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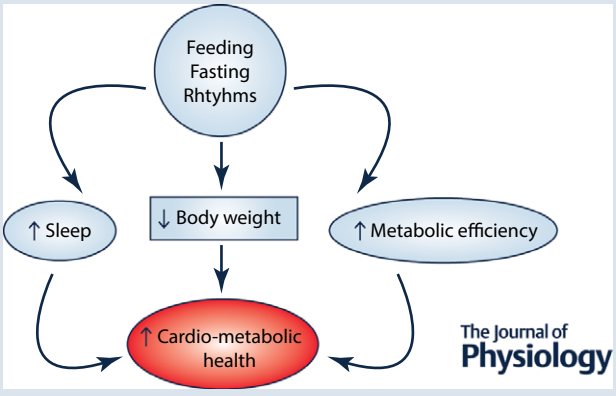
TOPICAL REVIEW

Time-restricted feeding for prevention and treatment of cardiometabolic disorders

Girish C. Melkani[1] and Satchidananda Panda[2]

[1] *Department of Biology, Molecular Biology and Heart Institutes, San Diego State University San Diego, CA 92182, USA*
[2] *Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA*



Abstract The soaring prevalence of obesity and diabetes is associated with an increase in comorbidities, including elevated risk for cardiovascular diseases (CVDs). CVDs continue to be among the leading causes of death and disability in the United States. While increased nutritional intake from an energy-dense diet is known to disrupt metabolic homeostasis and contributes to the disease risk, circadian rhythm disruption is emerging as a new risk factor for CVD. Circadian rhythms coordinate cardiovascular health via temporal control of organismal metabolism and physiology. Thus, interventions that improve circadian rhythms are prospective entry points to mitigate cardiometabolic disease risk. Although light is a strong modulator of the neural circadian clock, time of food intake is emerging as a dominant agent that affects circadian clocks in metabolic organs. We discovered that imposing a time-restricted feeding (TRF) regimen in which all caloric intakes occur consistently within ≤ 12 h every day exerts many cardiometabolic benefits. TRF prevents excessive body weight gain, improves sleep, and attenuates age- and diet-induced deterioration in cardiac performance. Using an integrative approach that combines *Drosophila melanogaster* (fruit fly) genetics with transcriptome analyses it was found that the beneficial effects of TRF are mediated by circadian clock, ATP-dependent TCP/TRiC/CCT chaperonin and mitochondrial electron transport chain components. Parallel studies in rodents

Girish Melkani is an Assistant Professor (Research) at San Diego State University. His lab research focuses on protein unfolding, and myofibrillar and cardiac biology, addressing key signalling pathways linked with metabolic dysregulation, protein misfolding and myofibrillar based cardiometabolic diseases **Satchidananda Panda** is a professor at the Salk Institute for Biological Studies. His lab focuses on transcriptional regulation of circadian rhythms in behaviour, physiology and metabolism. His lab has established time-restricted feeding (TRF) protocols in mice and *Drosophila*. Using high resolution genome-wide temporal gene expression maps in several mouse and *Drosophila* tissues under various metabolic conditions, his lab is investigating the molecular underpinning of TRF benefits. Using complimentary expertise, the two authors are exploring the impact of daily rhythms on cardiac muscle physiology, circadian rhythm, nutrition, sleep and other metabolic disorders.

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have shown TRF reduces metabolic disease risks by maintaining metabolic homeostasis. As modern humans continue to live under extended periods of wakefulness and ingestion events, daily eating pattern offers a new potential target for lifestyle intervention to reduce CVD risk.

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Corresponding author G. C. Melkani: Department of Biology, Molecular Biology Institute and Heart Institute, San Diego State University, LS-350, 5550 Campanile Drive, San Diego, CA, 92182-4614, USA. Email: gmelkani@mail.sdsu.edu

Abstract figure legend Imposing feeding-fasting rhythms with time-restricted feeding (TRF) paradigm, circadian rhythm coordinates metabolism and physiology. TRF leads to improved sleep and metabolic efficiency as well as reduced body weight. Synchrony between these parameters orchestrates suppression of cardiometabolic disorders.

Abbreviations ALF, *Ad libitum* feeding; CVD, cardiovascular disease; ETC, electron transport chain; ROS, reactive oxygen species; TRF, time-restricted feeding; TRiC, TCP-1 ring complex.

