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Temporal partitioning of adaptive responses of the murine heart to fasting

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ABSTRACT

Recent studies suggest that the time of day at which food is consumed dramatically influences clinically-relevant cardiometabolic parameters (e.g., adiposity, insulin sensitivity, and cardiac function). Meal feeding benefits may be the result of daily periods of feeding and/or fasting, highlighting the need for improved understanding of the temporal adaptation of cardiometabolic tissues (e.g., heart) to fasting. Such studies may provide mechanistic insight regarding how time-of-day-dependent feeding/fasting cycles influence cardiac function. We hypothesized that fasting during the sleep period elicits beneficial adaptation of the heart at transcriptional, translational, and metabolic levels. To test this hypothesis, temporal adaptation was investigated in wild-type mice fasted for 24-h, or for either the 12-h light/sleep phase or the 12-h dark/awake phase. Fasting maximally induced fatty acid responsive genes (e.g., *Pdk4*) during the dark/active phase; transcriptional changes were mirrored at translational (e.g., PDK4) and metabolic flux (e.g., glucose/oleate oxidation) levels. Similarly, maximal repression of myocardial p-mTOR and protein synthesis rates occurred during the dark phase; both parameters remained elevated in the heart of fasted mice during the light phase. In contrast, markers of autophagy (e.g., LC3II) exhibited peak responses to fasting during the light phase. Collectively, these data show that responsiveness of the heart to fasting is temporally partitioned. Autophagy peaks during the light/sleep phase, while repression of glucose utilization and protein synthesis is maximized during the dark/active phase. We speculate that sleep phase fasting may benefit cardiac function through augmentation of protein/cellular constituent turnover.

1. Introduction

Time-of-day-dependent oscillations in biological processes are essential for maintenance of homeostasis, as well as cellular and organ function. In association with behavioral patterns, such as sleep/wake and fasting/feeding cycles, mammals exhibit daily rhythms in various circulating factors (hormones and nutrients), neural stimulation (e.g., autonomic and sympathetic tone), and cardiovascular parameters (e.g., blood pressure and heart rate) [12,20,33]. Disruption of these oscillations, through behavioral, environmental, and/or genetic means, invariably precipitates pathology. In humans, sleep deprivation and shift work both adversely impact neurohumoral factor oscillations, and significantly increase the risk of developing cancer, obesity, diabetes, and cardiovascular disease (CVD) [6,15,19,36]. This has led to speculation that maintenance/restoration of biological rhythms (through pharmacological or behavioral interventions) may be effective in the prevention/treatment of various pathologies. One such strategy includes time-of-day-restricted feeding. For example, restricting food intake only to the active period attenuates high fat diet induced body weight gain, adiposity, insulin resistance, and hyperlipidemia in rodents; this is associated with an augmentation of biological rhythms at tissue-specific (e.g., transcriptional, translational) and whole body (e.g., neurohumoral, behavioral) levels [1,3,4,16]. Similar cardiometabolic benefits have been observed in humans following restriction of caloric intake prior to the evening [22,26]. Recently, we have reported that restricting food intake to the active period (particularly towards the early portion

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of the active period) attenuates high fat diet induced cardiac dysfunction [25,35]. Collectively, these observations suggest that prevention of food intake during the inactive/sleep phase may be beneficial for various cardiometabolic parameters, including contractile function of the heart. However, the mechanisms mediating cardioprotection remain largely unknown.

Considerable information is available regarding daily oscillations in cardiac processes; this is particularly true for cardiac metabolism. Upon awakening, cardiac contractility increases in association with foraging for food, predation avoidance, and reproduction. Accordingly, myocardial oxidative metabolism must increase at this time, in order to meet demands for energy. During the initial stages of foraging, catabolic processes active during the sleep phase fast would therefore be expected to remain active, providing substrates for continued search of food. Once the forage for food is successful, anabolic processes should become more active, thereby facilitating storage of excess nutrients. Consistent with these concepts (reviewed in [41]), we have previously reported that myocardial glucose utilization increases during the active period in ad libitum fed mice, likely meeting the energetic demands of the heart in response to elevated workload [8]. Myocardial triglyceride synthesis also increases towards the end of the active period in ad libitum fed mice, thereby facilitating storage of excess nutrients (possibly in anticipation of the upcoming sleep phase fast) [34]. More recently, we have found that protein synthesis increases at the end of the active phase, which, we hypothesize, promotes replacement of damaged proteins in anticipation of the next active period [41]. However, despite appreciation that organisms likely evolved to anticipate periods of food scarcity, the temporal regulation of many of these metabolic processes during fasting is less well understood, particularly in the mouse.

