Molecules and Cells

Journal Club

Sleep Hungry for Cellular Cleanup!

Circadian autophagy modulates fruit fly lifespan.

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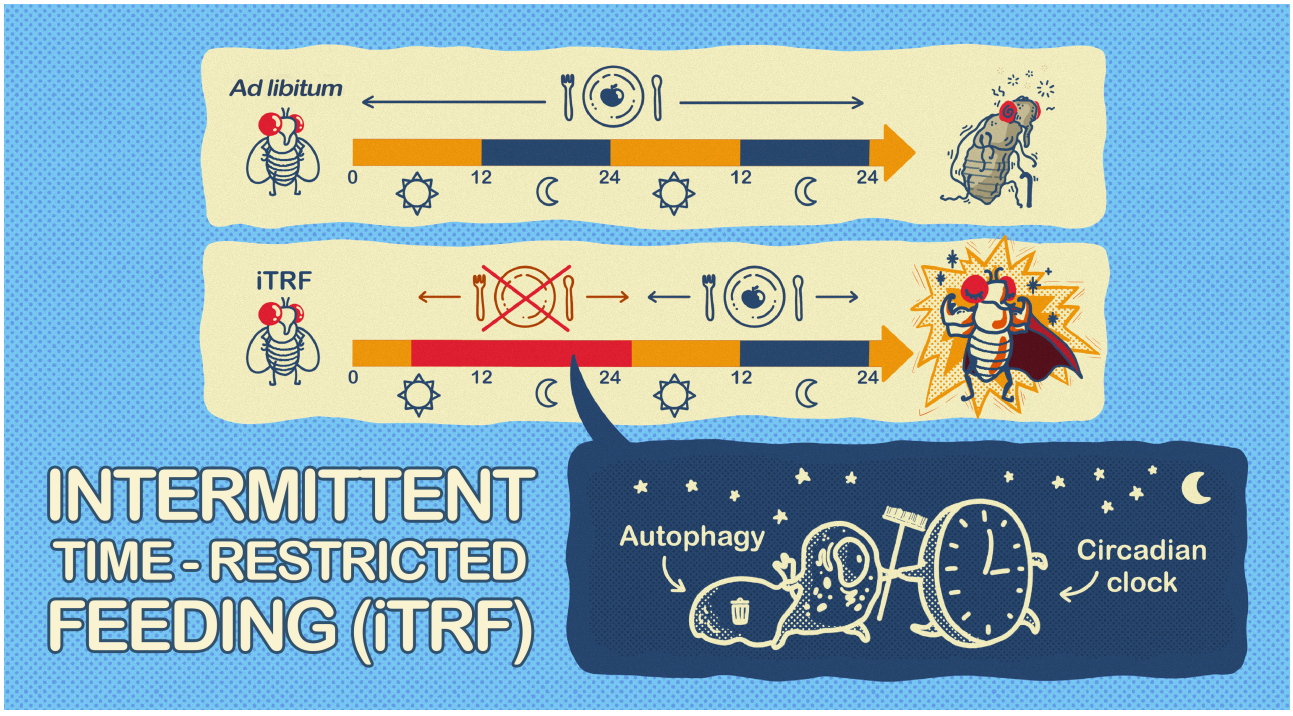

Enhanced autophagy during night/fasting phase has a key modulator for health and life span extension. Compared with flies in *ad libitum* control group, the flies in 20 h of night/fasting (iTRF, intermittent time-restricted feeding) group exhibits significantly elevated beneficial impacts on healthspan and lifespan through upregulated autophagy activity during night/fasting phase.

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Time-restricted feeding (TRF) is an emerging dietary approach for managing weight and improving metabolic parameters such as blood glucose levels and blood pressure; it also has anti-aging effects (Regmi and Heilbronn, 2020). In TRF, the daily eating window is restricted to a certain period during the active phases of the day rather than restriction of calorie or nutrient intake. Although it has attracted increasing attention owing to its numerous benefits, the underlying mechanisms by which TRF mediates these favorable effects, particularly in terms of coordinating with the circadian clock, have not been completely elucidated. Moreover, clinical studies to determine the effectiveness and specific mechanisms of TRF under various conditions are limited, particularly in terms of day or night-biased fasting and genetic differences, especially regarding lifespan. Considering the high homology between the genomes of *Drosophila melanogaster* and humans (Ugur et al., 2016), *D. melanogaster* has been used as an effective and useful model to investigate the significant modulators of TRF-mediated advantageous effects on healthspan (remaining healthier for longer) and lifespan (living longer) (Acosta-Rodriguez et al., 2021; Piper and Partridge, 2018; Suh et al., 2020).

