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Circadian rhythms and cardiovascular disease

Danielle Feuvray
University of Paris-Sud 11 and UMR CNRS 8162, France

Correspondence: Professor Danielle Feuvray, University Paris-Sud 11 and CNRS UMR 8162, Marie Lannelongue Hospital, 92350 Le Plessis Robinson, France.
E-mail: danielle.feuvray@u-psud.fr

Circadian rhythms are daily, approximately 24-hour oscillations in physiology and behavior such as food consumption, blood pressure, and metabolism. These rhythms are believed to give an adaptive advantage by allowing an organism to anticipate changes in the environment and regulate its physiology accordingly. It is well recognized that aspects of both cardiovascular physiology and the clinical manifestation of cardiovascular disease display diurnal variations. The central loci of the mammalian clock are two small clusters of hypothalamic neurons called the suprachiasmatic nuclei (SCN), which constitute the master pacemaker that orchestrates rhythmic patterns of behavior and physiology throughout the organism. For many years, neurons of the SCN were believed to contain the unique clock controlling circadian rhythmicity of peripheral tissues via neural and humoral signals. Surprisingly, the cloning and characterization of mammalian clock genes have revealed that most tissues in the body also contain autonomous circadian clocks that are necessary for the rhythmic expression of clock output genes. These peripheral oscillators, which share many of their molecular components with the master oscillator, can also be distinguished by the expression of specific transcription factors. The molecular oscillator is composed of interlocking positive and negative transcriptional and translational feedback loops that drive the circadian expression of genes.

In this issue of Heart and Metabolism, the Basic Article by Drs Tsai and Young offers a detailed review regarding diurnal variations in myocardial cell metabolism. A striking example is given by the modulation in the responsiveness of the heart to fatty acids, the primary fuel source for the healthy myocardium. Elegant studies in rodent hearts have shown that diurnal variations in the responsiveness of the heart to fatty acids are mediated by the cardiomyocyte circadian clock. Variations are preserved in single cells ex vivo, provided that the circadian clock within the cardiomyocyte is fully functional. Interestingly, the rhythmic expression of genes regulated by the circadian clock is disturbed in various pathological states. The New Therapeutic Approaches article by Drs Duez and Staels points out that recent data demonstrate, not only that the expression of certain nuclear receptors is driven by the circadian clock, but also that they participate in the circadian control of metabolism. In particular, the nuclear receptor Rev-erba is involved in the regulation of the core clock machinery and, consequently, may play a central part in orchestrating the temporal coordination of metabolism in several cell types. The recent identification of natural (heme) and synthetic ligands for Rev-erba suggests that it may represent a potential target for the treatment of metabolic diseases that are related to disturbance of circadian rhythms. Whether Rev-erba is expressed in a circadian manner in the heart, cells of
the vascular wall, or cardiac myocytes remains to be elucidated.

In the Main Clinical Article of this issue of *Heart and Metabolism*, Dr Dominguez-Rodriguez and colleagues give a superb overview of the available evidence linking disruption of circadian rhythms to cardiovascular disease. There is indeed a universal appreciation of the presence of diurnal variations in the response of the cardiovascular system in both physiological and pathophysiological circumstances. Serious adverse cardiovascular events appear to be conditioned by time of day. Therefore biological responses, which are under the control of the molecular clock (within both cardiac myocytes and vascular smooth muscle cells), may interact with environmental cues to influence the clinical manifestation of cardiovascular disease.

The Refresher Corner by Drs Reiter and Tan points out that melatonin, a hormone produced mainly in the pineal gland, may have a protective role in reducing cardiac ischemia-reperfusion injury, as shown in experimental models. Melatonin, an endogenous signal of darkness, is an important component of the body’s internal time-keeping system. This is subtly illustrated by the jet-lag syndrome described in the Case Report by Dr Jackson. Melatonin regulates major physiological processes. Reiter and Tan also underline that, in addition to its relevant antioxidant activity, melatonin exerts many of its physiological actions by interacting with specific membrane receptors. There are circadian variations in the expression of the melatonin receptor and, possibly, signal transduction pathways in various organs, including the heart. Further investigations are needed to clarify the potential importance of the use of melatonin in situations of oxidative damage to the heart in humans.

This issue of *Heart and Metabolism* provides a contemporary description of various aspects by which circadian rhythms may control the coordination of metabolic processes. It also highlights that disturbances of the circadian clock may predispose or lead to metabolic disorders and therefore influence the phenotype of cardiovascular disease.

*See glossary for definition of these terms.*
Diurnal variations in myocardial metabolism

Ju-Yun Tsai and Martin E. Young
USDA/ARS Children’s Nutrition Research Center, Baylor College of Medicine, Department of Pediatrics, Houston, Texas, USA

Correspondence: Dr Martin E. Young, USDA/ARS Children’s Nutrition Research Center, Baylor College of Medicine, Department of Pediatrics, 1100 Bates Street, Houston, Texas 77030, USA.
Tel: +1 713 798 7567; fax: +1 713 798 7101; e-mail: meyoung@bcm.edu

Sponsorship: This work was supported by the National Heart, Lung, and Blood Institute (HL-074259; M. E. Y.), the USDA/ARS (6250-51000-044; M. E. Y.), Kraft Inc. (M. E. Y.), and DeBakey Heart Fund (J.-Y. T.). Ju-Yun Tsai is in the Graduate Program in Cardiovascular Sciences.

Conflicts of interest: None.

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.

Heart Metab. 2009;44:5–9.

Keywords: Chronobiology, circadian clock, fatty acids, heart, triglyceride

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.**

<table>
<thead>
<tr>
<th>Metabolic parameter</th>
<th>Time of peak in oscillation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>General oxidative metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen consumption</td>
<td>Middle of active phase</td>
<td>[4,13]</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>Middle of active phase</td>
<td>[4,13]</td>
</tr>
<tr>
<td>Lactate release</td>
<td>Middle of sleep phase</td>
<td>[13]</td>
</tr>
<tr>
<td>Glycolysis capacity</td>
<td>Middle of active phase</td>
<td>[13]</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>Active-to-sleep phase transition</td>
<td>[13]</td>
</tr>
<tr>
<td>Fatty acid metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Oxidation</td>
<td>No oscillation observed</td>
<td>[4,13]</td>
</tr>
<tr>
<td>Non oxidative metabolism</td>
<td>Middle of sleep phase</td>
<td>[13]</td>
</tr>
<tr>
<td>Triglyceride content</td>
<td>Sleep-to-active phase transition</td>
<td>[13]</td>
</tr>
<tr>
<td>Transcriptional response to fatty acids</td>
<td>Middle of active phase</td>
<td>[14,15]</td>
</tr>
<tr>
<td>Lipid peroxidation</td>
<td>Middle of active phase</td>
<td>[16]</td>
</tr>
<tr>
<td>Amino acid/protein metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Sleep-to-active phase transition</td>
<td>[17]</td>
</tr>
<tr>
<td>Amino acid content</td>
<td>Middle of sleep phase</td>
<td>[19]</td>
</tr>
<tr>
<td>Coenzyme metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAD⁺ concentrations</td>
<td>Middle of active phase</td>
<td>[18]</td>
</tr>
</tbody>
</table>
Acid uptake demonstrates 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-acylglycerol-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.

**Basic article**

Diurnal variations in myocardial metabolism

![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; FABP, fatty acid transport protein; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LDL, low-density lipoprotein; LPA, lysophosphatic acid; LpL, lipoprotein lipase; MAC, 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.**
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]. Therefore, 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, pkd4), 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 this 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).

Acknowledgments

We wish to thank Douglas Burrin (Baylor College of Medicine) for his help with rat heart amino acid analysis by HPLC.

REFERENCES

Diurnal variations in myocardial metabolism


Disruption of normal circadian rhythms and cardiovascular events

Alberto Dominguez-Rodriguez, Pedro Abreu-Gonzalez and Juan Carlos Kaski

Department of Cardiology (Coronary Care Unit), University Hospital of Canarias, Tenerife, Spain, Department of Physiology, University of La Laguna, Tenerife, Spain, and Cardiac and Vascular Sciences, St George’s University of London, UK

Correspondence: Prof. J.C. Kaski, Cardiovascular Biology Research Centre, Division of Cardiac and Vascular Sciences, St George’s University of London, Cranmer Terrace, London SW17 0RE, UK.

E-mail: jkaski@sgul.ac.uk

Conflicts of interest: None.

Abstract

The intrinsic properties of the heart and the vascular tree exhibit marked oscillations over 24 h. Diurnal variations in the response of the cardiovascular system to environmental stimuli are mediated by the complex interplay of extracellular (ie, neurohumoral factors) and intracellular (ie, circadian clock) influences. The intracellular circadian clock comprises a series of transcriptional modulators that together allow the cell to ‘‘perceive’’ the time of day, thus enabling suitable responses to expected stimuli. These molecular timepieces have been identified and characterized within both vascular smooth muscle cells and cardiomyocytes, giving rise to a multitude of hypotheses regarding the potential role of the circadian clock as a modulator of physiological and pathophysiological cardiovascular events. This article summarizes the evidence available at present linking circadian rhythm disruption and cardiovascular disease.

Keywords: Circadian clock, diurnal variations, cardiovascular disease

Introduction

Circadian clocks have been identified in every mammalian cell investigated to date, including key components of the cardiovascular system, such as cardiomyocytes and vascular smooth muscle cells. There is universal appreciation of the presence of diurnal variations in cardiovascular function, in both physiological and pathophysiological circumstances. The recent identification of a molecular “machinery” within cells in the cardiovascular system, with the potential to modulate an array of cellular processes, has sparked increasing interest among researchers. Historically, diurnal variations in blood pressure, heart rate, and cardiac output, in addition to major cardiovascular events (ie, myocardial infarction and sudden death), have been attributed primarily to the occurrence of circadian changes in the autonomic nervous system – that is, sudden increase in sympathetic activity. However, it has become apparent that changes in the ability of the cardiovascular system to respond to neurohumoral stimuli in an appropriate and timely manner are likely to be of equal importance.