Introduction

A recent World Health Organization (WHO) report revealed that diabetes and obesity-related diseases have soared in every country, nearly quadrupling over the last 35 years, to 422 million adult cases (http://www.who.int/diabetes/global-report/en/) (Steinberger *et al.* 2009; Rodriguez-Colon *et al.* 2010). Obesity and diabetes are associated with a number of comorbidities, including elevated risk for CVD (Steinberger *et al.* 2009; Rodriguez-Colon *et al.* 2014; Morris *et al.* 2016). CVD continues to be among the leading causes of death and disability in the United States. The leading risk factor for cardiac and cardiometabolic diseases are age, shift work, an energy-dense diet, diabetes and obesity (Durgan & Young, 2010; Azadbakht *et al.* 2013; Morris *et al.* 2016). These seemingly unrelated risk factors have one common connection – circadian rhythm disruption. Circadian rhythms are ∼24 h cycles in behaviour, physiology and metabolism that arise from coordinated regulation of numerous pathways in different organs. Almost every organ in animals exhibits a circadian rhythm in gene expression and function. Unbiased gene expression studies are revealing that more than half of the genome shows daily rhythm in expression in a tissue-specific manner (Hatori *et al.* 2012; Sherman *et al.* 2012; Chaix *et al.* 2014, 2016; Panda, 2016; Zarrinpar *et al.* 2016). Conversely, genetic perturbation of the circadian clock in model organisms increases the incidence and severity of cardiometabolic diseases (Steinberger *et al.* 2009; Durgan & Young, 2010; St-Onge *et al.* 2016). Similarly, lifestyle perturbation of the circadian clock, as occurs among shift workers or in experimental models of shiftwork in animals, can disrupt the circadian clock and trigger obesity, diabetes, mitochondrial diseases and CVD (Maury *et al.* 2010; Gill *et al.* 2015; Morris *et al.* 2016; St-Onge *et al.* 2016). Therefore factors that affect circadian rhythms offer new avenues to understand the aetiology, prevention and treatment of cardiometabolic diseases. While the circadian rhythm in sleep and wakefulness is primarily synchronized to the ambient light–dark cycle, the timing of food intake (and conversely the fasting period) appears to affect the robustness of circadian rhythms in metabolic organs. This has led to the hypothesis that the daily cycle of eating and fasting is a determinant of circadian function in cardiac tissue. Accordingly, consolidating all caloric intake to a few hours without altering the daily intake of quality or quantity of nutrients supports robust circadian rhythms. This newly emerging approach of time-restriction of caloric intake or time-restricted feeding (TRF) appears to impart both preventative and therapeutic effects on metabolic diseases in experimental animals. In this review, we will introduce recent work on circadian rhythms and TRF with specific reference to cardiometabolic diseases.

Circadian clocks, rhythms and cardiometabolic diseases. Circadian rhythms in animals emerge from cell-autonomous, self-sustaining, ∼24 h transcriptional feedback loops (Panda *et al.* 2002; Vanin *et al.* 2012; Harfmann *et al.* 2015; Chaix *et al.* 2016). The specific molecular components and mechanisms of circadian rhythms first identified in *Drosophila* are largely conserved in mammals (Helfrich-Forster, 2000; Panda *et al.* 2002; Vanin *et al.* 2012; Hardin & Panda, 2013). In *Drosophila*, the transcriptional activators Clock (Clk) and Cycle (Cyc; also known as dBmal1) dimerize and transcriptionally activate the Timeless (Tim) and Period (Per) genes. The formation of the Per–Tim heterodimer, in turn, inhibits the activities of Clk–Cyc (Helfrich-Forster, 2000; Panda *et al.* 2002; Hardin & Panda, 2013; Mendoza-Viveros *et al.* 2017). The molecular circadian clock generates daily rhythms in a large number of genes and proteins by (a) regulating transcription from *cis*-regulatory sites, (b) regulating transcription factors, which then regulate indirect clock targets, (c) affecting post-transcriptional regulatory processes, and (d) functionally interacting with signalling and transcriptional regulators (Mendoza-Viveros *et al.* 2017). As a result, in most animal tissue examined, several hundreds or even thousands

of transcripts show daily rhythm in their expression. Functional annotation of these rhythmic transcripts revealed that nutrient metabolism and basic cellular functions are under circadian modulation (Chaix *et al.* 2016; Zarrinpar *et al.* 2016).

Importantly, expression and function of many of the clock components are intimately interlinked with cellular metabolism. Binding of Clk–Bmal1 (mammalian homologues of Clk–Cyc) to DNA is influenced by redox state. Furthermore, some of the clock components are post-translationally modified by phosphorylation, acetylation and glycosylation (Bass & Lazar, 2016), which in turn affect their stability and function. Enzymes mediating these modifications respond to cellular energy states and thereby constitute nodes through which the circadian clock is integrated with energy status. Additionally, several secondary metabolites in mammals affect circadian rhythm through their impact on chromatin modification and by serving as ligands for clock components (Chaix *et al.* 2016; Longo & Panda, 2016; Manoogian & Panda, 2016; Panda, 2016; Zarrinpar *et al.* 2016; Mattson *et al.* 2017). Such reciprocal interaction between metabolism and the circadian system suggested that the circadian system can 'sense' metabolic state of the cell and in turn regulate the timing of expression of a large number of genes working in seemingly disparate pathways to optimize physiology.

