Diurnal variations in myocardial metabolism

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Abstract

Diurnal variations in the myocardium have been described at several levels, including gene expression, cellular signaling, metabolism, contractile function, and dysfunction. Regarding myocardial metabolism, carbohydrate, fatty acid, amino acid/protein, and coenzyme metabolism have all been shown to oscillate in the heart in a manner dependent on the time of day. The purpose of this review is to highlight our current understanding of diurnal variations in myocardial metabolism, with specific emphasis on fatty acid metabolism. Mechanistic studies have revealed control of myocardial fatty acid metabolism by an intramyocellular mechanism, known as the cardiomyocyte circadian clock. Whether disruption of myocardial metabolism diurnal variations during disease states contributes towards the etiology of contractile dysfunction is currently unknown.
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Introduction

Diurnal variations (ie, fluctuations over the course of the day, in the presence of environmental cues, such as the light/dark cycle) are observed in myocardial biology [1]. Gene expression, cellular signaling, metabolic fluxes, and contractile function of the heart oscillate dramatically as a function of time [1–7]. For example, robust diurnal variations in heart rate and cardiac output are observed in both animal models and humans [5–7]. Similarly, the incidence of adverse cardiac events (eg, arrhythmias, sudden cardiac death, myocardial infarction) exhibits dramatic diurnal variations [8]. Myocardial contractile function and metabolism are inseparably interlinked [9]. Furthermore, significant evidence suggests that myocardial metabolism profoundly influences outcome after an adverse cardiovascular event (eg, myocardial infarction) [10]. It is therefore not surprising that myocardial metabolism also exhibits robust diurnal variations [11,12]. These oscillations in myocardial metabolism are attuned not only to sleep/wake cycles, but also to feeding/fasting cycles [11]. The purpose of this review is to provide a brief overview regarding diurnal variations in myocardial metabolism, with a specific emphasis on fatty acid metabolism. We will also highlight our current knowledge regarding the contribution of the cardiomyocyte circadian clock towards myocardial metabolism diurnal variations.

Diurnal variations in myocardial metabolism

General metabolism

Diurnal variations in metabolism have been observed across various species, at several levels. In mammals, oscillations in metabolism that are dependent on the time of day are observed at whole-body, organ, and cellular levels. To date, studies focusing on diurnal variations in myocardial metabolism have been carried out primarily in rodents. The rat heart exhibits profound diurnal variations in carbohydrate, fatty acid, amino acid, and coenzyme metabolism (summarized in *Table I*) [4,13–19]. For example, during the active phase, a propensity exists for glucose units (derived both extracellularly and from glycogen) to be fully oxidized [4,13]. This in turn would aid the myocardium in meeting the high energetic demand at this time. Evidence also exists suggesting that amino acid and protein turnover exhibit clear diurnal variations. For example, Rau and Meyer [17] have reported that net protein synthesis increases near the sleep-to-wake (light-to-dark) phase transition in the rat heart, which is independent of feeding/fasting cycles. Total free amino acid concentrations are increased in the rat heart during the sleep (light) phase, suggesting that myocardial concentrations do not simply increase postprandially [19]. High-performance liquid chromatography (HPLC) analysis reveals myocardial levels of arginine, serine, and tyrosine significantly elevated at this time (M. E. Young, unpublished observations). These observations lead to the hypothesis that myocardial accumulation of amino acids during the sleep phase allows anticipation of increased protein synthesis at the sleep-to-wake transition.

To date, our most complete understanding of the mechanisms driving diurnal variations relates to myocardial fatty acid and triglyceride (triacylglycerol [TAG]) metabolism. The following section highlights our current knowledge in this field.

Fatty acid and triacylglycerol metabolism

Fatty acids are the primary energy source for the healthy myocardium, generating approximately 70% of the ATP utilized for contraction [20]. However, fatty acids are more than just a fuel and, when in excess, they exert cardiotoxic effects [21]. Significant sources of fatty acids for the heart include circulating non-esterified fatty acids, circulating lipoproteins, and endogenous myocardial TAG [22]. All these sources exhibit diurnal variations, as do both the acute and chronic responsiveness of the myocardium to fatty acids. Acutely, fatty acids depress myocardial contractile function and efficiency of the rat heart to the greatest extent during the sleep/resting phase [13]. In terms of metabolic flux, although fatty acid oxidation rates do not exhibit diurnal variations ex vivo, channeling of fatty acids into distinct non-oxidative pathways does, including phospholipid, diacylglycerol, and TAG synthesis [13]. Chronically, for the rat, transcriptional responsiveness of the heart to increased fatty acid concentrations peaks during the active phase [14].

