Commentary

MOLECULAR METABOLISM

Tuning in to the rhythm of clock genes in skeletal muscle★

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Circadian rhythms and metabolic homeostasis are closely entangled biological processes. *Clock genes* refer to a group of genes which regulate a variety of biological responses through their periodic expression. Genes encoding the core clock mechanism include the positive transcriptional regulators brain and muscle Arnt-like protein-1 (BMAL1) and circadian locomotor output cycles kaput protein (CLOCK) and the transcriptional repressors PERIOD (PER) and CRYPTOCHROME (CRY) [1]. Together, these factors ultimately activate or repress their own expression and therefore constitute a self-sustained transcriptional feedback loop. Impairment of the clock system has been associated with various diseases including obesity, cardiovascular dysfunction and cancer [2]. In mice, inactivation of *Bmal1* and *Clock* was shown to impair both glucose tolerance and insulin secretion [3]. Notably, inactivation of the clock system in pancreatic islets leads to overt diabetes mellitus, emphasizing the importance of clock genes in beta-cell function.

While the critical role of clock genes in metabolism is now well appreciated, many questions remain to be solved about the peripheral and tissue-specific action of circadian regulators in insulin-sensitive tissues. For instance, mice deficient in hepatic expression of *Bmal*1 exhibited hypoglycemia in their resting phase whereas whole body *Bmal* knockout mice had normal resting blood sugar levels [4]. Presumably such differences stem from failed synchronization of the intact central circadian timing system, which resides in the brain's suprachiasmatic nucleus (SCN), and peripheral, cell-autonomous clocks in the liver. In fact, peripheral pacemakers of the clock system may largely determine basic metabolic functions in response to environmental inputs.

In this issue of *Molecular Metabolism*, Schiaffino and coworkers present convincing evidence for the physiological importance of a functional muscle clock [5]. Using Cre-loxP recombination technology, the authors inactivated *Bmal1* specifically in skeletal muscle of mice, either constitutively (mKO) or through an inducible construct (imKO). While whole-body knockout of *Bmal1* was associated with reduced activity, degenerative loss of skeletal muscle mass and accelerated aging, *Bmal1* mKO mice had normal life span and the weight of certain muscle types was even increased. The circadian rhythm of locomotor activity was unaltered in the knockout mice, but surprisingly activity levels were increased during the dark phase in *Bmal1* mKO mice. Importantly, insulin-stimulated glucose uptake into skeletal muscle was substantially reduced in the knockout mice whereas whole-body glycemia was unaltered. In *Bmal*-deficient muscles, the abundance of the insulin-regulated glucose transporter protein GLUT4 was reduced to approximately half of the normal levels, similar to the levels found in heterozygous muscle-specific GLUT4 knockout mice [6]. Interestingly, expression of the RabGAP protein TBC1D1, a direct target of AKT and AMP-dependent kinase and a potential regulator of insulin- and contraction-stimulated GLUT4 translocation was also substantially reduced in skeletal muscle of mKO mice. Ablation of *Tbc1d1* or knockout of the related *Tbc1d4* (AS160) also results in reduced abundance of GLUT4 protein in muscle whereas mRNA levels of GLUT4 in skeletal muscle were not altered [7,8]. In skeletal muscle of mKO mice, the mRNA of GLUT4 was also unchanged, indicating that the loss of GLUT4 in *Bmal1* mKO mice likely results from post-translational events, perhaps missorting and degradation of the protein. As both GLUT4 and TBC1D1 display oscillation in their expression in muscle, this study makes a case by linking diurnal expression of signaling proteins to the regulated trafficking of insulin effectors. Analysis of muscle metabolites further revealed a reduced activity of pyruvate dehydrogenase (PDH), leading to impaired glucose oxidation and redirection of glycolytic intermediates to alternative metabolic pathways. Altogether, these results point towards a central role of the circadian clock for skeletal muscle metabolism by regulating metabolic switching between fasting and feeding state.

