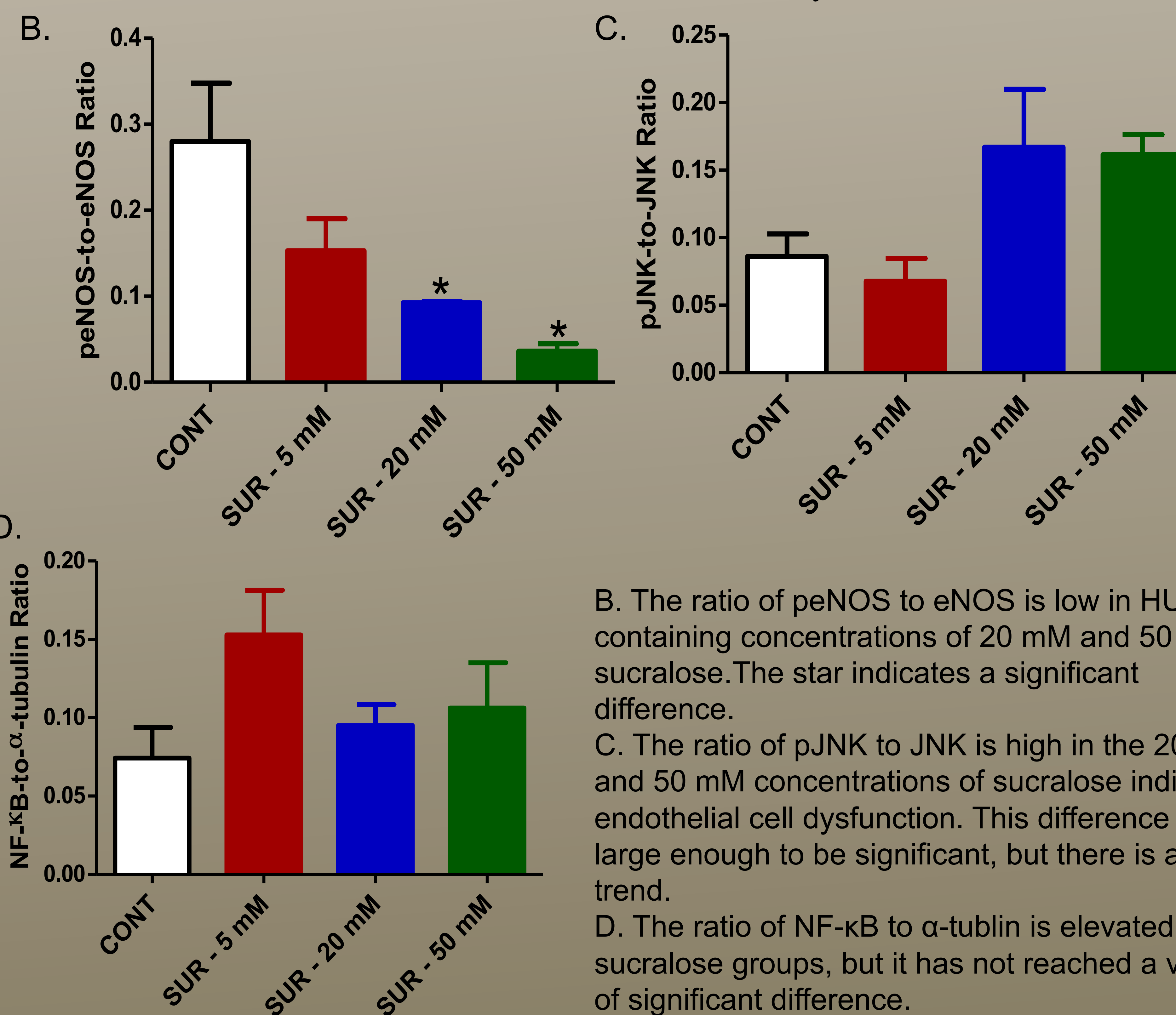


Fig. 2 DCF staining shows high levels of oxidative stress in HUVECs incubated with 20 mM sucralose 50 mM sucralose.

Quantitative Results for Western Blot Analysis



B. The ratio of peNOS to eNOS is low in HUVECs containing concentrations of 20 mM and 50 mM sucralose. The star indicates a significant difference.

C. The ratio of pJNK to JNK is high in the 20 mM and 50 mM concentrations of sucralose indicating endothelial cell dysfunction. This difference is not large enough to be significant, but there is a trend.

D. The ratio of NF-κB to α-tubulin is elevated in all sucralose groups, but it has not reached a value of significant difference.

Conclusions

- Sucralose at higher concentrations induces oxidative stress and reduces eNOS expression in cultured HUVECs.
- Sucralose at higher concentrations also increases inflammatory markers.
- Based on these findings it is likely that sucralose consumption may lead to endothelial dysfunction.

References

- Stefanie Dimmeler, Ingrid Fleming, Beate Fisslthaler, Corinna Hermann, Rudi Busse & Andreas M. Zeiher. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. International Weekly Journal of Science. May 6, 1999.
- Guigliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Europe PubMed Central. 1996.