In mammals, circadian oscillations in thousands of transcripts have been described in tissues of the cardiovascular system, including the atrium, ventricle, aorta and endothelial cells (Rudic *et al.* 2005; Bray *et al.* 2008; Koike *et al.* 2012). Accordingly, circadian mutant mice exhibit compromised cardiovascular functions (reviewed in Paschos & FitzGerald, 2010). For example, over-expression of the dominant negative circadian $clock^{\Delta 19}$ mutant (CCM) in mouse cardiomyocytes disrupts normal circadian gene expression and cardiac function. Hearts of CCM mice exhibit increased fatty acid oxidation, lactate release, elevated fractional shortening, bradycardia and a longer R–R interval (Bray *et al.* 2008). Additionally, circadian mutant mice also exhibit a plethora of subtle cardiovascular defects, which might increase susceptibility to CVDs (Paschos & FitzGerald, 2010). A clock controlled gene – KLF15 – offers a mechanistic link between circadian rhythm and cardiac function. Circadian clock regulates rhythmic expression of KLF15, which in turn regulates circadian expression of Kv channel-interacting protein 2 (KChIP2), a key component required for transient outward potassium current. In the mouse heart, constitutive over-expression or knockout of transcription factor KLF15 causes loss of the rhythmic QT interval and enhanced susceptibility to ventricular arrhythmia (Jeyaraj *et al.* 2012). Whole animal or cardiac specific circadian clock mutant mice also show compromised metabolic homeostasis, which can contribute to CVD risks. In humans, misalignments of circadian rhythms in shift workers lead to increase risks of cardiac diseases (Morris *et al.* 2016). Overall, these studies have associated the circadian clock to cardiometabolic health and suggest that the maintenance of a robust circadian system may reduce CVD risks.

Time-restricted feeding paradigm and metabolic health in rodents. Animals and humans have a diurnal rhythm in food intake with the major part of the food consumed during the organism's natural wakeful period. Due to the reciprocal interaction between metabolism and circadian rhythms, this daily eating–fasting rhythm acts synergistically with the molecular circadian clock to drive daily rhythms in anabolic and catabolic metabolism and dependent physiology. *Ad libitum* access to an energy-dense high fat diet (HFD) can disrupt the feeding–fasting rhythms in animals with food intake erratically spread over a major part of the 24 h day. Such an erratic eating pattern dampens the normal circadian oscillator in metabolic organs including the liver. In experiments to test the contribution of an HFD vs. erratic eating pattern to HFD-induced obesity, mice were fed an HFD *ad libitum* or allowed to eat the same number of calories within a restricted time interval of 8 h. Surprisingly, under such a TRF protocol mice are significantly protected from diet-induced obesity and associated metabolic diseases. Recently, TRF benefits have been observed in mice fed up to 12 h every day (Hatori *et al.* 2012; Sherman *et al.* 2012; Chaix *et al.* 2014). Some of these benefits in rodents include improved glucose tolerance, reduced triglyceride, reduced cholesterol, reduced systemic inflammation and improved endurance. As hypothesized, TRF sustains a robust circadian clock in the liver and prevents metabolic reprogramming of the hepatic transcriptome that typically occurs in mice fed an HFD *ad libitum* (Hatori *et al.* 2012; Sherman *et al.* 2012; Chaix *et al.* 2014). Recently it has been shown that feeding mice during the daytime is associated with desynchronization of peripheral clocks and leads to obesity and other metabolic challenges (Yasumoto *et al.* 2016). Furthermore, additional studies have shown that TRF reduces adiposity in male C57BL/6 mice that are challenged with an HFD and that restricted feeding in middle-aged C57BL6/J mice alleviates the negative effects of an HFD on metabolic health, including body weight, liver weight and glucose tolerance (Duncan *et al.* 2016; Sundaram & Yan, 2016). Although TRF yields several metabolic improvements in rodents, its impact on cardiac function, its genetic mechanism, and whether the phenomenon is relevant to non-rodent species is unclear.

