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CIRCADIAN RHYTHMS

Muscle circadian clock regulates lipid storage

> "the muscle clock promotes neutral lipid storage by BMAL1-dependent activation of *Dgat2*"

Brain and muscle ARNT-like protein 1 (BMAL1, also known as ARNTL) together with other clock proteins regulates the body's circadian rhythm, which controls energy homeostasis. The muscle clock regulates insulin sensitivity and glucose metabolism; however, the role of the muscle clock in lipid metabolism is unknown. Now, new research by Kenneth Dyar, N. Henriette Uhlenhaut and colleagues shows that the muscle clock promotes daily cycles of lipid storage and inhibits the breakdown of proteins and lipids via a BMAL1-dependent pathway before the body wakes from sleep.

In a previous study, Dyar and colleagues showed that loss of BMAL1 in mice caused circadian rhythm dysregulation and impaired the expression of BMAL1 target genes, including *Rev-erbα* and *Rev-erbβ* (also known as *Nr1d1* and *Nr1d2*, respectively). BMAL1 and nuclear receptor REV-ERBα form vital positive and negative regulatory elements of the circadian clock. "What remained unknown from a mechanistic standpoint was the identity of the direct BMAL1 target genes and any physiological role of the muscle clock in the regulation of lipid and protein metabolism," explains Dyar.

To begin their search for the target genes of BMAL1 and REV-ERBα, the authors used a genome-wide approach to identify key gene targets of BMAL1 in transgenic mice with muscle-specific knockout of *Bmal1* (mKO mice). The investigators found gene expression changes in canonical clock-related pathways and dysregulation in fatty acid, triglyceride and phospholipid metabolism. The loss of BMAL1 also abolished the expression of both REV-ERBα and REV-ERBβ in the muscle of mKO mice. This loss was associated with increased expression of REV-ERBα target genes (as the repression exerted by REV-ERBα was lifted) and increased the expression of regulatory networks involved with lipid and amino acid metabolism.

To understand how the regulation of muscle metabolism is affected by the loss of BMAL1, Dyar and colleagues used metabolite profiling and lipidomics on muscle samples from the mKO mice. The levels of a large proportion of metabolites were altered in the mKO mice compared with wild-type mice. In particular, the loss of BMAL1 increased the levels and 24-hour oscillation patterns of lipids and amino acids. Additionally, muscle samples from mKO mice had reduced triglyceride storage compared with samples from wild-type mice and accumulated substantial levels of bioactive lipids (especially over the period of light–dark transition).

Furthering their understanding of the role BMAL1 has in regulating levels of triglyceride, the authors investigated whether there are any differences in body composition in mKO mice compared with wild-type mice and found substantial differences in both fat and lean tissue mass. Surprisingly, mKO mice had increased lean mass and reduced fat mass. "This is the combined consequence of increased muscle lipid metabolism, increased muscle protein turnover and decreased metabolic efficiency," explains Uhlenhaut.

Finally, to uncover the mechanisms responsible for the reduced levels of triglyceride in mKO muscles the authors analysed the expression profiles of genes involved in glycerophospholipid and triglyceride metabolism. Dyar and colleagues found that the reduced levels of triglyceride in mKO muscle were associated with reduced expression of *Dgat2* (a target gene of BMAL1 and REV-ERBα that encodes the enzyme that converts diacylglycerols to triglycerides in mouse muscle) and the blunting of its 24-hour oscillation pattern.

"Taken together these findings show that the muscle clock promotes neutral lipid storage by BMAL1-dependent activation of *Dgat2*, while inhibiting lipid catabolism and protein turnover by direct REV-ERBα-mediated repression of major target genes," concludes Dyar. "This repression causes an accumulation of bioactive lipids and amino acids and has major implications for how circadian misalignment, as occurs in shift work, is associated with increased metabolic disease risk, including muscle insulin resistance."

Ivone Leong

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