THE POTENTIAL ROLE OF PHOSPHOPROTEIN ENRICHED IN ASTROCYTES-15 (PEA15) IN THE REGULATION OF CIRCADIAN RHYTHM AND METABOLIC DISEASE.

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Introduction & Background

Phosphoprotein Enriched in Astrocytes-15 (PEA15) is a key regulator in the mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway. The MAPK/ERK pathway plays a critical role in the timing of the suprachiasmatic nucleus (SCN) of the hypothalamus which is central to circadian rhythm timing. The circadian rhythm controls energy homeostasis through the regulation of enzymes, hormones, and transport systems involved in metabolism. Disruption of the circadian rhythm contributes to metabolic disease, specifically obesity and insulin resistance. Because of the connection between metabolism and circadian rhythm, our lab explores the effect of PEA-15 loss of function on metabolic flexibility, with further studies exploring PEA15's effect in the circadian rhythm.

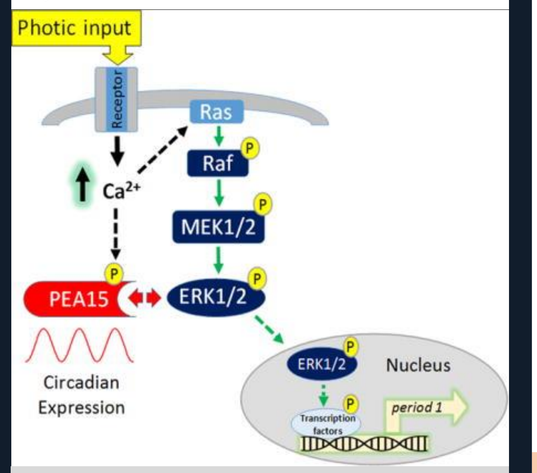


Figure 1. PEA-15's effect on MAPK/ERK pathway. PEA-15 blocks ERK-dependent transcription and proliferation through binding ERKs together, anchoring them to the cytoplasm, and preventing their localization in the nucleus. PEA-15 then functions as a regulator in the MAPK/ERK pathway. This pathway leads to transcription of the PER1 gene, a key regulator in circadian rhythm. PEA-15 gene expression is highly expressed in the hypothalamus, indicating it as a key regulator in the SCN timing system. Loss of function of PEA-15 causes increased ERK nuclear localization. As a result, cell proliferation increases, indicating that PEA-15 can redirect the biological outcome of MAPK kinase signaling.(1) Loss of circadian control has been shown in recent studies to be coupled with increased proliferation rates, where dysregulation of proliferation contributes to the pathology of many degenerative diseases.

Preliminary Results

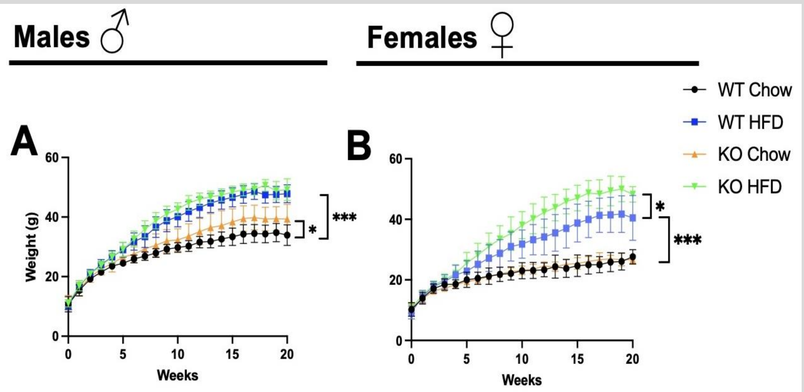


Figure 2. PEA 15 alters body weight in a sex-specific manner.

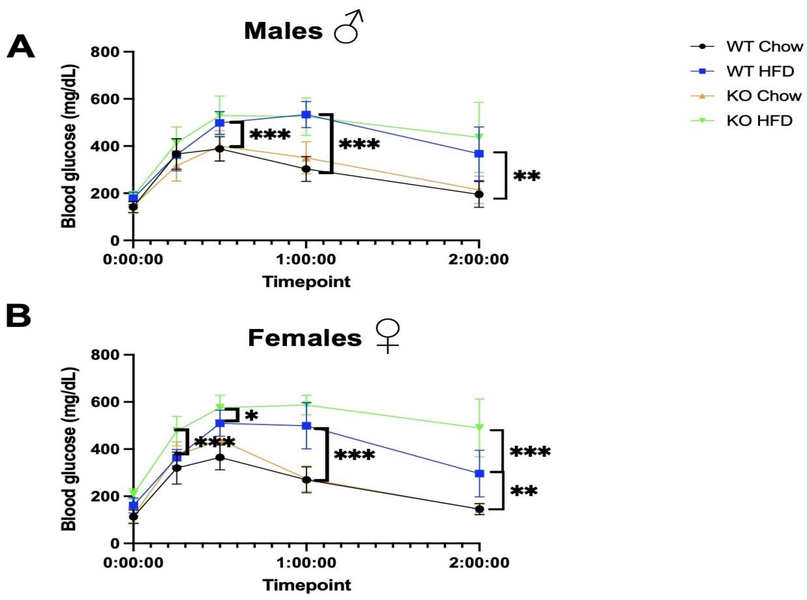


Figure 3. PEA15 alters glucose tolerance in a sex specific manner.

