Circadian Disruption of Core Clock Genes *Bmal1, Reverb-a, Per2, and Cry1* in Adipose Tissue Due to Western Diet-Induced Obesity

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The Western diet (WD) is characterized by consumption of a high proportion of fat and sugar and is linked to chronic metabolic diseases including cardiovascular disease, diabetes, and obesity. Approximately 30% of the genes in the mammalian organs follow light-induced circadian rhythms controlled by the suprachiasmatic nucleus (SCN) in the hypothalamus. Disruption of circadian rhythmicity can lead to obesity and chronic metabolic diseases; however, the converse has also been observed: WD-induced obesity can disrupt peripheral circadian rhythms.

Four core clock genes that control how the SCN regulates rhythmicity in the central clock and peripheral tissues (those beyond the hypothalamus) are *Bmal1, Reverb-a, Per2, and Cry1*. By examining core clock and clock-controlled gene expression in various peripheral tissues, we can validate that the WD regulates peripheral clocks in key metabolic tissues related to obesity. Thus, we examined core clock gene expression in epididymal white adipose tissue (eWAT), a type of visceral adipose tissue in mice with a role in whole-body energy and glucose homeostasis.

Male C57NL/6N mice were fed *ad libitum* a Chow diet (n=42) or Western high fat and sugar diet (WD+S) (n=42). After 16 weeks of feeding, the animals were sacrificed every 6 hours (n=7 per time point). Tissue RNA was analyzed with Real Time Quantitative Polymerase Chain Reaction (RT-qPCR) to examine core clock gene expression, with GADPH used as the housekeeping gene. The rhythmicity and expression levels were then compared between the Chow diet control group and the WD+S group and graphed according to the acrophase, which is the timepoint with the highest level of expression over the 24-hour period. To monitor changes in rhythmicity, acrophases were compared between the groups; a difference between these was denoted as a "phase advancement" in hour units. As shown in Figure 1, *Bmal1* and *Per2* had similar differences; both were phase-advanced by 4 hours and dampened, or less expressed, in the WD+S Group compared to the Chow. *Reverb-a* and *Cry1* also shared differences; both were phase-advanced by 12 hours (a directly opposing shift). The overall acrophase timeframe of these genes altogether doubled in range, from a 9-hour window (12-21 hours) to 18 hours (0-18 hours).

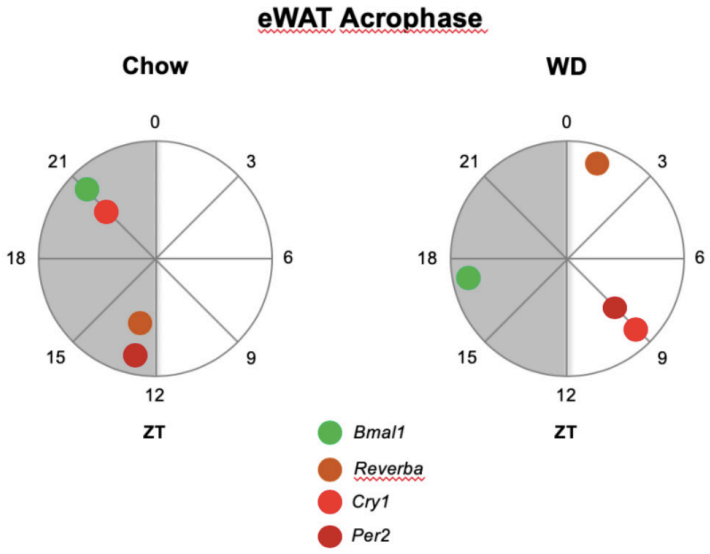

Figure 1: 24-hour clock graphs showing acrophase times of peak expression for each gene A. Acrophase of *Bmal1, Reverb-a, Cry1, and Per2* in Chow group B. Acrophase of *Bmal1, Reverb-a, Cry1, and Per2* in Western diet group.

In addition to eWAT, the core clock genes in the liver have previously been shown to be disrupted in Western diet-induced obesity. In the liver, we observed that *Bmal1, Per2, Reverb-a, and Cry1* were phase-advanced by 12 hours, 4 hours, 0 hours, and 1 hour, respectively. We hypothesize that the hippocampus in the brain, acting as a peripheral tissue, is disrupted in Western diet-induced obesity. Similar opposing shifts were observed in the hippocampus as was observed in the eWAT: *Bmal1* and *Reverb-a* were phase-advanced by

17 hours and 8 hours, respectively, and *Per2*, and *Cry1* were both phase-advanced by 12 hours.

The key impact of this research is that it shows WD+S disrupted the rhythmicity of the core clock in the adipose tissue, which is consistent with our hypothesis that peripheral rhythmicity is disrupted by Western diet consumption.

Statement of Research Advisor

Beatriz has performed key experiments demonstrating adipose tissue clock gene disruption in a mouse model of diet-induced obesity. Her contribution was critical to establishing that circadian disruption occurs in peripheral organs which was necessary for validating our model of obesity-linked circadian disruption.
– Michael Greene, Nutrition, Dietetics & Hospitality Management