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

Background: The human circadian rhythm is responsible for the regulation of many physical, emotional, behavioral, hormonal, and genetic properties. This circadian rhythm is itself regulated and maintained by the circadian genes, a set of genes whose interactions result in their having a rhythmic expression pattern. Disruption of the circadian rhythm has been shown to lead to a variety of pathologies spanning across multiple organ systems, such as cardiovascular, endocrine, and gastrointestinal issues, among others. Additionally, both nicotine consumption and a high fat diet have been shown to impact cardiovascular health. In this study, we aim to investigate the effects of high fat diet and nicotine consumption on circadian gene expression in human aortic smooth muscle cells (HAoSMCs).

Methods: HAoSMCs were cultured to 80% confluency before being serum shocked to synchronize the circadian rhythms of the cells. The cells were then cultured in the absence (control) or presence of 200μM palmitate (a saturated fatty acid), 0.5μM nicotine, or the combination of 200μM palmitate and 0.5μM nicotine. RNA was extracted from each treatment group every 4 hours for 24 hours. The RNA was then analyzed via quantitative Reverse Transcription PCR (RT-qPCR) to examine the expression of the following circadian genes — PER1, PER2, CRY1, CRY2, Clock, and ARNTL (also known as Bmal1). Angiotensin converting enzyme 1 and 2 (ACE1 and ACE2) were also studied, as they are the major regulators of cardiovascular function. Changes in gene expression over the 24-hour period were analyzed using the $2^{-\Delta\Delta CT}$ method, with data being normalized to the housekeeping gene Tbp. Additionally, the proliferation of HAoSMCs with the same treatments was examined using an MTT Cell Proliferation Assay at 48 hours.

Results: The RT-qPCR revealed that the palmitate and combination nicotine + palmitate treatments disrupted the circadian expression patterns of CLOCK, PER1, PER2, CRY1. Notably, ACE2 expression appeared to follow a circadian rhythm as well, with increased expression at 20- and 24-hour time points, which was blocked in the presence of palmitate. Interestingly, unlike ACE2, ACE1 did not experience any significant reduction in gene expression from any treatment at any time point. Meanwhile, the proliferation assay revealed that both nicotine and palmitate inhibited HAoSMC proliferation with palmitate exerting a significantly greater effect.

Conclusions: The results suggest that a diet consisting of large amounts of saturated fatty acids can alter the expression of some circadian genes in HAoSMCs, as well as being able to significantly downregulate the expression of the ACE2 gene in HAoSMCs. The results of the cell proliferation assay also show that continual exposure to fatty acids is highly detrimental to the survival of HAoSMCs. Overall, our study suggests that a diet high in saturated fatty acids can be extremely detrimental to cardiovascular health.