***Drosophila* as a genetic model organism for cardiovascular and metabolic diseases.** Due to parallel genetic functions between flies and vertebrates during

cardiogenesis, the *Drosophila* model has been well established to explore the genetic basis of deterioration of cardiac function that arises due to aging, diet, or genetic mutation (Ocorr *et al.* 2007; Birse *et al.* 2010; Wolf & Rockman, 2011; Melkani *et al.* 2013; Gill *et al.* 2015). The *Drosophila* heart is cylindrical in shape, with a conical structure at the anterior end. It resides within the dorsal abdominal cavity, extending from the first to the sixth body-wall segment along the midline. The primary function of the fly heart is to pump haemolymph. The pumping action is generated by the rhythmic diametric expansion (diastole) and contraction (systole) of the conical/cylindrical structure. By combining a semi-intact *in vivo* preparation with high-speed imaging, cardiac rhythms can be imaged for a sufficient period of time to quantitatively assess defining characteristics (Fig. 1), such as the heart period, and systolic and diastolic diameter (Gill *et al.* 2015), as well as the variability associated with these measurements from one heartbeat to another (Ocorr *et al.* 2007; Birse *et al.* 2010; Wolf & Rockman, 2011; Melkani *et al.* 2013; Gill *et al.* 2015). Despite some obvious limitations in modelling many heart diseases and therapeutic interventions, several genetic and non-genetic risks for heart diseases in humans also increase disease risks in *Drosophila*. In the adult fly, like in humans, cardiac arrhythmias appear and intensify with age (Ocorr *et al.* 2007; Birse *et al.* 2010; Wolf & Rockman, 2011; Melkani *et al.* 2013; Gill *et al.* 2015). Likewise, nutritional challenges that compromise cardiac function in humans (e.g. high-fat or high-sugar diets) have similar effects in flies (Birse *et al.* 2010; Na *et al.* 2013; Gill *et al.* 2015). In addition, the short life-span and advanced genetics of *Drosophila* allow