Myocardial fatty acid utilization is regulated at several levels, including uptake, binding, activation, and channeling into oxidative or non-oxidative pathways, in addition to secretion (*Figure 1*). Uptake of long-chain fatty acids into the cardiomyocyte involves several proteins, including fatty acid translocase (FAT/CD36), fatty acid transport proteins (FATP), fatty acid binding proteins (FABP), and long-chain acyl coenzyme A (CoA) synthetases (ACSL) [23]. Uptake of fatty acids derived from lipoproteins also requires lipoprotein lipase (LpL) [22]. Many of these mediators of fatty

Table I. Diurnal variations in rat heart metabolism.

Metabolic parameter	Time of peak in oscillation	References
General oxidative metabolism		
Oxygen consumption	Middle of active phase	[4,13]
Glucose metabolism		
Glucose oxidation	Middle of active phase	[4,13]
Lactate release	Middle of sleep phase	[13]
Glycogenolysis capacity	Middle of active phase	[13]
Glycogen content	Active-to-sleep phase transition	[13]
Fatty acid metabolism		
β-Oxidation	No oscillation observed	[4,13]
Non oxidative metabolism	Middle of sleep phase	[13]
Triglyceride content	Sleep-to-active phase transition	[13]
Transcriptional response to fatty acids	Middle of active phase	[14,15]
Lipid peroxidation	Middle of active phase	[16]
Amino acid/protein metabolism		
Protein synthesis	Sleep-to-active phase transition	[17]
Amino acid content	Middle of sleep phase	[19]
Coenzyme metabolism		
NAD^+ concentrations	Middle of active phase	[18]

