

This review is the first article in a thematic series on **Circadian Rhythm and Cardiovascular Function**, which includes the following articles:

Molecular Time: An Often Overlooked Dimension to Cardiovascular Disease

Circadian Clocks and The Transcriptome  
Functions of The Cardiomyocyte Circadian Clock  
Circadian Clocks and Vascular Function  
Molecular Clocks and Cardiometabolic Syndrome  
Circadian Proteins and Genotoxic Stress

*Martin Young, Guest Editor*

## Molecular Time An Often Overlooked Dimension to Cardiovascular Disease

Tami A. Martino, Michael J. Sole

**Abstract:** Diurnal rhythms influence cardiovascular physiology such as heart rate and blood pressure and the incidence of adverse cardiac events such as heart attack and stroke. For example, shift workers and patients with sleep disturbances, such as obstructive sleep apnea, have an increased risk of heart attack, stroke, and sudden death. Diurnal variation is also evident at the molecular level, as gene expression in the heart and blood vessels is remarkably different in the day as compared to the night. Much of the evidence presented here indicates that growth and renewal (structural remodeling) are highly dependent on processes that occur during the subjective night. Myocardial metabolism is also dynamic with substrate preference also differing day from night. The risk/benefit ratio of some therapeutic strategies and the appearance of biomarkers also vary across the 24-hour diurnal cycle. Synchrony between external and internal diurnal rhythms and harmony among the molecular rhythms within the cell is essential for normal organ biology. Cell physiology is 4 dimensional; the substrate and enzymatic components of a given metabolic pathway must be present not only in the right compartmental space within the cell but also at the right time. As a corollary, we show disrupting this integral relationship has devastating effects on cardiovascular, renal and possibly other organ systems. Harmony between our biology and our environment is vital to good health. (*Circ Res.* 2009;105:1047-1061.)

**Key Words:** circadian rhythm ■ cardiomyopathy ■ hypertension ■ gene expression ■ cardiovascular physiology

*“Our body is like a clock; if one wheel be amiss, all the rest are disordered, the whole fabric suffers: with such admirable art and harmony is a man composed.”*

—Robert Burton, 1621

The last century has seen a detailed dissection of the molecular events underlying human biology. Although the physiology of organ systems, particularly the cardiovascular, was known to exhibit rhythmic activity over the

24-hour day, cell biochemistry was considered by most as a continuous activity localized in the different compartments of cellular space. The recent discovery of actual molecular clockwork mechanisms inside virtually all of our cells has added time as a critical fourth dimension of cellular physiology.

Cardiovascular tissues show significant daily variation in physiological processes, molecular gene, and/or protein expression. An increasing number of experimental and clinical

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From the Department of Biomedical Sciences (T.A.M.), Ontario Veterinary College, University of Guelph; Toronto General Hospital (M.J.S.); and University Health Network, Heart and Stroke Richard Lewar Centre of Excellence, University of Toronto, Ontario, Canada.

Correspondence to Tami A. Martino, Department of Biomedical Sciences (T.A.M.), OVC, University of Guelph, Guelph, ON, Canada, N1G2W1; E-mail tmartino@uoguelph.ca or Michael J. Sole, Toronto General Hospital, Room 4N-488 585 University Ave, Toronto, ON, M5G2N2, Canada; E-mail michael.sole@uhn.on.ca

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Non-standard Abbreviations and Acronyms	
<b>ACE</b>	angiotensin-converting enzyme
<b>BP</b>	blood pressure
<b>GSK</b>	glycogen synthase kinase
<b>HR</b>	heart rate
<b>L:D</b>	light:dark
<b>OSA</b>	obstructive sleep apnea
<b>PAI</b>	plasminogen activator inhibitor
<b>PCR</b>	polymerase chain reaction
<b>SCD</b>	sudden cardiac death
<b>SCN</b>	suprachiasmatic nucleus
<b>SHR</b>	spontaneously hypertensive rat
<b>TAC</b>	transaortic constriction
<b>VIP</b>	vasoactive intestinal peptide
<b>WKY</b>	Wistar-Kyoto rat

studies reveal that coordination of these rhythmic processes is a key fundamental mechanism underlying healthy organ growth and renewal. This review will focus on circadian rhythms and cardiovascular disease, a relatively recent area of research, which holds great promise. Other reviews in this series will discuss the cardiomyocyte circadian clock, the vascular clock and function, and the clock and cardiometabolic syndromes. Thus, we will assume a basic understanding of the molecular circadian system (Figure 1 and excellent reviews<sup>1-3</sup>). Also, we will take a translational approach, focusing primarily on myocardial disease excluding any in-depth discussion of the important areas covered in several earlier reviews on heart and vascular circadian clocks.<sup>3,4</sup>