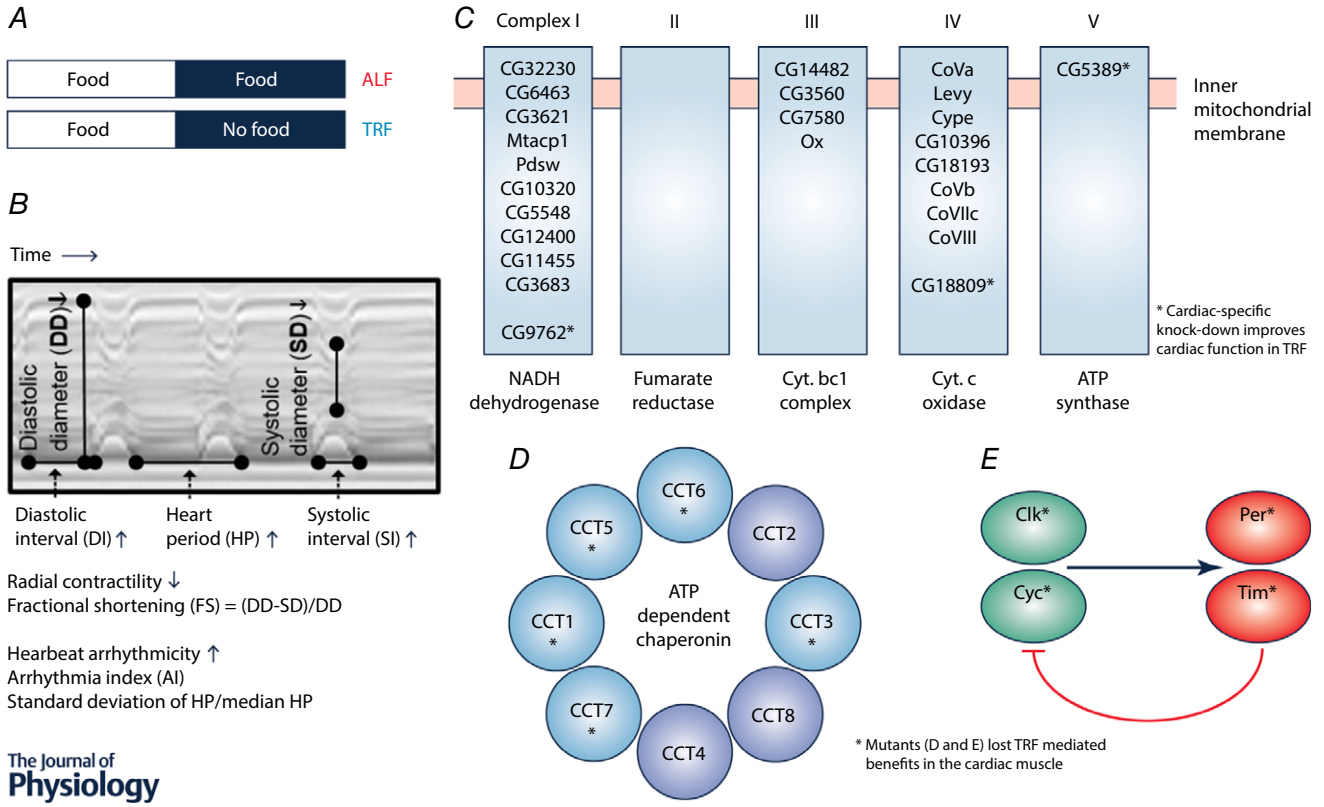

Figure 1. Relationship among potential pathways linked with TRF-induced cardiac benefits and their impact in ameliorating cardiometabolic disorders
A, as reported before (Gill *et al.* 2015), flies are maintained on a 12:12 h light–dark cycle. The TRF flies have access to food for 12 h whereas ALF flies have access to food for 24 h. *B*, cardiac parameters were calculated from mechanical (M)-mode traces (showing the movement of the heart tube edge (*y*-axis) over time (*x*-axis)). Age-associated alterations of cardiac parameters shown with upward and downward arrows. *C*, TRF down-regulates expression of mitochondrial ETC components in *Drosophila* hearts and cardiac-specific knock-down of ETC genes *CG5389* (Mitochondrial ATP synthase), *CG9762* (NADH dehydrogenase) and *CG18809* (spliceosome-associated protein-18) delays age-associated cardiac defects. *D*, up-regulation of cytoplasmic chaperonin (TCP/TRiC/CCT) was cardiac specific under TRF and mutation of TCP/TRiC/CCT eliminated the TRF benefit. *E*, mutation of circadian cock genes (*Clk*, *Cyc*, *Per* and *Tim*) eliminated the TRF benefit. Therefore, circadian genes are required for cardioprotection under TRF. Synchrony between feeding–fasting and light–dark cycles synergistically optimize metabolism by driving anabolic and catabolic processes at appropriate times of the day, which in turn lessens ROS and sustains cyto-architecture (Gill *et al.* 2015).

it to serve as an excellent model system for examining gene networks. *Drosophila* serves as an admirable model system for basic discoveries in metabolic syndrome, circadian rhythms, energy metabolism, mitochondrial homeostasis and cardiac physiology (Ocorr *et al.* 2007; Birse *et al.* 2010; Wolf & Rockman, 2011; Melkani *et al.* 2013; Gill *et al.* 2015). As in other model organisms and in humans, age, energy-dense diets and disruptions of circadian rhythm compromise cardiac performance in the fruit fly (Ocorr *et al.* 2007; Birse *et al.* 2010; Wolf & Rockman, 2011; Melkani *et al.* 2013; Gill *et al.* 2015), suggesting that conserved pathways (including the circadian clock) mediate cardiac muscle function.

Imposing feeding–fasting rhythms with TRF in *Drosophila*. Adult flies (*Drosophila melanogaster*) sleep more during the night and have a short siesta-like sleep during the day (Helfrich-Forster, 2000; Klarsfeld *et al.* 2003; Grima *et al.* 2004; Picot *et al.* 2007; Vanin *et al.* 2012). However, they do occasionally wake up during the night and when food is available *ad libitum*, as in most experimental situations, they consume a measurable amount of food at night (Gill *et al.* 2015). As flies age, the circadian organization of activity and rest deteriorates, with increased activity and reduced sleep during the night (Gill *et al.* 2015). Therefore, the flies offer a tractable model to test the role of the feeding–fasting rhythm on overall health during aging.

To test the role of a daily feeding–fasting cycle on animal health, *ad libitum* feeding (ALF) and TRF cohorts are carefully maintained. Flies (Oregon-R) are collected as soon as they emerge and are maintained on ALF for a few days before assigning them to different eating pattern regimens. At ∼2 weeks of age, they are assigned to one of the two paradigms (Fig. 1*A*). The ALF group is essentially the standard practice in fly labs where the flies are group housed in vials where a semi-solid food is available 24 h per day. The flies are maintained on a 12 h light–12 h dark cycle at 22°C in humidified incubators. TRF flies are held in these identical housing conditions and in standard vials with semi-solid food for 12 h during the daytime and are switched to vials with 1.1% agar (no food) at night. The procedure is repeated every day and the ALF flies are also switched to food vials to control for any unintended consequences of transferring flies between vials every day. As seen in rodents, both ALF and TRF groups consume the same amount of calories every day. Such an experimental set-up controls for genotype, age, nutrition quality and quantity and allows for systematic analyses of the impact of TRF and ALF on body weight, flight ability, cardiac performance and underlying molecular changes at different ages (Gill *et al.* 2015).