Intracellular circadian clocks provide a means by which the heart and vasculature can “anticipate” diurnal variations in stimuli, such as autonomic nervous activity, ensuring an optimal response. The attenuation or malfunction of this molecular mechanism could therefore impair the ability of the heart, the vasculature, or both, to respond appropriately to environmental stimuli, which in turn may contribute to the development of cardiovascular disease. This article summarizes current knowledge regarding circadian clocks within the cardiovascular system, the biological processes they influence, and how a
disturbance of these circadian rhythms can lead to cardiovascular disease.

The central circadian clock

Almost all living organisms have developed biological rhythms linked to the day/night or light/dark cycles of the sun [8]. The impact that such rhythms that follow the time of day and season of the year exert on a variety of physiological functions in humans has been recognized for a long time [8]. The internal oscillator, or control station regulating the body’s circadian clock, is the suprachiasmatic nucleus, a tiny structure (comprising approximately 70,000 neurons) located in the hypothalamus, above the optic chiasm [9]. The suprachiasmatic nucleus processes external signals such as ambient light and inputs from the brain to regulate a variety of cyclic functions, including body temperature, sleep/wake cycles, and secretion of hormones such as cortisol, melatonin, thyroxin, and vasopressin [8].

Evidence gathered over the past 15 years suggests that melatonin influences several functions of the cardiovascular system. Similar to other organs and systems, the cardiovascular system exhibits diurnal and seasonal rhythms in heart rate, cardiac output, and blood pressure [10], which are likely to be modulated by the suprachiasmatic nucleus and, possibly, the melatoninergic system. The circadian pacemaker within the suprachiasmatic nucleus stimulates the pineal gland to produce melatonin at night. This process is set by the phase-shifting actions of light, such that normal physiological plasma concentrations of melatonin during the day are very low, but they begin to increase at night, from around 22.00 h onwards, with a peak at approximately 03.00 h, and a return to daytime values by 09.00 h. Endogenous production of melatonin is approximately 30 μg per day [11], associated with peak plasma concentrations of approximately 100 pg/mL, although these are highly variable among individuals, and decrease with age [12].

The enterochromaffin cells within the gastrointestinal tract also synthesize melatonin; this probably accounts for the normal low daytime plasma concentrations of this hormone, and is not under the circadian control of the suprachiasmatic nucleus [13].

Melatonin and cardiovascular disease

In previous studies, our group of investigators has reported a relationship between melatonin and light/dark variations in the production of inflammatory systemic markers such as interleukin-6, C-reactive protein, matrix metalloproteinase-9, and soluble vascular cell adhesion molecule-1 [14–17]. We have also demonstrated, in a prospective study in patients with ST-segment elevation myocardial infarction, that melatonin may have a prognostic role. These findings, taken together, appear to suggest that patients who develop adverse events (heart failure or cardiac death) have significantly lower nocturnal concentrations of melatonin than patients who do not experience such events [18]. Moreover, in a recent study in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention, we observed a relationship between melatonin concentrations and ischemia-modified albumin, a marker of myocardial ischemia. Our data suggest that melatonin acts as a potent antioxidant, reducing myocardial damage induced by ischemia-reperfusion [19].

Expression of circadian clock genes in the heart

Oscillations in gene expression in the heart have been examined extensively in mouse models, using both real-time polymerase chain reaction and expression array analysis [20,21]. Genes encoding both core clock components and clock-controlled genes, relevant to cardiac function, have demonstrated dramatic oscillations in heart tissue isolated at intervals throughout the circadian cycle [22]. Included in these oscillating transcripts are genes relevant to carbohydrate utilization, mitochondrial function, and fatty acid metabolism [23].

Although the circadian clock within the heart drives cardiac physiology, the function of this clock can be disrupted in pathological conditions. In a model of experimentally induced cardiac hypertrophy, the core molecular oscillator continues to cycle, but the amplitude of oscillations in transcription factors such as D-element binding protein are blunted [24], and the circadian cycle of expression of metabolic genes is lost. Hence, the tissue would be less prepared to respond to increases in physiological demand, predisposing it to metabolic abnormalities. Streptozotocin-induced diabetes in the rat is another model of contractile dysfunction that alters clock gene expression in the heart; clock component oscillations show normal amplitude, but their phase is advanced by approximately 3 h in this model [25]. Thus the impact of disease states on the cardiac circadian clock seems to be at the levels both of circadian clock genes and of clock-controlled output genes relevant to tissue-specific functions.

Circadian rhythms within the cardiovascular system

Intracellular circadian clocks exist within at least two major cells types in the cardiovascular system, namely
cardiomyocytes and vascular smooth muscle cells. This molecular mechanism is present in all mammalian cell types, but it has not been fully characterized in, for example, endothelial cells [26]. Circadian clocks within individual cells of the cardiovascular system can influence physiological cardiovascular responses – for example, increasing sympathetic nervous activity before awakening – thereby ensuring an appropriately rapid response when required. In the in-vivo setting, a complex interplay between environmental influences and intrinsic mechanisms (ie, central and peripheral circadian clocks) exists that contributes to changes in cardiovascular function over the course of the day (Figure 1) [27].

Circadian rhythms in blood pressure that occur in humans [28–31] are lowest at night, reaching a trough at around 03.00 h and a peak at around 09.00 h. A second peak in blood pressure is often seen early in the morning (19.00 h) [11]. Day-to-night differences in physical and mental activity appear to be major determinants of the circadian rhythms in blood pressure [28,32]. In humans, shift workers show an essentially complete resynchronization of blood pressure rhythms within the first 24 h of the shift rotation [33,34]. Rhythms in heart rate appear to be driven largely by diurnal variations in autonomic nervous system activity [35,36]. Several lines of evidence suggest that the circadian rhythms of these two cardiovascular parameters might be differentially regulated. Hu et al [37] have reported that, in humans, circadian rhythmicity in heart rate variability persisted, peaking in the early hours of the morning, even when their analyses controlled for sleep/wake and behavior cycles. Such studies expose an intrinsic component influencing normal cardiovascular function. It could be hypothesized that diurnal variations in autonomic stimulation (driven by the suprachiasmatic nucleus) and circadian-clock-driven diurnal variations in responsiveness of the heart to autonomic stimulation act together as major determinants of heart rate circadian rhythms. Environmental modulation of the synchronization between peripheral and central clocks may contribute to the development of cardiovascular disease; however, this remains speculative at present. A loss of synchronization of this type occurs in patients with diabetes mellitus, obesity, and sleep apnea, and in shift workers, all of whom are associated with increased risk for cardiovascular disease [27].

It has been shown that shift workers experience physical and psychological changes during the night. The exact mechanisms leading to these changes and their clinical impact are poorly understood at present. Stress-related biological variables, such as cortisol and body temperature, have a circadian pattern characterized by increased values during daytime, when the individual is active (awake), and lower values during the sleeping hours [38]. Some studies have shown a strong influence of physical activity levels on the circadian changes in heart rate and blood pressure [39–41].

Furlan et al [42] reported that continuous weekly changes in time of maximum and minimum output in the cardiac sympathetic and vagal autonomic control may play a part in the excessive rate of cardiovascular disease in shift workers. Circadian changes in autonomic activities have been postulated to be one of the reasons for the increased incidence of ischemic heart disease, stroke, and sudden death [6] in these individuals.

The circadian clock in physiological and pathophysiological states

Circadian clocks are altered in various animal models of cardiovascular disease. Young et al [23,24] found that the rhythmic expression of genes regulated by the circadian clock (eg, dbp, hlf, tef, pdk4, and ucp3) is significantly attenuated in the rat heart during left ventricular hypertrophy induced by pressure overload. Consistent with these observations, Mohri et al [43] reported that oscillations in circadian clock genes are severely attenuated in the hypertrophic hearts of Dahl rats fed a high-salt diet (an animal model of hypertension). In contrast, Naito et al [44] reported an augmentation of circadian clock gene oscillations in...
the aortae and hearts from a different rat model of hypertension, the spontaneously hypertensive rat, which is associated with amplified rhythms in pai-1.

In humans, circadian rhythms in pathophysiological cardiovascular events are also well documented. We have known for a number of years that circadian fluctuations affect, and perhaps even orchestrate, a variety of pathophysiological states. The onset of myocardial infarction, sudden cardiac death, and stroke, are all increased between the hours of 06.00 h and 12.00 h. These responses may be related, at least in part, to increased sympathetic activity after an individual gets out of bed, the interaction between catecholamines and platelets thus affecting atherosclerotic plaque pathophysiology [6]. Circadian variations have also been observed in relation to hemodynamic responses, including blood pressure, myocardial blood flow, and heart rate, and cardiovascular events. In addition, circadian alterations have been documented in the response of platelets to aggregating stimuli, the concentration of plasma fibrinogen and coagulation factors, and the activity of the fibrinolytic system [6].

Implications for research

The existence of circadian clocks within components of the cardiovascular system has far-reaching implications, which extend beyond the clinical setting. Given the diversity of diurnal variations in the intrinsic properties of the cardiovascular system, which manifest at several levels, namely gene and protein expression and cellular and organ function, extreme caution is required when research studies are being designed. Both in-vivo and in-vitro studies may be affected by circadian variations, therefore considering time of day may be important in the design of research experiments. Performance of experiments at an inappropriate time of the day or the omission of suitable time controls may lead to erroneous conclusions or uninterpretable data. Such temporal considerations will undoubtedly help to reduce discrepancies between studies performed in different laboratories, and also discrepancies between gene and protein expression measurements and animal and human models.

Conclusions

Circadian rhythms are regulated by three components: (1) the circadian pacemaker or “clock”, (2) an “input” mechanism, which allows the clock to be reset by environmental stimuli, and (3) an “output” mechanism, which regulates physiological and behavioral processes. For many years, it has been accepted that neurons in the suprachiasmatic nucleus were responsible for the control of circadian rhythms in peripheral tissues, acting via neural and humoral signals (eg, melatonin). It is currently believed that cells in other systems, including the cardiovascular system (ie, cardiomyocytes and vascular smooth muscle cells), are under the influence of circadian clocks similar to that in the suprachiasmatic nucleus.