Further studies are needed to follow up on the molecular mechanisms that integrate chronobiology and metabolism in peripheral insulin-sensitive tissues. The relation of reduced *Tbc1d1* levels and diminished GLUT4 protein in skeletal muscle remains to be investigated, as the lack of an effect on expression of the related *Tbc1d4*. Because inactivation of *Bmal1* in skeletal muscle alters circadian expression of most other core clock and clock-associated genes, it also remains to be determined which factors are ultimately responsible for the altered expression of insulin effectors and signaling proteins. Even though whole-body glycemia of *Bmal1* mKO mice apparently was normal, a subtle desynchronization of the central circuitry in the SCN and the periphery could lead to impaired glucose disposal and might impose a risk for developing type 2 diabetes. An open question remains whether over-expression of *Bmal1* might improve insulin action in skeletal muscle.

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Thus, adjusting the clock in insulin-resistant skeletal muscle could constitute a future option for treating metabolic dysfunction. Hopefully time will tell, so we stay tuned for more exciting research on clock genes.

REFERENCES

[1] Ueda, H.R., Hayashi, S., Chen, W., Sano, M., Machida, M., Shigeyoshi, Y., Iino, M., Hashimoto, S., 2002. System-level identification of transcriptional circuits underlying mammalian circadian clocks. Nature Genetics 37:187–192.

[2] Richards, J., Gumz, M.L., 2012. Advances in understanding the peripheral circadian clocks. FASEB Journal 26 (9):3602–3613.

[3] Marcheva, B., Ramsey, K.M., Buhr, E.D., Kobayashi, Y., Su, H., Ko, C.H., Ivanova, G., Omura, C., Mo, S., Vitaterna, M.H., Lopez, J.P., Philipson, L.H., Bradfield, C.A., Crosby, S.D., JeBailey, L., Wang, X., Takahashi, J.S., Bass, J., 2010. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 466 (7306):627–631.

[4] Lamia, K.A., Storch, K.F., Weitz, C.J., 2008. Physiological significance of a peripheral tissue circadian clock. Proceedings of the National Academy of Sciences of the United States of America 105:15172–15177.

[5] Dyar, K.A., Ciciliot, S., Wright, L.E., Biensø, R.S., Tagliazucchi, G.M., Patel, V.R., Forcato, M., Peña Paz, M.I., Solagna, F., Anders, G., Albiero, M., Moretti, I., Eckel-Mahan, K.L., Baldi, P., Sassone-Corsi, P., Rizzuto, R., Bicciato, S., Pilegaard, H., Blaauw, B., Schiaffino, S., 2013. Muscle insulin sensitivity and glucose metabolism are controlled by the intrinsic muscle clock. Molecular Metabolism 3 , http://dx.doi.org/10.1016/j.molmet.2013.10.005.

[6] Zisman, A., Peroni, O.D., Abel, E.D., Michael, M.D., Mauvais-Jarvis, F., Lowell, B. B., Wojtaszewski, J.F., Hirshman, M.F., Virkamaki, A., Goodyear, L.J., Kahn, C.R., Kahn, B.B., 2000. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. Nature Medicine 6 (8):924–928.

[7] Dokas, J., Chadt, A., Nolden, T., Himmelbauer, H., Zierath, J.R., Joost, H.G., Al-Hasani, H., 2013. Conventional knockout of Tbc1d1 in mice impairs insulin- and AICAR-stimulated glucose uptake in skeletal muscle. Endocrinology 154 (10):3502–3514.

[8] Lansey, M.N., Walker, N.N., Hargett, S.R., Stevens, J.R., Keller, S.R., 2012. Deletion of Rab GAP AS160 modifies glucose uptake and GLUT4 translocation in primary skeletal muscles and adipocytes and impairs glucose homeostasis. American Journal of Physiology Endocrinology and Metabolism 303 (10): E1273–E1286.

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