In *Drosophila* TRF also exerts beneficial cardiometabolic effects, thus illustrating the relevance of eating pattern on metabolic health across species (Hatori *et al.* 2012; Sherman *et al.* 2012; Chaix *et al.* 2014, 2016; Panda, 2016; Zarrinpar *et al.* 2016). We have shown that cardiac physiological parameters of 3-week-old flies under ALF and TRF were identical. Flies with hearts under ALF showed an age-dependent increased incidence of cardiac arrhythmias, prolonged systolic and diastolic intervals as well as increased heart period under ALF at 5 weeks of age (Fig. 1 and Table 1). The mean diastolic diameter decreases with age, which results in lowering cardiac contractility (Gill *et al.* (2015) and Fig. 1*B*). These cardiac parameters further deteriorate in 7-week-old flies. Interestingly, cardiac contractility was sustained under TRF in both 5- and 7-week-old flies compared to their ALF counterparts. Additionally, compared to ALF, age-associated increased cardiac arrhythmias, heart period, and systolic and diastolic intervals were also attenuated under TRF in 5- and 7-week-old flies. Overall, the cardiac phenotypes of 5- or 7-week-old TRF flies were similar to those of 3- or 5-week-old ALF flies. Furthermore, when flies are introduced to TRF later in life (at 5 weeks) they still showed improvement of some cardiac parameters (Gill *et al.* 2015), compared to age-matched ALF flies. In addition to attenuation of age-associated cardiac defects, the TRF regimen suppresses cardiac defects arising from a high fat diet (Gill *et al.* 2015). Overall, TRF increased the cardiac health-span of *Drosophila*.

TRF also improved several health parameters (Table 1) that are known to contribute to cardiovascular disease risks. Flies under TRF were protected from age-dependent body weight gain, had improved flight index (muscle function), and sustained consolidated nightly sleep even into their middle ages. Such pleiotropic effects reflect TRF impacts on tissues throughout the body including the brain. Accordingly, systematic gene expression studies could identify potential correlative molecular changes.

Cardiac metabolism, proteostasis and cardiometabolic health. Rodent studies have shown the metabolic benefits of TRF and correlative changes in the expression of several established pathways (Hatori *et al.* 2012; Sherman *et al.* 2012; Chaix *et al.* 2014, 2016; Duncan *et al.* 2016; Sundaram & Yan, 2016; Zarrinpar *et al.* 2016). However, the impact of TRF on cardiometabolic function and the genetic basis of TRF benefits that attenuate cardiac aging dysfunction are not yet explored in the rodent. In *Drosophila*, unbiased measurement of global changes in gene expression during 24 h periods in ALF and TRF flies have begun to shed light on probable mechanisms (Fig. 1). The diurnal gene expression pattern of *Drosophila* head, body and heart yielded several clues. First of all, these gene expression changes under TRF were not similar to the well-established reporter of caloric restriction (CR) in *Drosophila* (Farhadian *et al.* 2012), thus indicating that the underlying mechanism is likely to be different from that of

Table 1. Summary of altered physiological parameters and cardiac-specific genes under TRF

Parameters	ALF	TRF
Food intake	*Ad libitum* 24 h	12 h during the day
Activity	Equivalent	Equivalent
Sleep	Deteriorates with age (⇊)	Improved (⇈)
Flight ability		⇈ Relative to ALF
Body weight	Vary	Constant
Circadian gene expression (head and body)		⇈ Relative to ALF
Cardiac expression of ETC components (Fig. 1*C*)		⇊ Relative to ALF
Cardiac expression of TCP components (Fig. 1*D*)		⇈ Relative to ALF
Cardiac physiology (age- and diet-induced)	Deteriorates	Sustained

CR. In both head and body, TRF improved the robustness and synchrony of rhythmic transcripts (Gill *et al.* 2015). Several gene expression studies have shown that hundreds of transcripts display daily rhythm in head and body of ALF flies, with their peak expressions generally distributed throughout day and night. The ratio between peak and trough levels of these transcripts is a measure of robustness or amplitude of oscillation (Gill *et al.* 2015). TRF improved the amplitude of oscillation of many cycling transcripts in both head and body. The timing of peak expression of these genes (phase) also coalesced at dawn and dusk time. Such changes reflect TRF and might consolidate rhythmic transcripts encoding anabolic and catabolic processes to two distinct parts of the 24 h days. The increased amplitude implies both induction and repression of genes that are tied to nutrient or xenobiotic metabolism are more efficiently controlled under TRF (Gill *et al.* 2015). Since the circadian clock is necessary for robust and synchronous oscillations in a large number of transcripts (Gill *et al.* 2015; Longo & Panda, 2016; Mattson *et al.* 2017), circadian rhythm mutants may fail to drive genome wide expression rhythms and may not respond to TRF (Fig. 1*E*). Accordingly, fly strains carrying hypomorphic or loss of function alleles of *clk*, *cyc*, *per* and *tim* failed to show TRF-induced benefits on cardiac function (Gill *et al.* 2015). Many of these mutants also showed compromised cardiac function at birth, which parallels the cardiac defects found in clock mutant mice (Paschos & FitzGerald, 2010).