Many aspects of cardiovascular physiology are subject to diurnal variations, and serious adverse cardiovascular events, including myocardial infarction and sudden cardiac death, appear to be conditioned by the time of day. It has therefore been suggested that biological responses, which are under the control of the “molecular clock”, may interact with environmental cues to influence the phenotype of human cardiovascular disease. Thus numerous mediators of cardiovascular disease display a circadian variation, and evidence for molecular clock regulation of these mediators is beginning to unfold. The mechanisms that regulate the circadian clock and the clinical implications of disturbances in circadian rhythm remain a fertile field of investigation.

REFERENCES

Metabolic imaging with cardiac magnetic resonance spectroscopy

Stefan Neubauer
University of Oxford Centre for Clinical Magnetic Resonance Research, Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford, UK

Correspondence: Professor Stefan Neubauer, Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK.
Tel: +44 (0)1865 851085; fax: +44 (0)1865 222077; e-mail: Stefan.neubauer@cardiov.ox.ac.uk

Sponsorship: The author is funded by the British Heart Foundation, the Medical Research Council, the Wellcome Trust, and the National Institute for Health Research Biomedical Research Centre Programme.

Conflicts of interest: None.

Abstract
The only non invasive technique for the assessment of cardiac metabolism in patients that does not use radiation is magnetic resonance spectroscopy (MRS). By interrogating signals from phosphorus-31 and hydrogen-1, spectroscopy offers a wealth of metabolic information on the heart muscle. This review focuses on the two areas of greatest potential for MRS: heart failure and ischemic heart disease. MRS has demonstrated deranged cardiac energetics in patients with heart failure, and this is probably a major mechanism contributing to contractile dysfunction. In ischemic heart disease, altered energetics are a highly sensitive indicator of the presence of myocardial ischemia, offering the prospect of a non invasive biochemical stress test for the heart. Although major technical development is required for the future, cardiac MRS holds great potential for clinical application.


Keywords: 31P-Magnetic resonance spectroscopy, cardiac metabolism, cardiac energetics, heart failure, ischemic heart disease

Introduction
Cardiovascular magnetic resonance is now a well established technique in clinical cardiology and is used to assess cardiac anatomy, function, perfusion, and viability. However, this method only uses the signal from protons in fat and water molecules for image generation. In contrast, magnetic resonance spectroscopy (MRS) allows us to use the signals, not only from protons, but also from other nuclei such as phosphorus-31 (31P) and others (eg, sodium-23, carbon-13) [1–3]. 31P-MRS is the most widely used form of spectroscopy, and it allows detection of adenosine-5’-triphosphate (ATP) and phosphocreatine (PCr), the high-energy phosphate compounds in the heart. ATP is the direct energy source for all energy-consuming reactions in the heart, whereas PCr is the major compound for energy storage and transport [4]. In principle, many research questions and clinical problems could be addressed by assessing cardiac high-energy phosphate metabolism; however, MRS is currently limited by the fact that MRS signals are approximately a million times weaker than the signals interrogated in magnetic resonance imaging, as a result of the lower concentrations of metabolites and lower magnetic resonance sensitivity of non proton nuclei. Thus the limited temporal and spatial resolution of the method currently limits its clinical application. In spite of these challenges, MRS studies have already contributed enormous insight into the pathophysiology of human heart disease.

Technical considerations
The first reports of human cardiac 31P-MRS are from the 1980s [5]. MRS can be performed on the same
magnetic resonance systems used for imaging, typically at a field strength of 1.5–3 Tesla (T). There are, however, additional hardware and software requirements, such as a $^{31}$P surface coil, a broadband radio-frequency transmitter, spectroscopy pulse sequences, and post-processing software [1,3]. Unlike magnetic resonance imaging studies, most MRS studies have been performed with patients in the prone position, to bring the heart closer to the surface coil. Before starting spectral acquisition, the magnetic field is homogenized with shimming, as MRS places high requirements on field homogeneity. Next, proton scout images are obtained for positioning the spectroscopic voxels over the heart. A range of different localization techniques (eg, 3-dimensional chemical shift imaging) makes it possible to obtain signal almost exclusively from the heart, excluding contaminating $^{31}$P signal from neighboring skeletal muscle and liver [3]. Because of the inherent low resolution of MRS, a large number of acquisitions have to be signal averaged, to obtain a magnetic resonance spectrum with an acceptable signal-to-noise ratio. A typical $^{31}$P spectrum from a normal individual is shown in Figure 1. Resonances are identified for the three phosphorus atoms of ATP ($\alpha$, $\beta$, and $\gamma$ ATP), PCr, and also 2,3-diphosphoglycerate (2,3-DPG) from blood and phosphodiesters (PDE) from phospholipids. Using line-fit algorithms, the PCr : ATP ratio can be calculated. This is an exquisitely sensitive index of the energetic state of the heart: it decreases within seconds of the onset of ischemia [6], because, when oxygen demand outstrips oxygen supply, PCr concentrations decrease long before ATP concentrations start to decrease. There is, however, a second mechanism that can decrease the PCr : ATP ratio: the reduction in the total creatine pool that occurs in heart failure [4]. Typical technical specifications for cardiac $^{31}$P-MRS at 1.5 T are an acquisition time of 20–40 min, voxel sizes of 20–70 ml and a PCr : ATP ratio variability of approximately 15%. Further developments in MRS methodology will improve these parameters, with a goal of voxel sizes of less than 10 ml, acquisition time less than 10 min, and a measurement variability of less than 10%.

Heart failure

Deranged cardiac energy metabolism is a hallmark of the failing heart [4,7]. Accordingly, PCr : ATP ratios are reduced in heart failure, correlating with the New York Heart Association (NYHA) functional class [8] and left ventricular ejection fraction [9]. Most importantly, they are a strong predictor of prognosis, and in one study the PCr : ATP ratio was a better predictor of long-term survival than NYHA class or left ventricular ejection fraction (Figure 2) [10]. Although PCr : ATP ratios are powerful indicators of the extent of energetic derangement in heart failure, they still underestimate the true extent of metabolic derangement. Recent spectroscopy techniques have made the absolute quantification of ATP and PCr possible, showing approximately 50% reduction in PCr and 35% reduction in ATP, with a concomitant 25% decrease in the PCr : ATP ratio, in heart failure [11]. An even more sensitive indicator of deranged energetics may be the dynamic rate of turnover of ATP. Recently, ATP turnover (ATP flux through the creatine kinase reaction) was measured in volunteers and patients with heart failure; for an 18% reduction in PCr concentrations, a 50% reduction in the rate of turnover of ATP was demonstrated [12]. Thus the measurement of ATP turnover rates holds promise as a highly sensitive indicator of energetic derangement in heart failure.

Figure 1. In-vivo human cardiac phosphorus-31 ($^{31}$P) magnetic resonance spectroscopy, 3-dimensional chemical shift imaging sequence. Left: Short-axis hydrogen-1 magnetic resonance image of the heart with a superimposed grid of spectroscopic voxels. The interrogated cardiac voxel (blue square) is placed in the interventricular septum to avoid contamination from skeletal muscle. Saturation bands are placed over the chest wall skeletal muscle to suppress further any skeletal muscle signal. Right: Example of a cardiac $^{31}$P-magnetic resonance spectrum in a healthy individual. Resonances for 2,3-diphosphoglycerate (2,3-DPG), phosphodiesters (PDE), phosphocreatine (PCr), and the three phosphorus atoms of adenosine-5'-triphosphate (ATP) (from left to right: $\gamma$, $\alpha$, and $\beta$-ATP) are detectable. 3T Siemens TIM-Trio system. Acquisition matrix size 16 x 16 x 8 voxels, field of view 240 x 240 x 200 mm.
An important area is the use of MRS for monitoring energetic changes in the heart after novel forms of treatment of heart failure. Our initial study indicated that conventional treatment of heart failure with β-blockers, angiotensin converting inhibitors, and diuretics for 3 months significantly improved the PCr:ATP ratio, together with clinical improvement [8]. Most recently, a study of the use of the investigational drug, trimetazidine, in patients with heart failure revealed that trimetazidine was associated with a 33% increase in the PCr:ATP ratio, concomitant with improvements in NYHA class and left ventricular ejection fraction [13]. Although currently available studies on this subject are clearly limited by their small size, the concept of monitoring novel drug therapy for heart failure with regards to its effects on cardiac energetics is an extremely appealing one.

Ischemic heart disease

A decrease in PCr concentration and concomitant increase in inorganic phosphate are among the most sensitive indicators of myocardial ischemia, occurring within seconds of the onset of oxygen deprivation [6]. This has led to the concept of a biochemical stress test for patients with suspected coronary disease [1]. Indeed, patients with left anterior descending coronary artery stenosis show significant decreases in PCr:ATP ratio during increased cardiac work, with the ratio returning to normal after recovery. After patients had undergone revascularization by percutaneous coronary intervention or bypass surgery, such decreases could no longer be demonstrated [14]. Furthermore, similar reductions in the PCr:ATP ratio during physical exercise have been observed in female patients with syndrome X [15]. In this cohort, over a period of 3 years, an abnormal 31P-MRS stress test has been demonstrated as a strong predictor of future cardiovascular events [16]. Thus 31P-MRS stress testing is, in principle, an extremely attractive technique for detecting myocardial ischemia at a tissue level, monitoring the anti-ischemic efficacy of medical or interventional therapy, and, potentially, as a tool with which to assess prognosis. The main challenge with studying ischemic heart disease with MRS is that it is spatially heterogeneous, requiring high spatial resolution, so that true clinical applicability will only be achievable once the improvements in technical specifications outlined above have been achieved.
A second application of MRS in ischemic heart disease is the assessment of viable myocardium. Normal, stunned, and hibernating myocardium show (near) normal concentrations of high-energy phosphates, whereas scar tissue has almost negligible concentrations of high-energy phosphates [17]. Thus, in principle, MRS would be well suited to the assessment of viability [1]. However, with the success of new magnetic resonance imaging techniques such as gadolinium-based late enhancement for viability imaging (spatial resolution 16 microliter), it now seems questionable that this particular application will ever become a success for MRS.