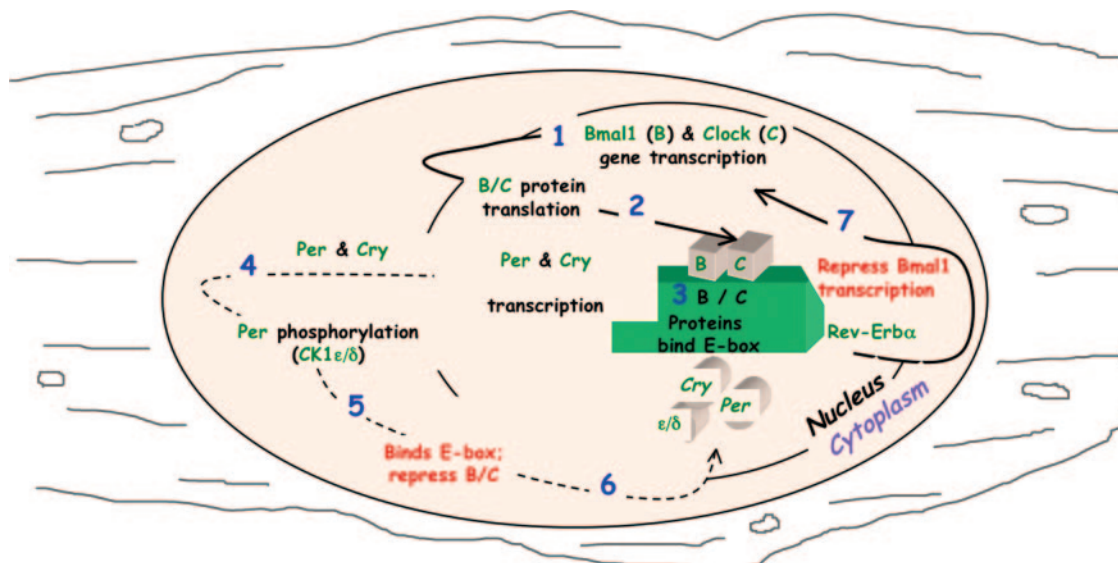
“Circadian” classically refers to the endogenous 24-hour cycle maintained in the absence of light; the term “diurnal” refers to conditions under which there is both an endoge-

nously generated circadian cycle and one modulated by external cues (zeitgebers) from the environment, predominantly light. Light is the main zeitgeber for the master clock, which is located in the suprachiasmatic nucleus (SCN) of the hypothalamus; the clocks in mammalian peripheral tissues are of course opaque to light and thus their coordination or modulation depends on central neural and hormonal signals derived from the SCN or peripheral zeitgebers such as activity and feeding. Humans are diurnal and live under normal 24-hour light and dark cycling (not dim or dark or “pure” circadian) conditions. Before the advent of substantive artificial lighting a century ago, our lives were synchronized to the natural light/dark rhythms defined by sunrise and sunset; we were active in the light of day, and slept at night. In contrast most rodents used in circadian (and other) research are nocturnal, ie, the rodent night is their subjective human day.

Circadian clock gene expression is a property of virtually all tissues except perhaps the testes.<sup>1,5-11</sup> The core genetic constituents of the cellular circadian clock: *clock*, casein kinase 1 $\epsilon$  (*CK1 $\epsilon$* ), period (*per1*, *per2*), *arntl* (*bmal1*), *rev/erb-a*, and cryptochromes comprise an autoregulatory feedback loop that cycles approximately every 24 hours. Neurohormones particularly relevant to the cardiovascular system, such as melatonin, glucocorticoids, catecholamines, growth hormone, atrial natriuretic factor, angiotensin II, aldosterone, and renin exhibit diurnal variation<sup>12-16</sup> and possibly synchronize peripheral tissue molecular circadian clocks with the SCN.<sup>17-22</sup>

### Diurnal Patterns of Heart Rate and Blood Pressure As Risk Factors for Cardiovascular Disease

The rhythmic changes in heart rate (HR) and blood pressure (BP) over the day night cycle are regarded as reflections of the diurnal variation in sympathovagal balance and have been used as clinical tools to monitor the function of the autonomic



**Figure 1.** These 7 steps comprise the basics of the cellular molecular circadian clock mechanism. This transcriptional/translational autoregulatory feedback loop cycles every 24 hours to “keep daily time” and thus coordinates hundreds or thousands of physiological processes so that they can occur during an optimal time of day.

nervous system. HR and BP are lowest at nighttime, during sleep, and in the early morning hours, coinciding with the period of vagal dominance and begin to rise before the time of waking in anticipation of the demands of our daytime activities; they decrease again in the evening anticipating sleep. Normal blood pressure across the diurnal cycle exhibits a 10% decrease at night, with a pressure surge in the morning just before and on awakening.

Blunting of normal heart rate variability has been associated with an increased risk of sudden cardiac death, particularly after myocardial infarction and in diabetic patients. Until recently, this variation has been solely attributed to diurnal rhythms of the autonomic nervous system; it now appears that there is an important contribution by the molecular clock within the cardiomyocyte. Cardiomyocyte specific clock mutant (CCM) mice exhibit an attenuation of diurnal heart rate variability in the absence of any overt conduction system abnormalities.<sup>23</sup>