In addition to rhythmic transcription, changes in tonic expression of genes may also contribute to TRF benefits. Analyses of longitudinal gene expression from the hearts of TRF and ALF *Drosophila* revealed > 400 transcripts showing either up- or down-regulation throughout the 24 h day. Functional annotation of these transcripts revealed two functional clusters: 19 different genes encoding the mitochondrial electron transport complex were down-regulated by 10–20% in TRF heart, while 7 out of 8 components of an ATP-dependent chaperonin were up-regulated (Fig. 1*C* and *D*). The eukaryotic cytoplasmic chaperonin T-complex protein (TCP)/TCP-1 ring complex (TRiC)/chaperonin containing TCP-1 (CCT) is a barrel-like structure composed of eight related subunit double repeats (Fig. 1*D*). They play a crucial role in proper folding of several proteins relevant for cardiac health including cytoskeletal components (Sternlicht *et al.* 1993; Kubota *et al.* 1995; Srikakulam & Winkelmann, 1999; Lundin *et al.* 2010). Recently, mutation of chaperone CCT7 (Ser525Leu) was associated with enhanced susceptibility for myocardial infarction in humans (Erdmann *et al.* 2013), possibly due to compromised folding and organization of cytoskeleton proteins. The increase in TCP component mRNA in TRF flies was not accompanied by a significant increase in mRNAs encoding cytoskeletal proteins. Rather, the TRF heart showed a 20–40% reduction in contractile protein mRNAs (Gill *et al.* 2015). The parallel increase in CCT chaperonins and reduction in cytoskeletal components are likely to indicate optimum cytoskeletal function in cardiac tissues of TRF flies. In *Drosophila*, P element insertion lines that potentially act as hypomorphic alleles of five different CCT components have been tested in a TRF paradigm (Fig. 1*D*). These mutations do not render any TRF benefits, thus suggesting up-regulation of CCT components is beneficial. Misregulation of cytoskeletal proteins has been associated with cardiac hypertrophy and it will be interesting to test if induction of TCP chaperone during TRF can attenuate several cardiac defects associated with mutation of contractile proteins.

Age- and diet-induced vulnerabilities to proteostasis and mitochondrial function, dampening of rhythms under ALF, as well as the gradual accumulation of lipotoxicity and reactive oxygen species (ROS) may eventually compromise the structural integrity of cardiac muscles (Gill *et al.* 2015). The high energetic requirements of cardiac muscle, its susceptibility to the ROS byproducts of the electron transport chain (ETC) and its proteostatic requirements for cardiac contractility are likely to render the cardiac muscles vulnerable to changes in cellular homeostasis. The mitochondrial ETC complexes constitute the principal site for production of ATP, heat and reactive oxygen species. A fine balance between ATP

and ROS production presumably improves cellular and organismal health (Camara *et al.* 2010; Feng *et al.* 2011; Farhadian *et al.* 2012). TRF modestly reduces expression of 19 ETC components in the heart throughout 24 h and of > 30 components for > 18 h every day (Fig. 1*C*). These genes encode components of all five complexes of the ETC, thus suggesting an overall reduction in ETC function may ensue. Reduced ETC function is associated with a health benefit (Camara *et al.* 2010; Durieux *et al.* 2011). While higher levels of ETC inhibitors are toxic, low to moderate levels are well tolerated by animals and can have health benefits (Camara *et al.* 2010). Down-regulation of three different ETC components (Fig. 1*C*) in *Drosophila* heart sustains cardiac health, even in ALF flies, thus suggesting that reduced ETC gene expression contributes to TRF benefits.

In summary, cardiac benefits linked with the TRF paradigm are mediated by the circadian clock, the TRiC chaperonin and mitochondrial ETC components (Gill *et al.* 2015). Whether these three functional clusters are part of the same pathway or they act in parallel remains to be investigated (Fig. 1*C, D* and *E*).