The purpose of the present study was to examine the temporal adaptation of the murine heart to fasting. We hypothesized that fasting during the sleep period elicits beneficial adaptation of the heart at transcriptional, translational, and metabolic levels. These studies revealed that in terms of glucose utilization and protein synthesis, the murine heart appears to exhibit greater responsiveness to fasting during the dark/active phase. In contrast, fasting-mediated alterations in autophagic markers are greatest during the light/sleep phase. Collectively, these observations support the concept that responsiveness of the heart to fasting is temporally organized. We speculate that sleep phase fasting may benefit cardiac function through augmentation of protein/cellular constituent turnover.

2. Materials and methods

2.1. Mice

We used wild-type mice, either on the C57Bl/6 J (24-h fasting time course) or FVB/N (12-h time-of-day-dependent fasting) background. All mice were male, were 16–20 weeks old (at the time of euthanasia), and were housed at the Animal Resource Program at the University of Alabama at Birmingham (UAB), under temperature-, humidity-, and light- controlled conditions. A strict 12-h light/12-h dark cycle regime was enforced (lights on at 6 AM; Zeitgeber Time [ZT] 0). The light/dark cycle was maintained throughout these studies; as such, physiologic diurnal variations were investigated in mice (as opposed to circadian rhythms). All mice had free access to water. When in the fed state, mice were provided a standard rodent chow. At the time of tissue and blood collection, mice were anesthetized with pentobarbital. All animal experiments were approved by the Institutional Animal Care and Use Committee of UAB.

2.2. Fasting protocols and non-invasive mouse monitoring

For the 24-h fasting time course study, a wire bottom floor was placed in the microisolator cage, thus preventing consumption of feces or bedding; ad libitum fed mice were also placed on a wire bottom floor, but were allowed access to chow. For the 12-h time-of-day-dependent fasting study (as well as a sub-set of the 24-h fasting time course studies), mice were housed in a computer-controlled Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments Inc., Columbus, OH), which enforced time-of-day-dependent food access in the absence of direct human intervention. More specifically, mice were allowed access to food either during the 12-h dark phase (i.e., light phase [LP] fasted mice) or during the 12-h light phase (i.e., dark phase [DP] fasted mice); these feeding/fasting regimes were enforced for a 9 day period, thereby allowing sufficient equilibration time. The CLAMS also continuously assessed food intake, physical activity (beam breaks), energy expenditure (indirect calorimetry), and respiratory exchange ratio (RER), as described previously [3]. In all studies, mice were singly housed and acclimatized to their housing conditions for at least 1 week prior to initiation of the experimental protocol.

2.3. Humoral factor analysis

Blood was collected at the time of euthanization, placed in EDTA-containing tubes, and centrifuged at 3000*g* for 10 min at 4 °C; resultant plasma was stored at −80 °C prior to assessment of insulin, non-esterified fatty acids (NEFA), and glucose levels using commercially available kits. Insulin was measured using a sensitive rat insulin RIA kit (EMD Millipore Corporation, Billerica, MA; catalog number SRI-13K). NEFA were measured on the Stanbio Sirrus analyzer (Stanbio Laboratories, Boerne, TX) using reagents from Wako Diagnostics, Mountain View, CA (catalog numbers are 999-34691, 995-34791, 991-34891, and 993-35191). Glucose was measured on the Stanbio Sirrus analyzer (Stanbio Laboratory, Boerne, TX) using a glucose oxidase reagent (manufactured by Stanbio Laboratory; catalog number 1071-250). Glucose levels were also determined in a drop of blood (prior to plasma preparation) through use of a FreeStyle Lite glucometer (Abbott Diabetes Care Inc., Alameda, CA).

2.4. Quantitative RT-PCR

RNA was extracted from hearts using standard procedures [5]. Candidate gene expression analysis was performed by quantitative RT-PCR, using methods described previously [13,17]. Specific Taqman assays were designed for each gene from mouse sequences available in GenBank, and have been reported previously [7,9,34,39,40]. All quantitative RT-PCR data were normalized to the housekeeping gene *cyclophilin* (this gene did not differ between experimental groups). Quantitative RT-PCR data is presented as fold change from the trough value in a specified group.

2.5. Immunoblotting

Qualitative analysis of protein expression and posttranslational modifications (e.g., lipidation, phosphorylation) was performed as described previously [8]. Lysates (5–30 μg) were separated on bis-acrylamide gels by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to a PVDF membrane and initially stained with ponceau for assessment of total protein loading. Membranes were subsequently probed with antibodies against PDK4 (Abcam #Ab38242), mTOR (Cell Signaling 2983), p-mTOR (Ser2448; Cell Signaling 2971), S6 (Cell Signaling 2317), p-S6$^{Ser240/244}$ (Cell Signaling 2215), LC3 (Cell Signaling 12,741), p62 (Abnova H00008878-M01), or anti-calsequestrin (Abcam 3516). Following visualization, bands were quantified with the freely available software, ImageJ (NIH). Proteins were normalized to calsequestrin, while phosphoproteins were normalized to their respective total proteins. Importantly, due to the nature of time course studies, in order to minimize the contribution that position on the gel might have on outcomes, samples were randomized on gels; samples were re-ordered post-imaging, only for the sake of