Hypertension is a risk factor for cardiovascular and renal disease; the diurnal pattern of BP cycling adds another dimension to our prognostic assessment. Patients with hypertension fall into 2 main groups of aberrant diurnal blood pressure profiles. One group parallels the cyclic variation in pressure exhibited by normotensives, including the nocturnal “dip” in blood pressure but at an elevated overall level. The second group, referred to as “nondippers,” fail to exhibit the normal 10% decrease in nocturnal BP, and a few even exhibit an increase. Nondippers have an increased risk for target organ damage, including greater left ventricular hypertrophy and increased risk of myocardial infarction and renal failure.<sup>24</sup> Although a number of extrinsic factors can affect diurnal BP profiles, such as sleep quality,<sup>25</sup> particularly obstructive sleep apnea (OSA), these changes in BP profiles are often mirrored by dysfunctional autonomic or sympathetic nervous activity. For example, hypertensive patients with aberrant rhythmic BP profiles exhibit significantly higher muscle sympathetic nerve traffic and similarly impaired baroreflex-sympathetic control, as compared to normotensive controls.<sup>26</sup> In patients with type 2 diabetes, nocturnal BP variability is an independent predictive factor for increased risk of adverse cardiovascular events.<sup>27,28</sup> Finally, recent studies have shown that vessels from *bmal1* knockout, *clock*, or *per2* mutant mice exhibit aortic endothelial dysfunction.<sup>29–31</sup> It appears likely that some of these abnormal blood pressure rhythms reflect intrinsic abnormalities of the vascular endothelial molecular clock. The molecular clockwork mechanism is also implicated directly in regulating daily variation in blood pressure and response to stress.<sup>32</sup>

### The Diurnal Timing of Cardiovascular Disease

The timing of onset of adverse cardiovascular events exhibits a diurnal rhythm. For example, onset of myocardial infarction in humans exhibits a daily rhythmic pattern with highest incidence between 6:00 AM and 12:00 PM<sup>33,34</sup>; the nadir occurs between 3:00 AM and 6:00 AM. Infarcts are approximately three times more likely to occur early in the morning as compared to late at night. Mukamal and colleagues<sup>35</sup> observed that patients with nocturnal infarcts had a far greater

risk of developing congestive heart failure; this was unrelated to Q-wave status,  $\beta$ -blocker treatment, or the time between symptom onset and treatment. Nocturnal infarcts occurred more frequently in patients with OSA as compared to those without the sleep disorder.<sup>36</sup>

Sudden cardiac death (SCD) is another adverse cardiovascular event exhibiting diurnal timing. The diurnal pattern of onset of arrhythmic SCD was first extrapolated on the basis of two large retrospective studies.<sup>37,38</sup> A database derived from mortality records of the Massachusetts Department of Public Health revealed a rhythm of occurrence of SCD that peaked in the early morning.<sup>37</sup> The Framingham Heart Study supported these data by revealing an identical SCD diurnal rhythm.<sup>38</sup> Prospective clinical studies have documented diurnal rhythms in ventricular refractoriness<sup>39</sup> and in defibrillation energy requirements<sup>40</sup>; each of these peaked in the early morning hours, providing additional corroboration for a diurnal variation in SCD.

Investigation of implanted cardioverter defibrillators has allowed the analysis of precursor threatening ventricular tachyarrhythmias. The diurnal distribution of ventricular tachyarrhythmia events was shown to exhibit a sharp increase in number of events in the early morning hours<sup>41</sup>; patients with markedly impaired ventricles at the time of implantation demonstrated much less diurnal variation.<sup>42</sup>

Rupture and dissection of aortic aneurysms also display diurnal rhythms. Again, patients were at significantly increased risk for these events in the early morning hours, with peak occurrence between 8:00 AM to 11:00 AM.<sup>43–45</sup>

There are several studies supporting the view that the timing of onset of adverse cardiovascular events is linked directly to the intrinsic clock mechanism, as opposed to the “stress of awakening.” For example, a retrospective study of SCD on the Hawaiian island of Kauai<sup>46</sup> revealed that the prevalence of SCD peaked from 6:00 AM to noon for Kauaians; however, it peaked from noon to 4:00 PM for recent visitors, corresponding to early morning in Japan. Krantz and colleagues<sup>47</sup> studied 63 patients with stable coronary artery disease using a well-validated structured diary with ECG monitoring; the results further supported the concept that an intrinsic diurnal mechanism influenced timing of onset of adverse cardiovascular events, rather than increased physical or mental activity. Hu and colleagues<sup>48</sup> used a mathematical analysis of heart beat dynamics; their data also supported the hypothesis that intrinsic diurnal influences on cardiac control, as opposed to extrinsic behavior may be involved in the diurnal pattern of adverse cardiac events in vulnerable individuals.

Finally, diurnal rhythms have also been documented for precursor risk factors such as vasomotor tone, platelet aggregability, and other factors involved in thrombosis or thrombolysis.<sup>49–52</sup> Plasminogen activator inhibitor (PAI)-1 is a primary regulator of the fibrinolytic cascade, and activity and mRNA abundance exhibit circadian variation which peaks in the morning,<sup>53</sup> consistent with the increased risk of myocardial infarction at this time.<sup>33,34</sup> The pattern is believed to be related to the core molecular clock mechanism. For example, clockwork components such as CLOCK:BMAL<sup>50,54</sup> and PERIOD<sup>255</sup> help regulate circadian variation in PAI-1 gene expression in cardiovascular tissues. Conversely, PAI-1

