The Effects of Nicotine and High Fat Diet on Circadian Gene Expression in Human Aortic Smooth Muscle Cells

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Introduction

- The 24-hour human circadian rhythm is responsible for the regulation of many biological processes.
- The circadian rhythm is, at its core, maintained by the CLOCK and ARNTL (also known as BMAL1) genes. These genes encode for transcription factors that induce the expression of the CRY1, CRY2, PER1, and PER2 genes. The proteins encoded for by these genes then inhibit the actions of CLOCK and ARNTL, creating a negative feedback loop that results in the 24-hour circadian rhythm **(Figure 1)**.

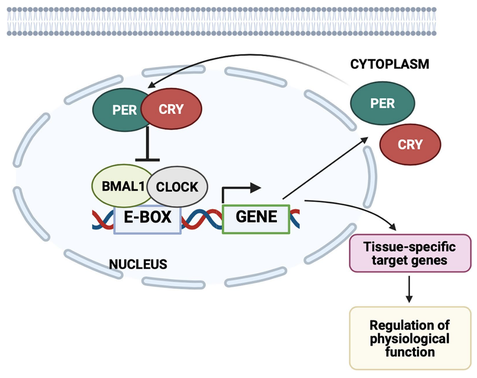

Figure 1. The negative feedback loop of the core circadian clock genes (Costello, Hannah M., and Michelle L. Gumz. "Circadian rhythm, clock genes, and hypertension: Recent advances in hypertension." *Hypertension*, vol. 78, no. 5, 4 Oct. 2021.)

- Disruption of the circadian rhythm has been linked to a variety of pathologies spanning multiple systems in the body, including the cardiovascular system. Similarly, both nicotine consumption and the consumption of a high fat diet, particularly a diet high in saturated fats, have been linked to cardiovascular dysfunction.
- In this study, we investigated the effects of nicotine and palmitate, a saturated fatty acid, on the proliferation and gene expression of human aortic smooth muscle cells (HAoSMCs).
- The genes analyzed in this study were the TBP gene (a housekeeping gene used to normalize data) and the circadian genes CLOCK, ARNTL, PER1, PER2, CRY1, and CRY2. The ACE1 and ACE2 genes were also analyzed due to their roles as major regulators of cardiovascular function

Methods

Synchronization of Circadian Rhythms

- The circadian rhythms of HAoSMCs were synchronized using the serum shock technique with a mixture of basal medium, antibiotics and antimycotics, and 50% fetal bovine serum.

Establishment of Treatment Groups

- HAoSMCs were cultured in the absence (control) or presence of 200μM palmitate, 0.5μM nicotine, or the combination of 200μM palmitate and 0.5μM nicotine.

RNA Extraction

- The RNeasy Plus Kit (QIAGEN) was used to extract RNA at 0-, 4-, 8-, 12-, 16-, 20-, and 24-hour time points.
- The RNA was then analyzed via quantitative Reverse Transcription PCR (RT-qPCR) using a SYBR Green Master Mix in conjunction with the iScript cDNA Synthesis Kit.

Measurement of Cell Proliferation

- An MTT Cell Proliferation Assay was conducted on cells from all four treatment groups at the 48-hour time point.

Results: Proliferation Assay

- The MTT Cell Proliferation Assay results show that both nicotine and palmitate inhibit proliferation of HAoSMCs, with palmitate inhibiting proliferation to a much greater degree (**Figure 2**).