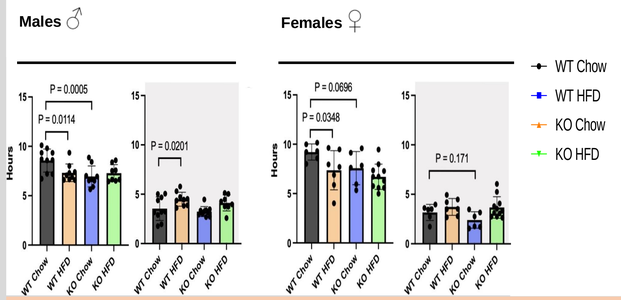


Figure 4. PEA15 alters sleep behavior.

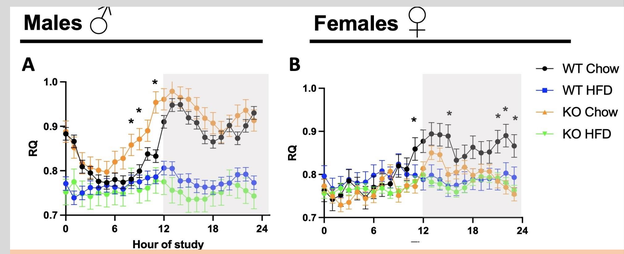


Figure 5. PEA15 shifts substrate utilization in a sex specific manner.

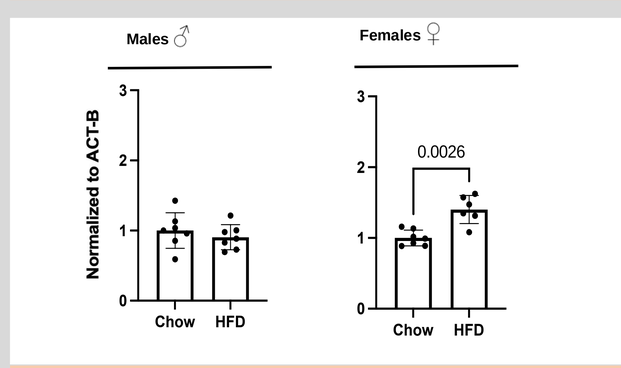


Figure 6. HFD increase PEA15 expression in the hypothalamus of the female mice but not in the male mice.

Proposed Study

Based on these results, we hypothesize that PEA15 loss of function disrupts circadian rhythm and contributes to metabolic disease. We will test this hypothesis through total RNA isolation followed by cDNA synthesis and evaluation of relative gene expression using custom-designed Taqman PCR array plates. We will determine changes in these 7 key regulators: Cry 1, Cry 2, Per1, Per2, Bmal, Clock, and NPAS2. These changes will be examined in the hypothalamus, liver, and adipose tissues of mice in the following treatment conditions: chow wild type (WT), chow knockout (KO), High Fat Diet (HFD) WT, and HFD KO. To evaluate sex as a biological variable, we will perform these studies in both male and female mice.

Materials and Methods

We will evaluate relative gene expression of the 7 key regulators of circadian rhythm in the hypothalamus, liver ,and adipose tissue of PEA15 KO mice and their WT littermate controls fed either a chow or HFD diet for 20 weeks. A total of 80 mice will be evaluated: 40 males, 40 females. We will take frozen tissues from the liver, hypothalamus, and adipose tissue and extract total RNA using Qiagen total RNA isolation kits. RNA will be converted into cDNA with reverse transcriptase, and PCR will be conducted through the custom circadian rhythm gene expression Taqman PCR array plates from Thermofisher. We will also evaluate three housekeeping genes to identify the appropriate controls: TPB, Actin, and YWHAZ. Gene expression will be normalized to the appropriately stable housekeeping gene and an ANNOVA will be performed to evaluate changes in gene expression between the treatment groups.

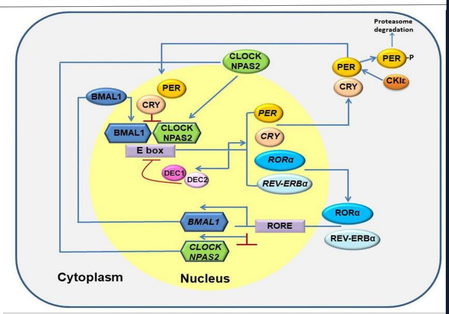
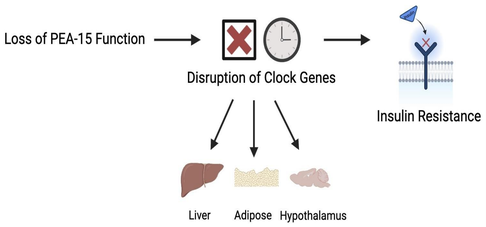


Figure 7. Schematic representation of circadian clock genes (2).

Expected Results



We hypothesize that the loss of function of PEA 15 disrupts the clock genes in adipose tissue, liver, and hypothalamus. This disruption, in turn, leads to central insulin resistance, ultimately contributing to the onset and progression of insulin resistance and metabolic diseases. Through our previous research, our laboratory has demonstrated that a high-fat diet leads to a notable increase in the expression of the PEA15 gene in the hypothalamus. Based on this finding, our current hypothesis anticipates a corresponding increase in gene expression in the adipose tissue and the liver as well. These results are expected to provide valuable insights into the role of PEA15 in the expression of clock genes across different tissues. Our research then will contribute to the broader understanding of circadian rhythm and their intricate connection to the development of metabolic diseases.

Acknowledgements

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References