Conclusions and future directions

Magnetic resonance spectroscopy is an extremely interesting technique allowing non invasive, non radiation measurements of cardiac metabolism in the patient. Its future success will undoubtedly depend on further technical development, but if high spatial and temporal resolution can be achieved in addition to high reproducibility of measurements, then widespread application of this method would be predicted. Technical improvement is most likely to come from substantially greater field strengths (eg, 7 T), new phased-array coils, and, at least for carbon-13, with hyperpolarization techniques [18], which are currently entirely experimental, but can, in principle, boost the magnetic resonance signal by a factor of up to 100,000. In addition to heart failure and ischemic heart disease, diabetes [19], obesity [20], and inherited cardiomyopathies [21] are other highly promising areas for novel research into the interrelations of cardiac metabolism and contractile dysfunction by MRS.

Summary

Magnetic resonance spectroscopy allows for the non invasive assessment of various aspects of cardiac metabolism without the use of ionizing radiation. The most promising areas are heart failure and ischemic heart disease. Before the method can find broad clinical application, further technical improvements are necessary that should lead to substantially improved temporal and spatial resolution, and reduced variability of measurement.

REFERENCES

Rev-erbα: a potential target for the treatment of circadian disorders

Hélène Dueza,b,c,d and Bart Staelsa,b,c,d

aUniversité Lille Nord de France, F-59000, Lille, France, bInserm, U545, F-59000, Lille, France, cUDSL, F-59000, Lille, France, and dInstitut Pasteur de Lille, F-59019, Lille, France

Correspondence: UR545 INSERM, Institut Pasteur de Lille, BP 245, 1 rue Calmette, 59019 Lille, France.
E-mails: Bart.Staels@pasteur-lille.fr, Helene.Duez@pasteur-lille.fr

Sponsorship: The authors acknowledge funding support from INSERM, the Région Nord Pas-de-Calais/FEDER, the Sixth EU Research Framework Program Diabesity (contract LSHM-CT-2003-503041), COST-action BM0602.

Conflicts of interest: None.

Abstract

Circadian variations are observed in many physiological processes, and alterations in these oscillations are closely linked to mood disorders, metabolic abnormalities and cardiovascular complications. The nuclear receptor Rev-erbα is a crucial component of the clock mechanism. It is an important metabolic regulator and a target for lithium, a drug used in the treatment of bipolar disorders. The recent identification of natural (heme) and synthetic ligands for Rev-erbα suggests that pharmacological modulation of Rev-erbα activity may be a potential therapeutic option in the treatment of circadian disorders including circadian-related metabolic and cardiovascular diseases.

Keywords: Rev-erbα, circadian rhythm, metabolism, mood disorders

Many physiological processes follow daily variations, amongst which alteration between rest and activity, blood pressure, body temperature, feeding behavior, and carbohydrate and lipid metabolism are a few examples. These variations are orchestrated by an internal biological clock that relies on interlocked transcriptional regulation, starting, in mammals, by the transcriptional activation of the per and cry genes by the heterodimer, CLOCK–Bmal1. In turn, the Per and Cry proteins repress CLOCK–Bmal1-activated transactivation, thereby allowing a new cycle to start. This loop allows rhythmic expression of the core clock genes [1]. In addition, CLOCK–Bmal1 activates the transcription of the nuclear receptor Rev-erbα, resulting in daily fluctuations of its expression which, in turn, results in the repression of Bmal1. This additional loop is believed to improve the robustness of the clock. Additional post-transcriptional steps, protein phosphorylation, sumoylation, and acetylation, participate in the fine adjustment of the circadian period to 24 h.

Circadian rhythms are driven by a “master” hypothalamic clock residing in the suprachiasmatic nucleus, which aligns our circadian behavior to the day/night cycle and seasonal variations in light intensity. In addition, autonomous, self-sustained oscillators exist in all peripheral tissues and are synchronized by the master clock via neuroendocrine signals. This arrangement allows resonance between the different clocks throughout the body. Nevertheless, other time cues – such as the time of availability of food – can entrain peripheral pacemakers – such as the one in the liver – independently of the master clock [2]. These peripheral oscillators are important to the timely tuning of local circadian physiology. In the liver, for instance, up to 20% of the transcripts cycle, including those encoding enzymes and transporters involved in the pathways of synthesis of fatty acids, cholesterol, and bile acids, among many others [3]. Interestingly, nuclear receptors that are involved in the regulatory control of numerous key steps of biological processes, such as lipid and glucose...
metabolism, display circadian variations in their expression, and thus are believed to entrain circadian variations in the expression of their target genes [4]. It is therefore not surprising that circadian misalignment between our internal timing system and the environment, for instance as a result of jet-lag or shift work, results in metabolic disorders. Shift work is associated with increased prevalence of obesity, dyslipidemia, altered glucose concentrations, and cardiovascular events [5]. Sleep cycles are equally important for the regulation of metabolism, and numerous data have revealed that sleep disorders are intricately linked to features of the metabolic syndrome [6].

Recent studies have revealed a direct link between the molecular clock and metabolic pathways. Turek et al [7] have shown that clock mutant mice display an altered circadian feeding pattern, are hyperphagic, become obese, and develop hyperlipidemia and hyperglycemia in response to being fed a high-fat diet. Furthermore, Bmal1−/− mice display altered circadian oscillations in plasma glucose concentration, in addition to glucose intolerance [8], and Bmal1 seems to participate in the control of adipogenesis [9].

Emerging evidence obtained in humans demonstrates that several clock polymorphisms are associated with body weight and increased susceptibility to obesity [10,11]. Bmal1 has also been associated with type 2 (non insulin-dependent) diabetes and hypertension in humans [12]. These data demonstrate a direct link between clock genes and metabolic abnormalities recapitulating the metabolic syndrome.

Rev-erbα is a nuclear receptor that acts as a constitutive transcriptional repressor by recruiting the nuclear receptor co-repressor (NCoR) and histone deacetylase 3 (HDAC3) [13]. Rev-erbα is an important clock component, and Rev-erbα-deficient mice exhibit a 0.5 h shorter circadian period and are more sensitive to phase shifts induced by light pulses than are wildtype controls. In vitro, stability of the Rev-erbα protein is important for synchronizing and maintaining circadian rhythms after a serum shock [14]. Besides its role in the molecular clock system, Rev-erbα exerts an important role in metabolic functions: it regulates lipid metabolism, and Rev-erbα−/− mice display increased plasma concentrations of triglyceride and very-low-density lipoproteins, in addition to increased liver expression of apolipoprotein C-III [15]. In addition, Rev-erbα−/− mice exhibit altered bile acid synthesis, and diurnal variations in the expression of genes involved in bile acid synthesis and regulation are either phase-shifted or dampened, indicating that Rev-erbα links bile acid physiology to the circadian clock [16]. Rev-erbα plays also a modulatory role in the regulation of adipogenesis [17,18]. It may therefore act as a gatekeeper between the molecular clock and metabolic regulatory networks.

Although Rev-erbα has long been considered as an orphan receptor, recent reports have demonstrated that the Rev-erbα–NCoR interaction is increased after heme ligand binding by Rev-erbα [19,20]. Moreover, Meng et al [21] have recently reported a novel synthetic Rev-erbα ligand that enhances Rev-erbα repressive transcriptional activity. Interestingly, this ligand can influence circadian rhythms, inducing phase advance or delay in cultured fibroblasts. Rev-erbα is therefore becoming an attractive target for the manipulation of the biological clock, and it is tempting to speculate that modifying Rev-erbα activity to gate the physiological response to the correct circadian time is a promising strategy in the treatment of metabolic and cardiovascular abnormalities secondary to circadian disorders.

Accumulating evidence supports a role of the endogenous circadian clock in the pathogenesis of mood disorders. Indeed, shift workers are at risk of developing, not only features of the metabolic syndrome, but also anxiety and emotional problems, and are frequent users of tranquilizer drugs [22]. Bipolar disorder affects about 1–3% of the general population and is characterized by alternation between manic and depressive episodes; it is usually more prevalent during the winter season when the light period is reduced. During manic episodes, affected individuals show major disturbances in circadian processes such as sleep, activity, and hormonal secretions [23]. Very recently, disturbances of circadian rhythms have also come to be understood as being at the basis of depression. Difficulty in falling asleep, early awakenings, shifts in the cycle of body temperature and endocrine activities have been observed in depressed patients and thought to be clinical manifestations of a strong disruption of circadian rhythms [24]. Acting on MT1, MT2, and 5HT2C receptors in the suprachiasmatic nucleus, agomelatine, a drug licensed for the treatment of depression in Europe in 2009, resets the internal biological clock and restores circadian rhythms, thus leading to a strong clinical efficacy on depressive symptoms [25,26]. Treatments currently successful in preventing or attenuating these episodes rely on a stable pattern of sleep and activity along with pharmacological treatment, whereas disruption of these rhythms worsens the manic episodes [27].