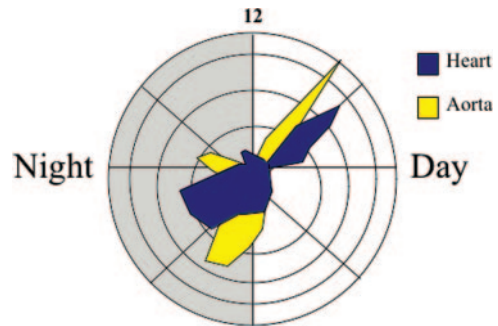
gene rhythms are reduced in the hearts of circadian mutant mice,<sup>56</sup> phase-altered with restricted feeding regimes,<sup>56</sup> and altered in atherosclerosis apoE<sup>-/-</sup> mice.<sup>57</sup> Diet patterns<sup>57,58</sup> and angiotensin (Ang) II receptor-mediated signaling molecules<sup>59</sup> modulate PAI-1 circadian expression patterns, in an organ-specific manner. Thrombomodulin also plays an important role in regulation of blood coagulation, and thrombomodulin mRNA and protein display circadian patterns and appear to be under control of circadian clock molecules in vascular endothelial cells.<sup>60</sup> Recently, Anea et al<sup>29</sup> demonstrated impairment of normal protective endothelial responses to vascular injury with intensified pathological remodeling and a predisposition to vascular thrombosis in *bmal1* knockout mice or *clock* mutant mice. Pathological responses in *clock* mutant mice were only evident under conditions of constant darkness when the intrinsic circadian defect was manifest; they were not present when the tissue molecular defect was overridden (rescued) by the central clock by housing the mice under normal light/dark (L:D) conditions. Mechanistically, these changes were associated with disruption of the normal phosphoinositide-dependent kinase 1, Akt, and endothelial nitric oxide synthase signaling pathways and were exacerbated by age. Westgate and colleagues<sup>61</sup> demonstrated the functional influence of circadian clock genes on regulation of diurnal thrombogenic responses to stimulus in vivo, using a photochemical injury model applied to the mouse femoral artery. Taken together, these data strongly link responses of the molecular clock in vascular tissue to day/night variation in cardiovascular events in humans.

### Cardiovascular Gene Expression Differs Day Versus Night

Cardiomyocytes do not replicate after development, although recent evidence supports a very low and continuing rate of renewal from as yet an unknown source of progenitor cells.<sup>62</sup> In contrast, cardiomyocytes turn over their protein contents and lipid membranes every few weeks, in effect renewing cell structure<sup>63</sup>; for example, the contractile protein myosin turns over with a cardiomyocyte half-life of approximately 15 days.<sup>64</sup> Until recently, clinical medicine had seen this biology as a continual cellular housekeeping activity, which occurred uniformly over the 24-hour day.

### Diurnal Gene Expression in Heart and Vasculature by Microarray/Polymerase Chain Reaction

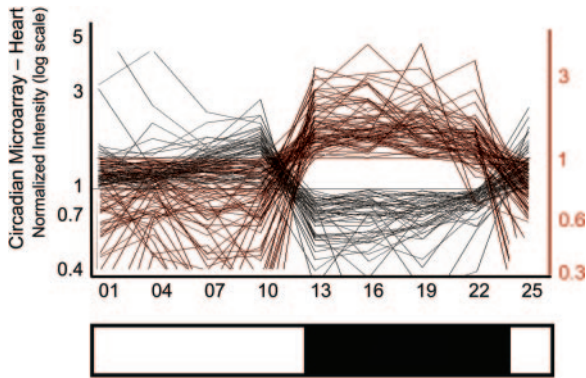
The first global microarray approach examining gene expression cycling in the murine heart was published in 2002.<sup>10</sup> The study investigated rhythmicity under circadian conditions, and found that a remarkable 8% to 10% of genes expressed in the heart undergo cyclic rhythms. As most life occurs under varying L:D conditions, and because the diurnal environment is most relevant to humans, it is important to understand global gene expression in the heart under diurnal conditions. Diurnal expression of cardiac core clock genes, such as *per* and *bmal1* in the rat heart, had been reported by Young and colleagues using quantitative polymerase chain reaction (PCR)<sup>11,65</sup>; their data were identical to that reported in a circadian environment.



**Figure 2.** Rhythmic gene expression in normal heart and aorta under L:D conditions is examined by microarray and bioinformatics and plotted on a radar diagram.<sup>8,67</sup> Phase histograms show only the genes with robust cycling profiles. Global gene expression in the heart shows a biphasic pattern with 2 major peaks, 1 in the light and 1 in the dark; the aorta reveals similar major light and dark peaks; however, these peaks occur at slightly different times than those for the heart; there is also a third minor peak in the aorta, which occurs in the dark. Presumably the specificity of peak and phase serves to ensure that cellular processes occur during a biologically optimal time of day for each tissue/organ. Modified from Martino et al.<sup>67</sup>

The behavior of the transcriptome however may differ under influence of L:D cycles. Therefore, we examined, for the first time, the cardiac murine transcriptome under normal 24-hour diurnal L:D conditions, using a microarray and bioinformatics approach.<sup>8</sup> Approximately 13% of the cardiac transcriptome was rhythmic under normal 24-hour diurnal L:D. The microarrays revealed that cycling of core clock mechanism genes was similar under diurnal L:D versus circadian conditions<sup>8,10</sup>; however, expression of many additional genes cycled or changed in the heart only under diurnal conditions, presumably in response to the zeitgeber triggers (light, activity, feeding) so relevant to our daily physiology.<sup>8</sup> In our studies, radar diagrams illustrated two principal peaks in cardiac gene expression; one in the light phase and a second in the dark phase (Figure 2). A third gene subset was identified that exhibited an abrupt change specifically at L:D transition times (Figure 3). The microarray gene expression profiles were also classified by the Gene Ontology Consortium; those that varied under diurnal conditions mapped to key biological processes such as cardiac metabolism, growth and remodeling, transcription, translation, and molecular signal pathways; many of these genes appear to be controlled by the cardiomyocyte circadian clock.<sup>23</sup>