Ulgherait et al. (2021) used genetically modified *D. melanogaster* under various fasting conditions to identify the crucial biological pathways that mediate improvement of health and longevity following intermittent TRF (iTRF). iTRF was defined as fasting from mid-morning for 20 h every alternate day with a recovery day of *ad libitum* diet between the fasting days. Starting from 10 to 40 days of age, *D. melanogaster* was subjected to *ad libitum* or iTRF to compare their health and longevity. *D. melanogaster* in the iTRF group exhibited an extended lifespan and improved muscle/neuronal function, which demonstrated the beneficial effects of iTRF on health and longevity. Moreover, food intake was increased in the iTRF group, which could exclude the contribution of the calorie restriction effect from the iTRF-mediated improvements in health and lifespan. The authors also discovered that extending the lifespan of *D. melanogaster* could occur independently of dietary protein restriction (DR), indicating that iTRF has additive effects on lifespan following DR. Thus, these data implicate the involvement of another pathway mediating iTRF-mediated lifespan extension other than calorie restriction and DR.

Because iTRF is a dietary strategy that involves managing the time of eating, the authors examined the 24-h oscillations of circadian clock gene expression during the iTRF periods. It has been found that circadian clock genes are associated with various biological pathways involved in the endocrine, immune, and cardiovascular systems as well as metabolism (Richards and Gumz, 2013). In case of *D. melanogaster* feedback loop, Clk (clock)-Cyc (cycle) heterodimers activate *per* (period) and *tim* (timeless) transcription, and the Per–Tim complex provides feedback to repress *clk-cyc* transcription. In turn, degradation of the Per–Tim complex leads to the release of Clk-Cyc to initiate the next transcription cycle (Tataroglu and Emery, 2015). The transcriptional levels of *clk* and *cyc* are upregulated during the daytime, whereas those of *per* and *tim* are upregulated during the night. Interestingly, iTRF upregulated the expression levels of core components for circadian clock regulation, specifically *clk* during daytime and *per* during the night/fasting phase. To test whether the circadian clock genes could regulate iTRF-mediated life extension, arrhythmic circadian mutant *D. melanogaster* such as Clk^{jrk}, cyc^{01}, and per^{01} were analyzed. The lifespan extension effect was found to be diminished in all mutants, indicating that the components of the circadian clock are essential for the regulation of iTRF-mediated beneficial effects. Intriguingly, lifespan extension was more significant during nighttime fasting than during daytime fasting, which suggests that the former can enhance the iTRF-mediated effects on longevity.

In particular, the expression levels of the autophagy-related genes *Atg1* and *Atg8a* (the mammalian homologues of ULK1 and LC3, respectively), were also elevated in the iTRF group during the fasting period. Autophagy is a self-digestion mechanism that is responsible for the removal of damaged organelles, malformed proteins, and nonfunctional long-lived proteins via lysosomal degradation of autophagosomes. This process is conserved between *D. melanogaster* and mammals (McPhee and Baehrecke, 2009). In energy-depleted conditions such as starvation, the Atg1 complex initiates autophagy and generates autophagosomes through the Atg8a-conjugation system for substrate degradation. Considering the role of autophagy in modulating aging via the degradation of dysfunctional cellular components and the prevention of their accumulation (Hansen et al., 2018), increased levels of autophagy-related genes could be linked to iTRF-mediated lifespan extension. To test this possibility, an RNAi-mediated loss of function experiment was conducted and showed that *atg1* or *atg8a* is associated with iTRF-mediated lifespan extension. Because iTRF enhances autophagy activity during the night/fasting phase, the authors explored TRF-mediated lifespan extension using a circadian-regulated *atg* expression system to examine whether enhanced autophagy at night is a key modulator of lifespan extension. *Per*-promoter regulated *atg* overexpression, which stimulates autophagy during the night/fasting phase, and increased the lifespan even in *ad libitum* conditions, whereas iTRF had no additive effects. Collectively, these findings suggest that autophagy activity during the night/fasting period is necessary and sufficient for iTRF-mediated health and lifespan extension. In agreement, Ulgherait et al. (2021) demonstrated that the enhanced longevity effect was a result of increased autophagy during the night/fasting phase using pharmacologically (RU486) inducible daGS-GAL4 system for *atg1* overexpression. In contrast, day-specific fasting in RU-inducible *atg1* overexpressed *D. melanogaster* did not extend the lifespan, further highlighting that the lifespan extension is enhanced by autophagy during night-specific fasting.

Ulgherait et al. (2021) demonstrated that a functional circadian clock is necessary for the beneficial effects of iTRF. Furthermore, the study emphasizes the crucial role of enhanced autophagy during the night/fasting phase in the process of iTRF-associated improvements in health and lifespan. Because the beneficial effects of enhanced autophagy were diminished in mutant *D. melanogaster* with arrhythmic circadian clock, it is suggested that the circadian clock is required for autophagy-mediated lifespan extension. However, additional studies are warranted to elucidate the major target

tissues and substrate molecules for iTRF-mediated autophagy in future studies. Nonetheless, this study demonstrates that the circadian system and autophagy play pivotal roles in mediating lifespan extension and that targeting these pathways could lead to anti-aging effects.

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CONFLICT OF INTEREST

The author has no potential conflicts of interest to disclose.

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