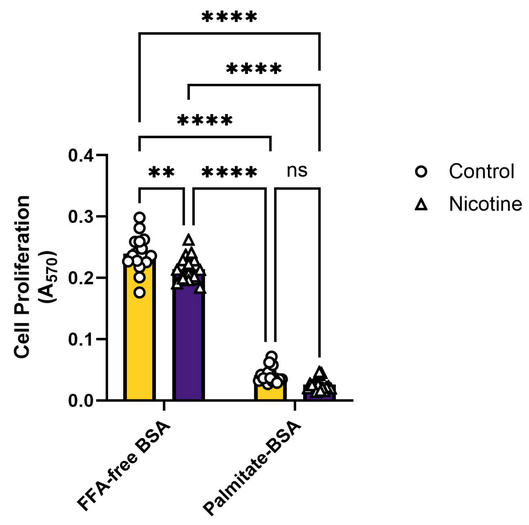

Figure 2. MTT Cell Proliferation Assay

Results: RT-qPCR

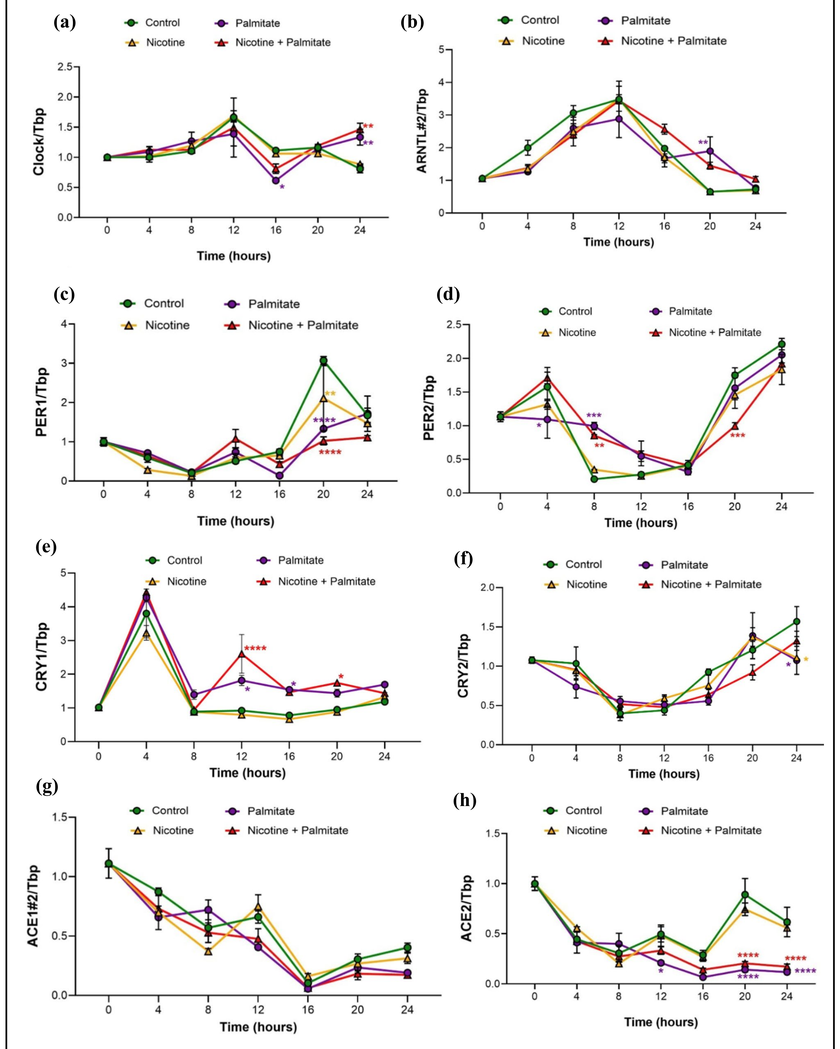

Figure 3. RT-qPCR results showing that treatment of HAoSMCs with palmitate or combination nicotine + palmitate alters expression of ACE2 and of circadian genes CLOCK, ARNTL, PER1, PER2, CRY1, and CRY2. Treatment with nicotine also alters expression of PER1 and CRY2.

Conclusion

- The results suggest that a diet consisting of large amounts of saturated fatty acids can decrease cell viability and alter the expression of some circadian genes, as well as the ACE2 gene, in HAoSMCs. Overall, the results suggest that a diet high in saturated fatty acids can be detrimental to cardiovascular health and function.
- Future studies investigating these effects should experiment with differing types and concentrations of fatty acids, and they should look into the mechanism underlying the toxicity and genetic alterations related to fatty acid treatment.