The observations of diurnal gene cycling have been extended to vascular tissues such as aorta<sup>66–69</sup> and vascular cells in culture.<sup>21,32,68,70–73</sup> Using microarrays and bioinformatics, we examined gene expression in the aorta under normal 24-hour diurnal L:D conditions.<sup>67</sup> The data revealed a major peak in the light and a major and second minor peak in the dark (Figure 2). Notably, these peaks occurred at different times than those in the heart. Rudic et al<sup>9</sup> also examined gene expression in the aorta under circadian conditions. They consolidated the data into functional cassettes to demonstrate that rhythms were especially relevant to genes important for vascular structural integrity and metabolism. Taken together, these studies established that gene expression in heart and



**Figure 3.** The subset of cardiac genes illustrated here show remarkably abrupt changes in expression only at the L:D transition times.<sup>8</sup> The black lines represent light-activated (or dark-repressed) genes: these genes exhibit increased expression in light with an abrupt decrease in expression at the onset of darkness; they remained repressed during the entire dark period only to be abruptly reexpressed when light returned. The red lines represent dark-activated (or light-repressed) genes which exhibit the opposite profile. Lights on zeitgeber time (ZT) indicates 0 and 24; lights off at zeitgeber time, 12; cardiac gene expression was assessed in 3 mice every 3 hours.

vasculature is rhythmic and dramatically different day versus night.

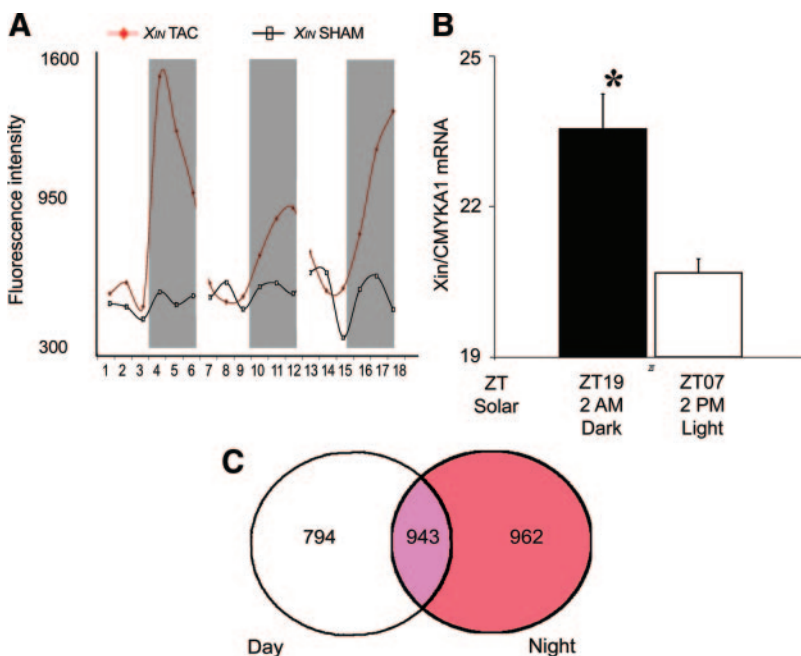
We also examined diurnal gene expression in murine heart and vasculature in a model of cardiovascular pressure overload following thoracic aorta constriction (TAC) in the mouse. Surprisingly, rhythmic expression patterns of core cycling genes (eg, *per2*, *bmal*, etc) were virtually superimposable in time, that is, the core-cycling transcriptome maintained the same period and phase in TAC pressure overloaded heart and vasculature as compared to normal controls.<sup>67</sup> In contrast, very different rhythmic profiles were observed for many other genes that were noncore clockwork. Some that were important for remodeling exhibited dramatically different 24-hour patterns in pressure overloaded hearts versus

controls (Figure 4A and 4B). Expression patterns of these genes exhibited statistically reproducible 24-hour profiles, although, notably, they did not conform to the classic rhythmic (or mathematical cosine-wave) diurnal pattern. Further investigation of these de novo gene patterns could provide novel insights into the diurnal molecular biology of the heart.

Young et al,<sup>11</sup> in the TAC rat, and Mohri et al,<sup>74</sup> in the salt-fed Dahl salt-sensitive hypertensive rat have also reported conservation of phase for clock gene expression. Young and coworkers suggest that phase alterations may occur in some pathologies, reporting a phase shift in the expression of circadian clock genes in the streptozotocin-induced diabetic rat.<sup>75</sup> Naito et al<sup>76</sup> studied the circadian variation of gene expression for various components of the renin-angiotensin system in spontaneously hypertensive rats (SHRs) and control Wistar-Kyoto (WKY) rats. The amplitude of mRNA expression of renin, angiotensinogen, angiotensin-converting enzyme (ACE), and angiotensin type 1a and type 2 receptors were greater in the SHRs as compared to WKY, particularly during the dark phase. The phase relationships of the expression of these genes between WKY and SHRs were complex.