Studies in human populations have identified several polymorphisms in clock genes that are associated with mood disorders. Although contrasting findings have been reported concerning a potential association between polymorphisms in the Rev-erbα gene and bipolar disorder [28,29], a polymorphism has been found in the 3’ flanking region of clock that associates with an increased number of manic episodes, insomnia, early waking time, and decreased need for sleep [30]. Interestingly, Roybal et al [31] have reported a
New therapeutic approaches

Rev-erbα: treatment target in circadian disorders?

remarkably similar overall human mania-like behavior in clock- mutant mice, (hyperactivity and depression-like behavior) [31], a phenotype normalized by chronic administration of therapeutic doses of lithium. Lithium is a commonly prescribed mood-stabilizing drug, lengthening the circadian period in rodents and humans, and this effect may be important for its therapeutic efficiency. Lithium targets glycogen synthase kinase 3β (GSK3β), a central regulator of the circadian clock. Interestingly, inhibition of GSK3β with lithium leads to rapid protosominal degradation of Rev-erbα, and activation of Bmal1 [14]. This effect was abrogated in cells overexpressing a mutant form of Rev-erbα that is resistant to lithium-induced degradation. This indicates that lithium targets the clock molecular machinery through the stabilization of Rev-erbα.

The involvement of Rev-erbα in the various aspects of circadian disorders discussed above is displayed schematically in Figure 1

Plasminogen activator inhibitor type 1 (PAI-1) plays a major regulatory role in the fibrinolytic cascade and promotes the development of atherothrombosis. Interestingly, PAI-1 plasma and tissue mRNA levels display circadian oscillations with a peak in the early hours, a time when the incidence of cardiovascular diseases such as myocardial infarction, and high blood pressure occur at higher frequency. Rev-erbα has been shown to repress PAI-1 gene expression, and this repressive effect is further enhanced by GSK3β-mediated phosphorylation and stabilization of Rev-erbα and blunted by lithium administration [32]. Altogether these data identify Rev-erbα as a major determinant of the circadian PAI-1 expression and a potential modulatory role of the morning susceptibility to myocardial infarction.

Conclusion

Rev-erbα is a gateway aligning the circadian system and physiology and, as such, it represents a potentially useful target for the treatment of aberrant circadian-related diseases.

See glossary for definition of these terms.

REFERENCES

New therapeutic approaches
Hélène Duez and Bart Staels


Beneficial effects of Vastarel MR in protecting patients with stable coronary artery disease against early morning ischemic burden

Stephane Coquempot
Suresnes, France

Correspondence: Stephane Coquempot, 35 rue de Verdun, 92284 Suresnes Cedex, France.
E-mail: stephane.coquempot@fr.netgrs.com

Abstract

Many biological and physiological functions are ruled by cyclical rhythms. The rates of manifestation of cardiovascular conditions such as angina pectoris, myocardial infarction, sudden cardiac death, and stroke also present a cyclical rhythm. Awareness of these circadian variations in biologic functions and in the occurrence of cardiovascular events makes it essential to develop pharmaceutical formulations that are capable of achieving optimal results by providing adequate blood concentrations at the time of maximum risk. Trimetazidine (Vastarel) MR is an example of a pharmaceutical product that has pharmacokinetic and 24-h anti-ischemic efficacy profiles that are adapted to match circadian rhythms in coronary artery disease.

Keywords: Circadian variation, coronary artery disease, trimetazidine MR

Introduction

Many biological and physiological functions are ruled by cyclical rhythms, and it has long been established that annual and lunar cycles have an impact on numerous metabolic pathways, endocrine and neuroendocrine systems, immunological responses, and many other physiological functions. Furthermore, beyond these annual and lunar variations, each and every day, many systems also undergo daily variations. In accordance with these cyclical daily variations in human functions, a number of diseases such as allergic rhinitis, asthma, rheumatoid and osteoarthritis, and peptic ulcer show circadian differences in terms of their severity. The rates of manifestation of many cardiovascular conditions such as angina pectoris, myocardial infarction, sudden cardiac death, and stroke also present with cyclical rhythms: in terms of circa-annual variation they are more frequent in autumn and winter, and in terms of circadian rhythm they are more frequent in the early morning hours. It has been shown that 30–40% of cases of sudden death [1], acute coronary syndromes, and strokes [2] occur between 06.00 AM and noon. More precisely, the figures indicate that, during this period there is a 40% greater risk of heart attack, a 29% increased risk of cardiac death, and a 49% increased risk of stroke compared with what would be expected if these events happened at random and were evenly distributed throughout the day [3].

In view of this knowledge of circadian variation in biologic functions and in the occurrence of
cardiovascular events, it is essential to develop pharmaceutical formulations that are capable of achieving optimal results by providing adequate blood concentrations at the time of maximum risk. The development of trimetazidine (Vastarel) MR, a modified release formulation of trimetazidine, is an example of a pharmaceutical product that has pharmacokinetic and 24-h anti-ischemic efficacy profiles that are specifically adapted to match circadian rhythms in coronary artery disease.

**Circadian variations in myocardial ischemia**

Many hemodynamic, environmental, and hematological changes are associated with awakening. Blood pressure and heart rate, for instance, have clearly established circadian rhythms, with a typical peak in the early morning and maximum daily values during the first 4–6 h after waking. Both blood pressure and heart rate decline from mid afternoon onwards, reaching their lowest values between midnight and 03.00 AM. These changes are mostly dependent on sympathetic nervous system activity, through the excretion of catecholamines and neuroendocrine activation: catecholamine and neurohormone concentrations, which diminish during sleeping hours and increase on awakening, are largely responsible for the variations in blood pressure, heart rate, and coagulability. Beyond the direct effect of increased catecholamine concentrations, it has been suggested that there is also increased end-organ responsiveness to catecholamines during the early morning hours, probably related to circadian variation in the autonomic control of the cardiovascular system [4,5].

The amplitude of the cardiovascular circadian rhythm in healthy people is of little clinical significance. However, in cardiovascular diseases, the amplitude of circadian changes is usually increased, and this has important consequences. Patients with stable coronary disease present with a clear early morning peak in the occurrence of symptomatic and silent ischemic episodes [6] (Figure 1). Beyond the time of awakening, it seems that the fact of getting up itself, and becoming active, also play major roles [7].

The available data clearly indicate that the vast majority of ischemic episodes occur within 2 h of individual’s getting up [8] (Figure 2). Indeed, getting up is linked with a significant imbalance between myocardial oxygen supply and demand. Oxygen supply is mainly restricted by a morning constriction of the coronary arteries that is possibly linked to several mechanisms, including sympathetic nervous system hyperactivity [9], a morning increase in plasma concentrations of cortisol and angiotensin II [10], an increase in blood viscosity [8], and an increased heart rate (shortening of diastole). The morning increase in sympathetic nervous activity itself leads to increases in several parameters that directly influence myocardial oxygen consumption, such as heart rate and myocardial contractility.

Finally, early morning is also the period of the day during which patients are the least well treated. This period corresponds to minimal plasma concentrations of medications, as (depending on the product’s dosing regimen), the last intake of drug will have taken place 12–24 h previously.

The significant rate of morning ischemic events is therefore a very strong argument in favor of driving the development of therapeutic treatments by focusing on this specific problem through a “chronotherapeutic” approach.

**Improvement of the 24-h anti-ischemic efficacy of trimetazidine with a modified release formulation**

This specific issue of a chronotherapeutic approach is precisely the reason why a modified release formulation of trimetazidine was developed. Trimetazidine is a metabolic antianginal drug that ensures an increase in myocardial energy production during...
ischemia. By selectively inhibiting 3-keto acyl coenzyme A thiolase (3-KAT), it partially inhibits free fatty acid oxidation during myocardial ischemia, consequently favoring glucose oxidation. This results in an increase in the amount of ATP available to ensure correct cardiac function [11].

Many trials in patients with stable angina had already demonstrated the clinical efficacy of trimetazidine 20 mg, either in monotherapy [12] or in combination with other drugs [13], compared with placebo [14] or other molecules [15]. Various data also provided clear-cut evidence of the benefits of prescribing trimetazidine in specific patient populations, such as those with diabetes [16,17] or ischemic cardiomyopathy [18–21]. However, even if it provides very satisfactory clinical results, trimetazidine 20 mg was not specifically designed to answer the problem of early morning ischemic events. This is the reason why a modified release formulation of the drug was developed.

The modified release formulation of trimetazidine, trimetazidine (Vastarel) MR, relies on a specific hydrophilic matrix that enables the progressive release of the active ingredient, trimetazidine, over time. It ensures a sustained effect with an increase in the minimum plasma concentrations of trimetazidine 12 h after the last intake of the drug.

In a cross-over design study that involved administration of trimetazidine MR twice a day or trimetazidine 20 mg three times a day and the measurement of plasma and urine concentrations of the drug over 4 days, trimetazidine MR provided better 24-h coverage than did trimetazidine 20 mg three times daily, with fewer fluctuations in concentrations of the drug. Moreover, at steady state, the minimum concentration at the end of the dosing interval \(C_{\text{min}}\) of trimetazidine MR was increased by 31% compared with that of trimetazidine 20 mg three times daily, and peak–trough concentration fluctuations were reduced from 121% to 86% (Figure 3) [22].

Beyond these pharmacokinetic features, the modified release formulation of trimetazidine also seems to bring a particularly well adapted answer to the clinical issue of circadian increases in ischemic events. In a pivotal study that assessed the antianginal and anti-ischemic efficacy of trimetazidine MR at trough plasma concentrations in 223 patients with stable angina, trimetazidine MR improved the time to 1-mm ST-segment depression \((P = 0.005)\) and delayed the time to onset of angina \((P = 0.049)\) after 2 months of treatment [23]. Six months after the beginning of treatment, patients receiving trimetazidine MR also showed a trend towards a greater decrease in the number of angina attacks per week than those receiving placebo (Figure 4).

This study confirmed that, thanks to its sustained anti-ischemic and antianginal efficacy, trimetazidine MR is able to protect the patients 12 h after the last dose was given (ie, during the early morning hours), at times when they are at increased risk of cardiovascular events.

**Conclusion**

The aim of chronotherapy is to deliver drugs at adequate concentrations during the time of greatest need. In the case of coronary artery disease, this represents the early morning post-waking period. With a twice daily dosing regimen and proven efficacy at trough plasma concentrations, trimetazidine MR is an antianginal treatment that is particularly well adapted for use in patients with stable coronary disease, preserving contractile energy over a cycle of 24 h.
Jet lag

Graham Jackson
Guy’s and St Thomas’ Hospitals NHS Trust, London, UK

Correspondence: Graham Jackson, Suite 301 Emblem House, London Bridge Hospital, 27 Tooley Street, London SE1 2PR, UK.
E-mail: gjcardiol@talk21.com
Conflicts of interest: None.