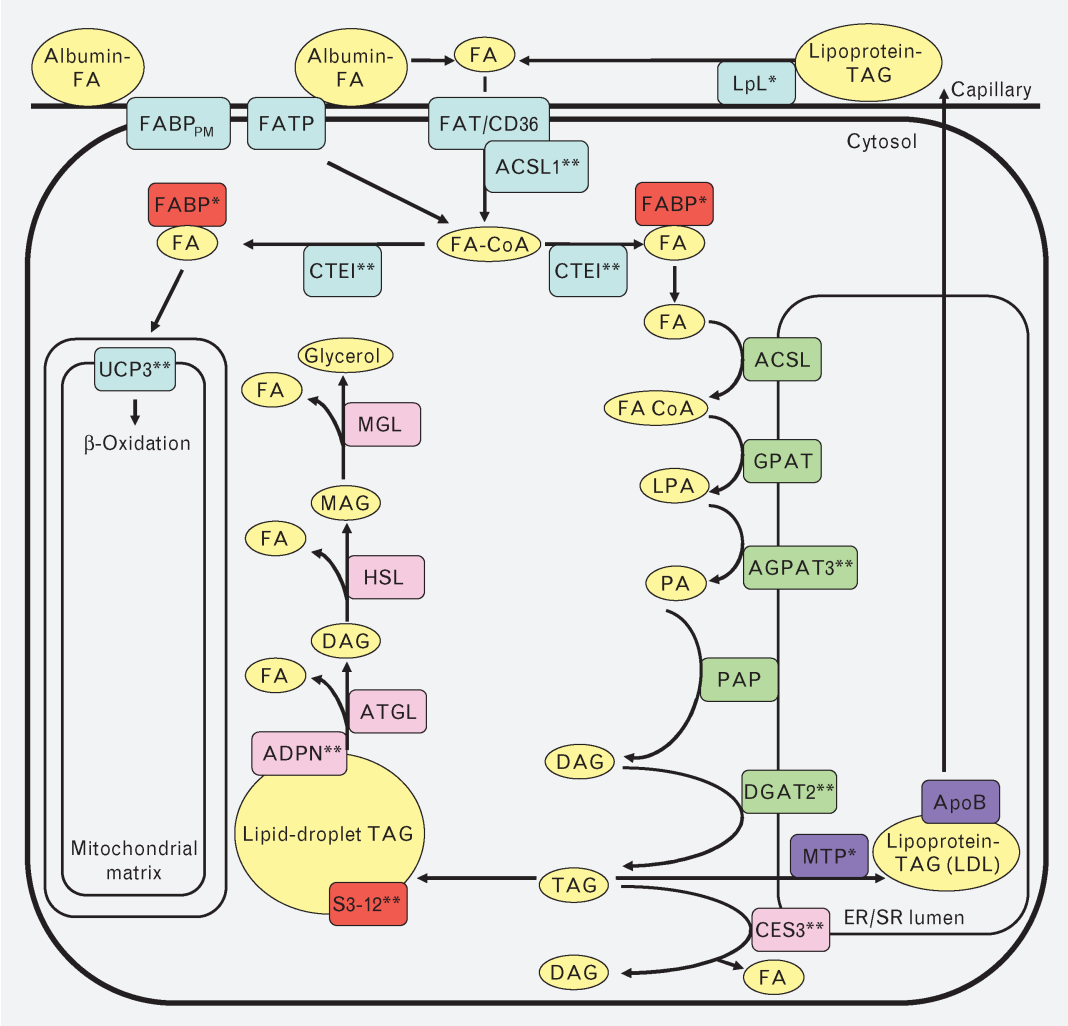

Figure 1. Schematic diagram of myocardial triacylglycerol metabolism. ACSL1, acyl coenzyme A synthetase long-chain 1; ADPN, adiponutrin; AGPAT3, 1-acylglycerol-3-phosphate O-acyltransferase 3; ApoB, apolipoprotein B; ATGL, adipose triglyceride lipase; CES3, carboxylesterase 3; CoA, coenzyme A; cTE1, cytosolic thioesterase 1; DAG, diacylglycerol; DGAT2, diacylglycerol acyltransferase; ER, endoplasmic reticulum; FA, fatty acid; FABP, fatty acid binding protein; FABP$_{PM}$, plasma membrane fatty acid binding protein; FAT/CD36, fatty acid transporter; FATP, fatty acid transport protein; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LDL, low-density lipoprotein; LPA, lysophosphatidic acid; LpL, lipoprotein lipase; MAG, monoacylglycerol; MGL, monoglyceride lipase; MTP, microsomal triglyceride transfer protein; PA, phosphatidic acid; PAP, phosphatidic acid phosphohydrolase; S3-12, adipocyte protein S3-12; SR, sarcoplasmic reticulum; TAG, triacylglycerol; UCP3, uncoupling protein 3. ∗Enzyme exhibits diurnal variation at the level of mRNA, protein, activity, or combinations thereof. ∗∗Diurnal variation mediated by the circadian clock. Adapted from Lewin and Coleman [23], with permission.

acid uptake demonstrate diurnal variations in expression/activity. LpL activity oscillates in the rat heart, peaking during the sleep phase [24]. In contrast, FABP peaks in the middle of the active/awake phase in the rat heart [25]. More recently, we have reported significant diurnal variations in the expression of *acsl1* and *acsl3* in mouse and rat hearts, respectively [26].

Once generated by ACSL, fatty acid CoAs are channeled into oxidative and non-oxidative pathways (*Figure 1*). Regarding the latter, TAG concentrations exhibit marked diurnal variations in the myocardium [13]. Enzymes involved in the Kennedy pathway of TAG synthesis include glycerol-3-phosphate acyltransferase (GPAT), 1-acyl-glycerol-3-phosphate acyltransferase (AGPAT), and diacylglycerol acyltransferase (DGAT) [27]. Recent gene expression microarray analysis of mouse hearts isolated every 3 h over a 24 h period revealed that *acsl1*, *agpat3*, and *dgat2* demonstrate significant diurnal variations [2].