### Diurnal Chromatin Remodeling

It is becoming increasingly evident that regulation of molecular gene rhythms involves chromatin remodeling. Histone modifications such as acetylation/deacetylation alter stability and condensation of histone-histone/DNA interactions, thus controlling access of transcription factors to DNA. In a recent landmark study it was shown that transcriptional regulation of the core clock mechanism in murine liver was accompanied by rhythms in H3 histone acetylation.<sup>77</sup> Mobilization of transcriptional coactivators and histone acetyltransferases is also rhythmic.<sup>71</sup> Remarkably, the CLOCK protein itself has also been shown to have histone acetyltransferase activity.<sup>78</sup> A molecular clock component can catalyze chromatin remodel-



**Figure 4.** A, Gene expression for a candidate biomarker *Xin* (or cardiomyopathy associated protein 1 *CMYA1*) is similar for TAC and sham-operated mice in the light but upregulated only in TAC in the dark. Results are plotted using fluorescence intensity (linear scale, raw data) from the microarray analysis of the hearts from 36 individual mice euthanized at 4-hour intervals.<sup>67</sup> White bars denote day (ZT03, -07, -11) and gray denotes night (ZT15, -19, -23). B, Validation by RT-PCR with significantly increased *Xin* expression in the dark (ZT19) vs light (ZT07);  $n=3/\text{time}$ .  $*P<0.05$  (Student's *t* test). C, TAC genes in which expression differs from sham, day vs night, and intersection between groups (see text).

eling, revealing unforeseen potential between chromatin remodeling and cell physiology. The interplay between chromatin remodeling and circadian clockwork has been recently well reviewed.<sup>79</sup> Finally, and relevant to this review, histone regulation is regarded as a crucial mechanism in cardiac gene expression, underlying growth, renewal, and remodeling, as has also been extensively reviewed.<sup>80</sup> However, temporal regulation of chromatin remodeling in normal versus disease heart remains to be elucidated. Another area of interest and intersect might be how environmental variables or stressors impact on these responses of the cardiovascular system.

## Potential for Biomarker Discovery

### Diurnal Gene Biomarkers

Diurnal variation in gene expression and the differences between nocturnal animals and diurnal humans must be considered in studies dissecting the molecular events underlying disease or studies focused on the search for biomarkers. For example, in the research laboratory, when assessing murine gene expression by microarray, comparison between normal and disease must control for the time of day when tissues are harvested. In our TAC experiments,<sup>67</sup> although core circadian clock gene cycling was not altered in the TAC mice, there was a substantial number of genes that did change expression with pressure overload. Of the genes analyzed, 2699 transcripts ( $\approx 12\%$ ) exhibited altered expression profiles in TAC hearts. Of these 1756 (65%) exhibited differences in gene expression between sham and TAC that were evident only either at night or during the day (Figure 4C); thus, sampling time would be critical to discovery of de novo biomarkers. Two additional approaches with potential for elucidating de novo circadian biomarkers delineating disease are the molecular timetable microarray approach by Ueda and colleagues,<sup>81</sup> and the cardiac-specific clock mutant mice developed by Young and colleagues.<sup>23</sup>

### Diurnal Proteomics Biomarkers

Proteins also exhibit diurnal cycling. Specific examples have been discussed elsewhere in this review, such as circadian clockwork proteins, some neurohormone proteins/peptides and/or their precursors, cytokines, and other factors. In addition to these individual proteins that cycle, there has been much recent attention paid toward identifying global proteomic cycling patterns. The idea is that by investigating an entire tissue proteome, de novo cycling proteins may be identified, including new candidate biomolecules relevant to tissue homeostasis and disease. Investigators use proteomic technologies (eg, 2D gels, mass spectrometry) to identify previously unrecognized cycling proteins in tissue; this is somewhat analogous to the global genomic cycling approach which investigated mRNA expression patterns using microarrays and PCR (see above). Global proteomic cycling was first demonstrated in murine liver<sup>82</sup> and subsequently evaluated in additional peripheral tissues including murine blood<sup>83,84</sup> and retina<sup>85</sup> and rat hypothalamus<sup>86</sup> and pineal gland.<sup>87</sup> Rhythmic protein cycling is likely dependant on an intact molecular clockwork mechanism, because abrogated protein expression patterns in liver were observed in normal versus circadian genetic mutant mice.<sup>82</sup> Posttranscriptional mechanisms such

as phosphorylation likely also contribute significantly to the proteomic cycling patterns in liver<sup>82</sup> and possibly other tissues. Thus, taken together, these data indicate that a significant percentage of the proteome likely cycles in most, or possibly all, body tissues providing additional diurnal biomarker candidates for clinical applications, in addition to the above noted gene based ones.