Abstract

Long-distance travel, whether for pleasure, business, or both, is associated with travel fatigue and jet lag. Symptoms reflecting the jet lag syndrome include disturbance of sleep, increased levels of fatigue and irritability during the arrival daytime, and a variable loss of concentration. Jet lag is caused by the slow adjustment of the body clock to the new time zone, leading to desynchronization of the daily rhythms with the new environment. Jet lag lasts for several days (about two-thirds of the number of time zones crossed traveling east, and half traveling west). Management includes behavioral measures and drugs, but no cure is available.


Case report

The time in Bermuda is 4 hours behind that in the UK, and traveling westerly from London takes 7 1/2 hours.
Using my British Airways Air Miles, my wife and I flew first class return, obviously in comfort. I am always cautious regarding avoiding dehydration (1 litre of water for every 5 hours flying time) and excess of both alcohol (wine only) and caffeine (avoided). Traveling west I do not sleep, but traveling east – as long as it is a night flight – I settle down as soon as is practical. On this occasion, we left London at 15.00 h, arriving on time at 18.30 h local time (22.30 h UK time). The travel fatigue that is associated with any long journey was not evident (comfortable travel, excitement on arrival) and our hotel was reached at 20.15 h. A quick “freshening up” was followed by dinner on the restaurant terrace, where we were greeted warmly (our fourth visit – nice to be recognized). A relaxing meal, good wine, and bed at 22.30 h. Awake at 03.00 h (why is it always 3 a.m.?) and grateful for the in-room filter-coffee maker at 05.00 h. A lazy day by the pool/beach followed, with slight fatigue only. Sleep disturbance persisted for 4 days, in spite of our taking no caffeine after 17.00 h (tea). The holiday was very enjoyable, with good weather, and very relaxing. Seven days later, we left Bermuda at 20.00 h, arriving back in London the next day at 06.30 h local time. A smooth and excellent flight and 2 hours’ sleep. The rest of the day was spent dealing with emails and the post, and checking with my secretary about the week’s events – from home, as planned – keeping going as long as possible to try to get back on UK time, bed at 22.30 h, and a complete night’s sleep to 06.00 h. No further symptoms or problems. Unusually, I had experienced far more sleep disturbance going west than east. In contrast, traveling east my wife adjusted after 1 day only, and traveling west 2 days of fatigue slowed her – we are all individuals where jet lag is concerned.

Discussion

Jet lag is a result of the body clock not being adjusted to the new time zone [1]. The rhythm most affected is sleep. As the body clock adjusts, jet lag resolves. As the number of time zones crossed increases, so the severity of the jet lag increases, with flights to the east more affected than those to the west. The world is divided into 24 time zones based around the Greenwich meridian in the UK, and the time changes by 1 hour for every 15 degrees traveled in either direction. Traveling over three time zones increases the risk of jet lag. Flying east, the body finds it more of a challenge to adjust to a longer day, so jet lag lasts (in days) for two-thirds the number of time zones crossed traveling east, compared with approximately a half the number traveling west. Speeding up the time frame may help prevent or reduce jet lag.
The best way to alleviate jet lag is to adjust the body clock to maximize the synchronization of the circadian phase to the rhythm of light and dark at the destination [2]. Traveling west, it is helpful to stay awake as long as it is light; traveling east, staying awake (avoiding bright light in the morning) and being out and about as much as feasible in the afternoon are to be recommended. Melatonin secreted by the pineal gland is turned off by light, and this pattern of behavior is designed to turn it back on naturally at the correct time: the release of melatonin is associated with it being time to sleep. Advice on general issues is summed up in Table I.

Pharmacological assistance is frequently advocated, using melatonin or a short-acting sedative such as the hypnotic agent, zolpidem. The effectiveness of melatonin (2–5 mg) taken at bedtime after arrival has been subject to a Cochrane review [3]. Melatonin may shift the time phase in addition to acting as a hypnotic, whereas zolpidem helps with sleep, but not with the circadian phase.

One Cochrane review found 10 randomized trials comparing melatonin with placebo in long-distance travelers; in eight of these, a clear reduction in jet lag was found after melatonin had been ingested. Five studies reported on global jet lag scores: on a scale of 0 = none to 100 = extreme, the mean was 48 among individuals receiving placebo and 25 among those receiving melatonin. The authors concluded: “Melatonin is remarkably effective in preventing or reducing jet lag and occasional short term use by adults appears to be safe”. They recommended 2–5 mg for up to 4 days after arrival, with no evidence of benefit if commenced beforehand. Regarding adverse effects, there are no safety data available for children or pregnant women, and melatonin should be avoided in those taking oral anticoagulants (eg, warfarin) or suffering from epilepsy.

In the USA, Thailand and Singapore, melatonin is considered to be a food additive and not a medication, so its purity is not regulated. Although in some countries (not the UK) it is considered a medication and requires a license, no preparation is marketed, so many rely on unregulated sources such as the Internet. The current situation is totally unsatisfactory, promoting counterfeit rather than genuine preparations.

### Summary

If a journey crosses fewer than three time zones, jet lag is unlikely to be significant. If the time away is fewer than 3 days it is advisable to try to keep on your home time. If the journey is more than three times zones, jet lag can be minimized by using light to promote body clock adjustment, taking melatonin to help with clock adjustment and sleep, and keeping alert during daytime (caffeine of your choice – mine’s a cappuccino!).

### Personal note

We were in the sky traveling smoothly at exactly the same time as the Air France flight was, tragically, not doing so. At times like these, we can only reflect on our vulnerability and offer sincerest condolences.

### REFERENCES


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**Table I. Advice for coping with travel fatigue.**

<table>
<thead>
<tr>
<th>Before the journey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plan the journey well in advance</td>
</tr>
<tr>
<td>Arrange for any stopover to be comfortable</td>
</tr>
<tr>
<td>Arrange documentation, inoculations, visas etc.</td>
</tr>
<tr>
<td>Make arrangements at the destination</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>During the journey</th>
</tr>
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<tbody>
<tr>
<td>Take some roughage (eg, apples) to eat*</td>
</tr>
<tr>
<td>Drink plenty of water or fruit juice (rather than tea, coffee, or alcohol)</td>
</tr>
<tr>
<td>Relax and rehydrate with non alcoholic drinks</td>
</tr>
<tr>
<td>Take a shower</td>
</tr>
<tr>
<td>Take a brief nap, if needed, but not enough to stop you getting to sleep at night</td>
</tr>
</tbody>
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*Many countries do not allow you to bring in fruit/foods.
Melatonin and cardiac pathophysiology

Russel J. Reiter and Dun X. Tan
Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas, USA
Correspondence to Russel J. Reiter, Department of Cellular and Structural Biology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA.
E-mail: reiter@uthscsa.edu
Conflicts of interest: None.

Abstract
Melatonin, an indole produced in several organs but most notably in the pineal gland, has a variety of effects that influence cardiac pathophysiology. Herein, we summarize the findings that illustrate the ability of melatonin to attenuate the severity of hypertension, limit myocardial damage, improve the function of the ischemic-reperfused heart, protect the heart from the toxicity of anthracycline drugs and from an immunosuppressant drug (cyclosporine A), and reduce cardiac hypertrophy and the associated pathophysiology caused by hyperthyroidism. The protective actions of melatonin at the level of the heart probably involve membrane melatonin receptors that exist on cardiomyocytes, in addition functions of melatonin as an antioxidant that are not receptor mediated. Whereas studies to date have been performed primarily in experimental animals, the uncommonly low toxicity of melatonin warrants tests of its utility in humans in cases of cardiac pathophysiology.

Keywords: Melatonin, heart, ischemia-reperfusion injury, hypertension, drug toxicity, cardiac hypertrophy

Introduction
Endogenously produced and exogenously administered melatonin may be of benefit to the cardiovascular system. Melatonin, N-acetyl-5-methoxytryptamine, is best known for its influence on circadian physiology. It was initially believed to be produced almost exclusively in the pineal gland [1], but recent data have indicated that it is likely to be produced in the heart also [2].

Melatonin may influence cardiovascular pathophysiology via both receptor-mediated [3] and receptor-independent [4] mechanisms. The classic melatonin membrane receptors (MT1 and MT2) are present in the heart and throughout the vascular system. Moreover, nuclear binding sites for melatonin exist [5]. The receptor-independent actions of melatonin relate to its ability, and that of its metabolites, to function as antioxidants [5–7].

Melatonin and hypertension
Removal of the pineal gland, a major source of circulating melatonin, causes a gradual, sustained increase in blood pressure. Moreover, melatonin treatment of spontaneously hypertensive rats decreases mean arterial pressure and heart rate, enhances relaxation of mesenteric arteries, and improves baroreflex responses [8]. Young healthy men and women have lower systolic, diastolic, and mean arterial pressure, and reduced blood norepinephrine (adrenaline) concentrations after taking melatonin.
The circadian rhythm of blood melatonin has a role in the normal daily fluctuation in blood pressure. Blood pressure is greatest during the daytime, when melatonin concentrations are least; these relative values are reversed at night [9]. Those individuals whose blood pressure decreases at night are referred to as “dippers”. Individuals who lack a nocturnal increase in blood melatonin concentrations do not experience a decrease in night-time blood pressure – that is, they are “non dippers”. Failure of a night-time reduction in blood pressure increases the 24 h mean pressure and exaggerates the strain on the cardiovascular system, which reportedly contributes to an increased risk of a cardiovascular accident and mortality [10].