Myocardial lipolysis is potentially mediated by a variety of lipases, including adipose triglyceride lipase (ATGL), adiponutrin (ADPN), hormone-sensitive lipase (HSL), triglyceride hydrolase (TGH), and carboxylesterase 3 (CES3) [28]. The findings of genetic studies have suggested that ATGL is rate-limiting for TAG hydrolysis [29]. ADPN is the nearest phylogenetic neighbor of ATGL, although controversy exists

regarding its lipase activity [30]. HSL has a greater affinity for DAG than for TAG [30]. Of these lipases, *adpn* and *ces3* oscillate in a diurnal manner in the mouse heart [2].

Reminiscent of the intestine and liver, the heart synthesizes and secretes lipoproteins (primarily low-density lipoprotein), which may play a significant part in regulating myocardial TAG stores [31]. A critical enzyme in lipoprotein secretion is microsomal transfer protein (MTP) [32]. Although diurnal regulation of MTP has not been investigated in the heart, it has been characterized in the intestine, where MTP mRNA, protein, and activity oscillate in a diurnal manner [32]. Thus myocardial lipoprotein secretion potentially exhibits a diurnal variation.

Contribution of myocardial metabolism diurnal variations by the cardiomyocyte circadian clock

Diurnal variations in myocardial metabolism are regulated by an interplay of extrinsic neurohumoral factors (eg, sympathetic activity, circulated insulin, thyroid hormone, and corticosteroid levels, as well as circulating fuels such as glucose, fatty acids, triglycerides, and ketone bodies) and intrinsic cell autonomous circadian clocks. Circadian clocks are defined as a set of proteins that generate self-sustained transcriptional positive- and negative-feedback loops with a free running period of approximately 24 h [33]. This molecular mechanism provides the selective advantage of anticipation, enabling the cell to prepare for an environmental stimulus before its onset (eg, modulates responsiveness to neurohumoral stimuli), and ultimately regulating function and metabolism in a manner that is dependent on the time of day. After characterization of the cardiomyocyte circadian clock, we developed a mouse model of genetic ablation of this molecular mechanism [15,34]. Using this model, termed ''cardiomyocyte-specific clock mutant'' (CCM), we defined roles for the cardiomyocyte circadian clock as a mediator of diurnal variations in myocardial gene expression, signaling, metabolism, and contractile function [2].

Microarray studies comparing wildtype and CCM mice identified various metabolic genes as being regulated by the cardiomyocyte circadian clock [2]. These included genes influencing glucose (eg, *pdk4*), fatty acid (eg, *acsl1*), and general oxidative (eg, *ndufa3/b3*) metabolism [2]. Consistent with these alterations in gene expression, CCM hearts exhibit alterations in fatty acid oxidation (increased) and glycogenolysis (decreased) [2]. In addition, through use of CCM mice, we have shown that diurnal variations in the transcriptional responsiveness of the heart to fatty acids are mediated by the cardiomyocyte circadian clock [15]. Additional evidence for the latter includes persistence of diurnal variations in FA transcriptional responsiveness in cultured adult rat cardiomyocytes (under which conditions neurohumoral influences are ablated) [15].

Not surprisingly, myocardial TAG metabolism is markedly altered in CCM hearts. Microarray studies revealed that the cardiomyocyte circadian clock regulates several genes involved in myocardial fatty TAG metabolism (eg, *agpat3*, *dgat2*, *adpn*, *ces3*; *Figure 1*) [2]. Consistent with these observations, fasting-induced myocardial TAG synthesis is abolished in CCM hearts [15]. More recently, we have found that diurnal variations in TAG synthesis observed in wildtype hearts perfused ex vivo are abolished in CCM hearts [35]. Taken together, these observations show that the cardiomyocyte circadian clock regulates myocardial TAG metabolism.

Summary

The myocardium exhibits profound oscillations in metabolism that are dependent on the time of day. Many of these oscillations appear to precede (as opposed to proceed) feeding/fasting and sleep/wake cycles. This anticipation is probably conferred by the cardiomyocyte circadian clock. Impairment of the cardiomyocyte circadian clock or diurnal variations in neurohumoral factors (eg, fatty acids), or both, will result in uncoupling of the synchronization of the heart with the environment, potentially accelerating contractile dysfunction through inappropriate responses (eg, channeling fatty acids into lipotoxic pathways).

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