### Single Nucleotide Polymorphism

A third potential approach to biomarker discovery investigates single-nucleotide polymorphisms in clockwork genes, looking for a causal relationship between the sequence polymorphisms and human cardiovascular (or related) disease. One area of particular interest is the region encoding the BMAL1 gene.<sup>88,89</sup> BMAL1 sequencing identified 19 polymorphisms including functional variants that affected transcriptional regulation in the promoter region, in hypertensive SHR versus normotensive WKY animals.<sup>90</sup> This is interesting because in the rat BMAL1 maps to a region on chromosome 1 bearing quantitative trait loci for blood pressure, type 2 diabetes mellitus, body weight, cardiac mass, and kidney mass. Moreover, in humans genome-wide scans for hypertension or type 2 diabetes indicate linkage in the BMAL1 region on chromosome 11.<sup>90,91</sup> Another study examined polymorphisms in 19 clockwork or related genes in humans, and results indicated that NPAS2 was linked with hypertension, and PER2 with high fasting blood glucose.<sup>92</sup> A third study identified single-nucleotide polymorphisms in 2 other circadian related genes (NPSR1, PDE4D) that were associated with sleep phenotypes<sup>93</sup>; this may also be relevant as sleep and its disturbances are associated with cardiovascular disease as discussed below.

## Cardiovascular Growth and Renewal Occurs During Sleeping Hours

We hypothesize that many of the crucial myocardial growth and renewal processes occur especially during the period normally allocated to sleep (the subjective night). This hypothesis is based on the cumulative observations from experimental and clinical studies noted above. Furthermore, it receives additional support from other studies as well. For example, in 1975, Rau et al<sup>94</sup> reported differential incorporation of labeled leucine into rat myocardial protein over 24 hours, which indicated that myocardial protein may be synthesized at the greatest rate late in the light period (rats asleep) with the least synthesis occurring 12 hours later (rats active). The authors perspicaciously concluded, "These preliminary studies are not conclusive but they support the hypothesis that there is a circadian rhythm of protein metabolism and abundance in the heart." Neurohormones with anabolic activity relevant to the cardiovascular system, such as growth hormone, atrial natriuretic peptide, aldosterone, angiotensin II, renin, and proopiomelanocortin exhibit diurnal variation<sup>13–16</sup> with specific gene and/or protein expression patterns during sleeping hours. Rat hearts isolated during the subjective day (dark phase) and perfused ex vivo exhibit greater cardiac power than those isolated during the subjective night<sup>95</sup>; the capacity for myocardial carbohydrate oxida-

tion and oxygen consumption was also increased during the subjective day.

Assessment of all the experimental molecular data described above are consistent with our hypothesis that myocardial renewal and growth is diurnal, with significant activity occurring during the subjective night when HR and BP are at their lowest and physiological stress is at minimum. Cell energy and resources then can be turned from coping with external physiological demands toward cellular repair and growth.

This hypothesis is also supported by clinical observations (including some of those described above, in the section Diurnal Patterns of Heart Rate and Blood Pressure As Risk Factors for Cardiovascular Disease and in the section The Diurnal Timing of Cardiovascular Disease). Nondipper hypertensives (nocturnal hypertension) exhibit an increased risk of target organ damage, with an increased risk of cardiovascular and renal disease<sup>24,96,97</sup> than similarly affected patients exhibiting a nighttime pressure decrease. Moreover, although infarcts are more common just before or in the morning after awakening, myocardial infarcts occurring during in the middle of nighttime sleep appear to be larger.<sup>35</sup> As noted above, angioplasties performed at night are less successful than those done during the day; perhaps this reflects a more deleterious immediate vascular response to injury following the procedure.<sup>98,99</sup> Furthermore, OSA adversely affects myocardial structure<sup>100,101</sup>; OSA results in an increase in transmural ventricular pressure applied in a setting of sleep disturbance and leads to autonomic neural and endocrine disruption (also in the section Disturbed Diurnal Rhythms and the Pathogenesis of Cardiovascular Disease).<sup>100,101</sup> Continuous positive airway pressure (CPAP), even though applied only during sleep, yields permanent long-term benefits including reverse (beneficial) cardiac remodeling.<sup>100–104</sup> Shift workers are subjected to repeated assaults on their sleep wake cycles; shift work triggers cardiovascular risk factors and is associated with increased cardiovascular morbidity and mortality.<sup>105–107</sup> Finally, conversion from conventional to nocturnal hemodialysis results in significant regression of left ventricular hypertrophy in patients with end stage renal disease.<sup>108</sup> Thus, clinical data also support the hypothesis that the heart is most susceptible to remodeling, or renews, significantly during the sleeping hours.

### **Disturbed Diurnal Rhythms and the Pathogenesis of Cardiovascular Disease**

#### **Sleep Disturbances, Sleep Apnea, and Cardiovascular Disease Progression**

Sleep patterns may be integrated with circadian/diurnal rhythms, and their disruption can play a significant role in cardiovascular disease progression. This has become particularly evident since the turn of the twentieth century, as we now live with daily disruptions caused by artificial light, intercontinental air flight, and 24-hour multinational communications including the Internet and email. We are no longer dependent on the natural day/night environment of our ancestors, indeed our activities can extend to 24 hours a day, 7 days a week. Day/night rhythms, shift work schedules, and

regular and adequate sleep are no longer prioritized as cornerstones of health. New central stimulants like modafinil suggest, the future “conquest of sleep.”<sup>109</sup> Modafinil does not appear to have a chronobiotic effect, at least in the hamster.<sup>110</sup> Such agents have a ready market, particularly the military, the National Aeronautics and Space Administration, the trucking and airline industry, and even the health professions. However, it would still seem that increasingly the scientific and clinical evidence indicates such an approach would not be prudent in humans, for studies have indicated an increased prevalence of cancer, obesity, diabetes, and adverse cardiovascular events in shift workers, transmeridian flight crews, and patients with sleep apnea and other sleep disturbances.<sup>105,106,111,112</sup>