In a double-blinded cross-over study, 16 men with essential hypertension given melatonin for 3 weeks, 1 h before sleep onset, exhibited reduced nocturnal systolic and diastolic blood pressures, by 6 and 4 mm Hg, respectively [11]. The mechanisms whereby melatonin influences blood pressure could involve any of the following: (i) a direct effect on neural centers governing cardiovascular status; (ii) reduction in catecholamine concentrations; (iii) relaxing smooth muscle in blood vessels; (iv) antioxidative actions. The ability of melatonin to modulate blood pressure may be a result of both receptor-mediated and receptor-independent processes.

**Melatonin and cardiac ischemic-reperfusion injury**

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are considered to be major contributors to cardiac damage during ischemia-reperfusion injury. Thus antioxidant strategies have often been considered in the prevention of ischemia-reperfusion damage. Of special interest in this regard are antioxidants that scavenge radicals at the mitochondrial level, which melatonin does [12]. Melatonin has frequently been tested for its ability to arrest cellular and molecular damage associated with a transitory interruption of the blood supply to the heart. These studies have uniformly shown that it is beneficial in reducing cardiac ischemia-reperfusion injury [4,13].

Several animal models have been used to document the protective actions of melatonin at the level of the heart. An isolated perfused heart model (Langendorff) in which the anterior descending coronary artery was temporarily ligated caused the heart to experience arrhythmias, including premature ventricular contraction and ventricular fibrillation upon reperfusion – alterations that often contribute to mortality [4]. When melatonin (1–250 μm) was infused during the ischemic and reperfusion episodes, the arrhythmias were prevented [14].

Not only cardiac pathophysiology, but also tissue destruction in the hypoxic-re-oxygenated heart is ameliorated by melatonin. Thus melatonin reduced infarct size, limited lipid peroxidation, reduced production of superoxide anion radical (O$_2^-$) and hydroxyl radical (⋅OH), decreased myeloperoxidase activity in hearts undergoing ischemia-reperfusion, and reduced the rate of death of the animals [4,13,15,16]. In an isolated working heart model, Dobsak et al [17] found that melatonin improved hemodynamic parameters, reduced postperfusion ventricular fibrillation, decreased apoptotic cardiomyocyte death, and reduced the formation of peroxyl radical (LOO$^\bullet$). In view of the reduction in tissue damage mediated by free radicals and the decrease in generation of ROS/RNS, the protective effects of melatonin in the ischemia-reperfusion heart were probably, at least in part, a result of the direct scavenging activity of melatonin or its stimulation of antioxidative enzymes, or both.

**Melatonin and cardiotoxic drugs**

Anthracyclines are chemotherapeutic drugs that cause irreversible myocardial damage, leading to life-threatening congestive heart failure. The cardiomyopathy associated with anthracycline usage is known to be mediated, in large part, by their ability to generate free radicals. As melatonin and its metabolites effectively reduce the oxidation of essential molecules [6,7], they would be expected to protect the heart from anthracycline-mediated toxicity.

When given to rats, doxorubicin alone (cumulative dose of 15 mg/kg) damaged the heart, as indicated by increased myocardial lipid peroxidation, ultrastructural damage to the heart musculature, reduced heart-to-body weight ratio, and decreased arterial pressure and left ventricular fractional shortening. Each of these changes was alleviated when melatonin was given in conjunction with the doxorubicin [18]. Subsequently, dozens of publications have confirmed the ability of melatonin to reduce the cardiotoxicity of doxorubicin [19]. It has also been shown to protect the heart from daunorubicin [20] and epirubicin [21].

Cyclosporine A (CsA) is a prototypic immunosuppressant drug commonly used to inhibit immune function in individuals receiving allotransplants, thereby reducing organ rejection. The toxicity of this molecule is most prominently manifested in the kidneys; however, collateral damage also occurs in the heart. When rats were treated for 21 days with subcutaneous CsA (15 mg/kg), increased concentrations of malondialdehyde, an oxidatively damaged lipid product, increased in the heart, whereas the activities of two antioxidative enzymes (superoxide dismutase
Melatonin and hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is a disease that causes a large number of deaths annually. Heart enlargement is normally a compensatory response, but the function of the hypertrophied cardiac muscle eventually becomes abnormal, leading to heart failure.

Hyperthyroidism is an endocrine condition that causes cardiac hypertrophy. Because the hyperthyroid state is associated with increased production of free radicals, Ghosh et al [24] compared the effects of two antioxidants, melatonin (2 mg/100 g body weight daily) and vitamin E (4 mg/100 g body weight daily), in potentially alleviating cardiac enlargement and the associated pathophysiology. When rats were treated with 3,5,3'-tri-iodo-L-thyronine (8 mg/100 g body weight daily) for 15 days, all the following changes were observed: cardiac enlargement, increased generation of lipid hydroperoxides and *OH, inhibition of copper/zinc superoxide dismutase, reduction in the glucose transporter GLUT4, downregulation of myocyte enhancer factor-2 (a regulator of GLUT4 expression), and increased B-type natriuretic peptide (a marker of heart failure). Each of these changes was reversed by concurrent administration of melatonin and partially prevented by vitamin E. Also, glucose uptake in the hypertrophic left ventricular cardiomyocytes was restored by both antioxidants.

These findings indicate that free radicals are a major contributor to cardiac pathophysiology under conditions of heart enlargement in hyperthyroidism. Vitamin E, at twice the dose of melatonin, was less effective in reversing the changes induced by 3,5,3'-tri-iodo-L-thyronine than was melatonin. The effects of melatonin may have been mediated by receptor-independent (free radical scavenging) or receptor-dependent processes [25].

Conclusions

Melatonin obviously has a variety of beneficial effects with reference to cardiovascular pathophysiology, including in the treatment of hypertension, ischemia-reperfusion injury, drug toxicity, and cardiac hypertrophy. Given the severity of these conditions and the uncommonly low toxicity of melatonin, clinical trials using this indole are highly justified. Unless the findings in animal investigations are totally misleading, it seems likely that melatonin will have similar protective effects at the level of the human heart.

REFERENCES


Increased concentrations of the inflammatory biomarker, high-sensitivity C-reactive protein, predict cardiovascular events. As statins decrease the concentrations of high-sensitivity C-reactive protein in addition to those of cholesterol, we hypothesized that people with increased concentrations of high-sensitivity C-reactive protein, but without hyperlipidemia, might benefit from treatment with statins. We randomly assigned 17,802 apparently healthy men and women with low-density lipoprotein (LDL) cholesterol concentrations less than 130 mg/dL (3.4 mmol/L) and high-sensitivity C-reactive protein concentrations of 2.0 mg/L or more to receive rosuvastatin 20 mg daily or placebo. They were followed for the occurrence of the combined primary endpoint of myocardial infarction, stroke, arterial revascularization, admission to hospital because of unstable angina, or death from cardiovascular causes. The trial was stopped after a median follow-up of 1.9 years (maximum 5.0 years). Rosuvastatin reduced LDL cholesterol concentrations by 50% and high-sensitivity C-reactive protein concentrations by 37%. The rates of the primary endpoint were 0.77 and 1.36 per 100 person-years of follow-up in the rosuvastatin and placebo groups, respectively (hazard ratio for rosuvastatin 0.56, 95% confidence interval [CI] 0.46 to 0.69; \( P < 0.00001 \)), with corresponding rates of 0.17 and 0.37 for myocardial infarction (hazard ratio 0.46, 95% CI 0.30 to 0.70; \( P = 0.0002 \)), 0.18 and 0.34 for stroke (hazard ratio 0.52, 95% CI 0.34 to 0.79; \( P = 0.002 \)), 0.41 and 0.77 for revascularization or unstable angina (hazard ratio 0.53; 95% CI 0.40 to 0.70; \( P < 0.00001 \)), 0.45 and 0.85 for the combined endpoint of myocardial infarction, stroke, or death from cardiovascular causes (hazard ratio 0.53, 95% CI 0.40 to 0.69; \( P < 0.00001 \)), and 1.00 and 1.25 for death from any cause (hazard ratio 0.80, 95% CI 0.67 to 0.97; \( P = 0.02 \)). Consistent effects were observed in all subgroups evaluated. The rosuvastatin group did not exhibit a significant increase in myopathy or cancer, but did have a greater incidence of physician-reported diabetes. In this trial of apparently healthy persons without hyperlipidemia but with increased high-sensitivity C-reactive protein concentrations, rosuvastatin significantly reduced the incidence of major cardiovascular events.

**Commentary**

An association between a biomarker (eg, C-reactive protein) and disease (eg, atherosclerotic diseases) may represent a causal relationship (causation), an increase in the biomarker as a consequence of the disease or its treatment (reverse causation), or an association that is spurious because both the biomarker and the disease are affected independently by another known or unknown factor (confounding). Several clinical studies have demonstrated the association between increased concentrations of these inflammatory markers and the risk of myocardial infarction, yet it has been debated whether the inflammatory state detected by these markers is a primary process that predisposes to atherothrombosis or a consequence of existing subclinical atherosclerosis.

What also is unclear is whether the widespread heightened cardiac inflammation is the culmination of an inflammatory process leading to plaque rupture, a consequence of plaque rupture, or both. Thus, akin to the ancient riddle of which came first, the chicken or the egg, C-reactive protein comes both before and after atherothrombosis.