Feeding–fasting rhythms, TRF and attenuation of cardiometabolic diseases. In addition to genes/pathways associated with TRF, other physiological processes such as fasting are known to influence genes associated with nutrient sensing that eventually improve physiology (Longo & Panda, 2016; Mattson *et al.* 2017). Although direct comparison of the TRF gene expression profile with the CR gene expression signature did not indicate CR playing a dominant role, we cannot rule out the benefits of overnight fasting. Daily feeding–fasting rhythms drive signalling pathways that interact with the circadian oscillator to increase the robustness or peak-to-trough differences of these transcriptional oscillations (Vollmers *et al.* 2009; Adamovich *et al.* 2014). Conversely, continuous food deprivation for 24 h dampens the circadian clock and drastically reduces the number of rhythmic transcripts in the liver (Vollmers *et al.* 2009). The combination of both a feeding–fasting cycle and a functional circadian clock acts synergistically to support robust rhythm in the expression and function of a large number of genes. These rhythmic outputs subsequently mediate anabolic and catabolic processes that are appropriate for specific phases of the feeding–fasting cycle (Longo & Panda, 2016). This general model of synergistic action between feeding–fasting and the circadian oscillator offers a framework to further examine some of the leading risks for cardiac diseases, including age, energy-dense diet and non-genetic circadian disruption.

Human relevance, limitations and future direction. The pleiotropic beneficial effects of TRF in both mammals and insects in mitigating multiple metabolic and cardiac risk factors without altering quality or quantity of nutrition has opened a potential lifestyle modification strategy to combat cardiometabolic diseases. Epidemiological study of 26,902 men (aged 45–82 years) has also shown that an aberrant eating pattern is associated with higher risk of CVD irrespective of dietary composition (Cahill *et al.* 2013). Results from 16 years of follow-up reveals that after controlling for diet and lifestyle, late night caloric intake increases heart disease risk in men by 55% (Cahill *et al.* 2013). This population-based study also revealed that skipping breakfast increases CVD risk by 27%. Overall, after controlling for genetic factors and dietary composition, an aberrant eating pattern is associated with CVD risks (Cahill *et al.* 2013). Various population-based or clinical studies have shown that sleep deprivation or poor sleep quality is associated with increased sympathetic nervous system activity and this increased activity is linked with elevation of hypertension and increased risk for CVD (Nagai *et al.* 2010; Grandner *et al.* 2016; St-Onge *et al.* 2016). In addition to epidemiological correlative studies, a controlled clinical study has also revealed that chronic circadian misalignment of rest activity and associated daily eating pattern even for a few days can increase cardiovascular disease risk in healthy adults (Morris *et al.* 2016). These cardiac factors include elevation of systolic and diastolic blood pressure and increased 24 h serum levels of interleukin-6, C-reactive protein, resistin and tumour necrosis factor-α (Morris *et al.* 2016).

While circadian disruption can occur through misalignment of sleep–wake and erratic eating pattern, assessing the daily eating pattern in humans and whether it can be modified to improve health is an emerging research topic. Such studies will also help to assess how much of the TRF benefits found in rodents and insects can be translated to humans. A recent study using a smartphone app to monitor eating time has revealed more than 50% of adults spread their daily caloric intake over 15 h or longer (Gill & Panda, 2015). Such extended eating in rodents along with obesogenic or high glycaemic diet predispose animals to metabolic diseases. Conversely, in a feasibility study in healthy adults, reducing the eating duration to 10–11 h without overt attempt to reduce calories or change nutrition quality showed weight loss, improved sleep and increased sense of energy (Gill & Panda, 2015). This preliminary observation prompts for more experimental evidence to established relationship among eating pattern, sleep and cardiometabolic health at the molecular level. Even though *Drosophila* and human hearts have some divergent functions, conserved pathways appear to govern form and function. Therefore, studies in model organisms including flies that allow specific perturbation of lifestyle and genetic makeup are important for mechanistic studies.

Modern humans, due to societal pressures, work schedules and night-time indoor illumination, stay awake

longer, which enables food consumption for longer durations of time. This extended duration itself, in addition to the caloric surplus, can be detrimental to health. Future studies will be useful in testing the effectiveness of TRF in preventing/delaying cardiac dysfunction associated with obesity and metabolic disease. Human nutrition research has primarily focused on two variables: energy intake (food, food type) and expenditure (exercise, thermogenesis, etc.). The daily feeding–fasting rhythm paradigm has translational potential for management of defective metabolism-induced cardiovascular disease in humans by controlling the timing of dietary intake. These are widely applicable to human health and could be implemented as a community-based approach to improve human cardiac disease linked with diabetes and obesity.

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Additional information

Competing interests

None.

Author contributions

Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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