**Mario Marzilli**

*C-terminal provasopressin (copeptin) is a strong prognostic marker in patients with heart failure after an acute myocardial infarction: results from the OPTIMAAL study*


The aim of this study was to compare the prognostic value of a novel and promising marker, copeptin, with those of B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) on death or a composite cardiovascular endpoint in patients who developed
heart failure after an acute myocardial infarction (AMI). From a subset of 224 patients (mean age 67 ± 10 years) of the Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan (OPTIMAAL) study, blood samples were drawn at a mean of 3 days after AMI when all patients had signs, symptoms, or both, of heart failure, or a left ventricular ejection fraction <0.35. Endpoints of interest were mortality (the primary endpoint of OPTIMAAL) and a composite cardiovascular endpoint, including death, myocardial infarction, stroke, or resuscitated cardiac arrest. Mean follow-up was 33 ± 7 months. Use of univariable Cox proportional hazards survival analysis revealed that higher concentrations of copeptin, BNP, and NT-proBNP were all significantly related to both the mortality and the composite cardiovascular endpoint (all \( P < 0.01 \)). In a multivariable Cox proportional hazards model, including all three biomarkers and other relevant covariates, a doubling of copeptin concentration was related to a 1.83 (range 1.26–2.64) times increased risk of mortality (\( P < 0.0001 \)) and a 1.35 (range 1.05–1.72) times increased risk of the composite cardiovascular endpoint (\( P = 0.018 \)). Receiver operating characteristic curves indicated that copeptin [area under curve (AUC) 0.81] was a stronger predictor of mortality than either BNP (AUC 0.66; \( P = 0.0063 \) compared with copeptin) or NT-proBNP (AUC 0.67; \( P = 0.0016 \) compared with copeptin). Finally, changes in copeptin concentrations after 1 month significantly added prognostic information to the baseline value. We conclude that copeptin is a strong and novel marker for mortality and morbidity in patients with heart failure after AMI. In this population, the predictive value of copeptin was even stronger than those of BNP and NT-proBNP.

**Commentary**

The use of the biomarkers, BNP and NT-proBNP, is increasingly common in the diagnosis and prognosis of patients with heart failure and in the risk stratification of adverse events in patients with a history of myocardial infarction. The blood concentrations of copeptin (the C-terminal portion of provasopressin), are also increased immediately after a myocardial infarction and are quickly and easily measured nowadays. Previous studies (eg, the Leicester Acute Myocardial Infarction Peptide [LAMP] study [1]) have already shown that the values of copeptin, NT-proBNP, and BNP measured during the hospital stay in patients with AMI were significantly greater in those who developed congestive heart failure, who were admitted to hospital again, and who died. These biomarkers overlapped each other, suggesting an additive effect of copeptin to those of BNP and NT-proBNP.

In the LAMP study, copeptin was even more predictive of death and of major adverse cardiovascular events than were BNP and NT-proBNP in patients with AMI, congestive heart failure, or moderate-to-severe left ventricular systolic dysfunction (or combinations thereof). This fact opens interesting possibilities as to adverse mechanisms of vasopressin acting through V1 and V2 receptors, and thus subsequent treatment protocols (eg, use of vaptans).

The findings of the OPTIMAAL study, reported in the paper by Voors et al, suggest that the role of these biomarkers remains weak in some ways. This study tested the effectiveness of two different therapeutic strategies – the first (drugs indicated for the treatment of heart failure according to current guidelines) based on the symptoms, and the second based on concentrations of BNP – in a population aged 60 years or older and with heart failure. Effectiveness (assessed in the 18 months following the start of the study) was judged in terms of overall survival, hospital-free survival, and hospital-free survival after heart failure. The study has not provided substantial evidence to show a superiority of BNP-guided treatment over symptom-guided treatment.

**REFERENCE**


Mario Marzilli

**Prognostic utility of ApoB/AI, total cholesterol/HDL, non-HDL cholesterol, or hs-CRP as predictors of clinical risk in patients receiving statin therapy after acute coronary syndromes: results from PROVE IT-TIMI 22**


The purpose of this study was to compare the prognostic utility of apolipoprotein (apo)B/AI, total cholesterol/high-density lipoprotein (HDL) ratio (TC/HDL), non-HDL cholesterol (non-HDL-C), or C-reactive protein (hs-CRP) as predictors of clinical risk among patients receiving statin treatment after acute coronary syndromes (ACS). In the PROVE IT-TIMI 22...
trial, patients with ACS were allocated randomly to groups to receive either pravastatin 40 mg or atorvastatin 80 mg. Cox regression models adjusting for confounders were used to assess the relationship between on-treatment lipids or hs-CRP and risk of death or acute coronary events. At 4 months, a 1-SD increment in apoB/AI (hazards ratio 1.10, 95% confidence interval [CI] 1.01 to 1.20), TC/HDL (hazards ratio 1.12, 95% CI 1.01 to 1.24), and non-HDL-C (hazards ratio 1.20, 95% CI 1.07 to 1.35) predicted events to a similar extent as did low-density lipoprotein (LDL)-C (hazards ratio 1.20, 95% CI 1.07 to 1.35). Risk prediction models that included LDL-C were not improved by the inclusion of apoB/AI, TC/HDL, or non-HDL-C. In contrast, the addition of hs-CRP significantly improved risk prediction models, irrespective of the lipid parameters included, with a 29–30% increased risk observed per 1-SD increment in log CRP. In this study of patients with ACS receiving statin therapy, on-treatment apoB/AI, TC/HDL, and non-HDL-C offered prognostic information similar to that obtained with LDL-C. However, the addition of hs-CRP to lipid-based measurements significantly improved risk prediction. On-treatment measurement of CRP may therefore offer additive prognostic information to that derived from lipid measurements in patients with ACS.

Commentary

The evaluation of risk and monitoring of treatment efficacy in hypercholesterolemic patients is traditionally based on the characterization of LDL-cholesterol values only. Of note, the measurement of atherogenic lipoproteins, such as intermediate-density lipoprotein and very-low-density lipoprotein, in addition to the balance between proatherogenic (apoB) and anti-atherogenic (apoAI) activity, should provide a more accurate definition of the risk profile in patients with ACS. The present large trial sought to evaluate whether intensive treatment with atorvastatin (80 mg/day), rather than standard-dose statin therapy (pravastatin 40 mg/day), in patients with ACS could affect the composite endpoint of death and non fatal acute coronary events.

Despite the encouraging observation that intensive treatment with atorvastatin (80 mg/day) reduced the ratios of apoB/apoAI and total cholesterol/HDL-C, in addition to the level of non-HDL-C, compared with standard-dose treatment in patients with ACS, measurements of the concentrations of each lipid provided information on risk prediction similar to that provided by LDL-C alone.

In contrast, the observations from this trial suggest that measurement of hs-CRP concentrations improves risk prediction and is only weakly related with each lipid parameter. Hs-CRP probably helps to identify the “synergy” between conventional risk factors and the inflammatory status of the body, thus improving the detection of dynamic processes related to coronary artery disease. It may therefore represent an independent, powerful risk predictor in patients with ACS.

Mario Marzilli

BNP-Guided vs Symptom-Guided Heart Failure Therapy. The trial of intensified vs standard medical therapy in elderly patients with congestive heart failure (TIME-CHF) randomized trial


Commentary

It sounds very attractive to have an objective index of efficacy and appropriateness when treating a serious illness. BNP is a peptide secreted by the heart in response to hemodynamic stress that increases sodium and chloride excretion and urine volume, decreases blood pressure, and reduces sympathetic nervous system activity and the activities of the renin–angiotensin system. In heart failure, BNP has been proposed as a marker of the presence and severity of the disease. In the emergency setting, it has been used to differentiate the cause of dyspnea, and at discharge after admission to hospital because of heart failure, it can predict adverse outcomes and re-admissions. What is more controversial is the use of BNP to monitor treatment.

Previous studies using BNP concentrations as a guide to treatment reported a significant reduction in events related to heart failure, including cardiovascular deaths or decompensation. Conversely, in this trial of intensified vs standard medical therapy in elderly patients with congestive heart failure (TIME-CHF) study, BNP-guided treatment had no advantage compared with symptom-guided treatment: the primary endpoint of 18 months of survival free of all-cause admission to hospital and the quality of life were similar between the two groups. In older patients, BNP-guided therapy was sometimes even harmful.

Thus the TIME-CHF trial places BNP-guided treatment of heart failure in perspective, and introduces
important caveats in the use of BNP in clinical practice. Treatment of heart failure is a matter of uptitration of medication and frequent re-assessment of the patient’s symptoms and signs – challenging the persistence and patience of the physician. There are no easy answers, no easy solutions, no short cuts. As for others biomarkers, it is not their reduction that counts, but how we obtain the reduction. Biomarker concentrations should no longer be considered as surrogate endpoints.

Mario Marzilli
Circadian clock
A “circadian” clock or rhythm is an internally generated biochemical, physiological, or behavioral cycle encompassing an approximate 24 hours duration in an organism, which allows the organism to anticipate and prepare for precise and regular environmental changes.

Cardiac magnetic resonance spectroscopy
Cardiac magnetic resonance spectroscopy is a specialized imaging technique associated with magnetic resonance imaging (MRI) that does not utilize ionizing radiation. It differs from MRI in that it actually provides biochemical information about the tissues of the human body in a non-invasive way, as opposed to only providing structural information with regards to the distribution of water and fat.

Melatonin
Melatonin (5-methoxy-N-acetyltryptamine) is the major secretory product of the pineal gland, produced in abundance during the dark cycle. It is involved in the regulation of the circadian rhythm of various physiological processes including blood pressure.

MicroRNA
Micro-RNAs are a class of highly conserved, endogenous, non-coding RNA molecules of approximately 22 nucleotides that silence gene expression at the post-transcriptional level by either promoting the degradation of messenger RNA (mRNA), or inhibiting the translation of protein from mRNA by translational repression.

Plasminogen activator inhibitor type 1 (PAI-I)
PAI-1 is a 50 kDa cytokine produced primarily by endothelial cells and adipocytes. PAI-1 functions as the principal inhibitor of both tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) by binding to the active sites of these enzymes and thereby preventing the cleavage of plasmin from plasminogen. PAI-1 is therefore a negative regulator of the fibrinolytic system.

Rev-erb alfa
Rev-erbA alpha is a nuclear receptor and transcriptional repressor that is highly expressed in the brain, skeletal muscle, liver, and adipose depots that participates in the development and regulation of circadian rhythms within these tissues.

Translational research
A novel research paradigm viewed as a bidirectional spectrum of basic, clinical, and patient-oriented research that aims to facilitate the movement (i.e. translation) of discoveries from basic laboratory research to clinical trials to point-of-care patient applications. The bidirectional framework of translational research also facilitates feedback of clinical outcomes to guide basic research directions.