OSA is a particularly relevant example in humans,<sup>100,101</sup> because the sleep disruption adversely affects many diurnal physiological rhythms, and this has profound cardiovascular consequences in humans including the development and/or exacerbation of heart failure<sup>113,114</sup> arrhythmias and other cardiovascular events.<sup>115</sup> One of the fundamental issues is that in the setting of sleep disturbance characterized by multiple apneic awakenings, this increases transmural ventricular pressure, ie, increased myocardial wall tension. Normal diurnal cycling is profoundly disturbed, with disruptions in sympathovagal balance and neurohormonal, cytokine, and immunocyte cycling. Nocturnal treatment with continuous positive airway pressure has beneficial effects, reinstating normal sleep, reversing adverse ventricular remodeling, ameliorating arrhythmias and hypertension, and normalizing plasma biochemical profiles.<sup>102–104</sup>

A role for sleep disturbance in cardiovascular disease progression is further indicated from experimental animal studies in which specific circadian clock genes have been disrupted. These studies also help to interrogate the underlying mechanisms involved, and disease is postulated to result from the development of significant obesity and altered metabolic status in these animals. In 1 study, it was revealed that *clock* mutant mice consumed similar amounts of food day versus night, as compared to normal nocturnal controls which consumed most of their food at night.<sup>116</sup> The *clock* mutant mice also exhibited altered diurnal variation in glucose and triglyceride, suppression of gluconeogenesis.<sup>117</sup> *Clock* mutants exhibited significant increased weight gain as compared to controls. In another study,<sup>118</sup> mice homozygous for the *per2* mutation exhibited a loss of diurnal rhythm feeding, along with abnormal activity rhythms, and a lack of corticosterone rhythm. On high-fat diets, the *per2*<sup>-/-</sup> mice developed obesity, as compared to wild-type littermate controls. Obesity and altered metabolic status are significant risk factors for cardiovascular disease.

#### **Disturbed Diurnal Rhythms Cause and Exacerbate Heart Disease, and Implications for Cardiovascular Remodeling at Night**

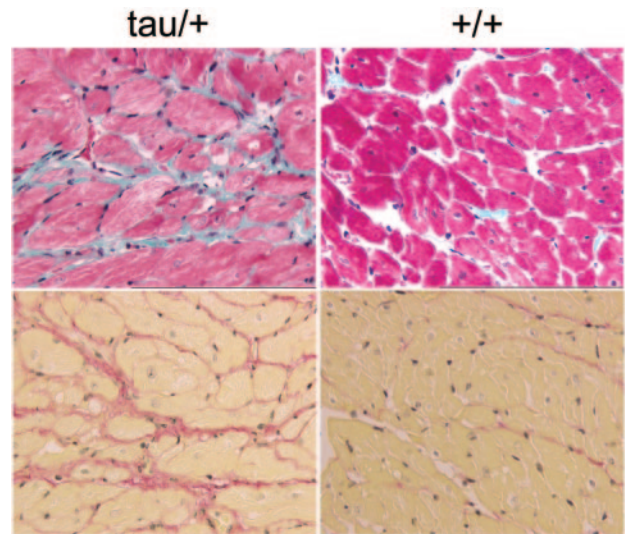
Most recently, diurnal rhythm disturbance has also been implicated as a specific etiologic cause of cardiovascular disease. Our first studies were performed with hamsters as these animals have a long well-defined history in rhythms research. Ralph and colleagues discovered a naturally occurring genetic mutation in hamsters, labeled *tau*, which altered

the intrinsic circadian period of these animals.<sup>119</sup> The *tau* mutation is in *CK1ε*, a “doubletime” homolog (*Drosophila*) that phosphorylates PER clock mechanism proteins.<sup>120</sup> Transplantation experiments in *tau* mutant hamsters demonstrated that the SCN of the hypothalamus was the “seat” of the master circadian clock,<sup>121</sup> an underlying mechanism driving the daily rhythms of behavior and physiology. The *tau* mutant allele reduced the free-running circadian period of the hamsters from ≈24 hours in the wild-type animals to 22 hours in the *tau*<sup>+</sup> heterozygotes. When the 22-hour heterozygotes were entrained to a 24-hour L:D cycle, they exhibited fragmented patterns of rest and activity and a reduced lifespan as compared to 24-hour wild types.

We hypothesized that the discordance between the environmental 24-hour day and the intrinsic, shortened circadian period of the *tau*<sup>+</sup> hamsters was etiologically linked to their reduced longevity, perhaps through heart disease. Previous studies by Penev and colleagues showed that TO-2 cardiomyopathic hamsters (not related to *tau* rhythm mutant hamsters, but rather an inbred, naturally occurring, cardiomyopathic strain) subjected to repeated disruption of their L:D cycle died earlier than their undisrupted counterparts<sup>122</sup>; this provided further support for this hypothesis and mirrored cardiovascular morbidity/mortality data in shift workers. Also mice subjected to phase advances of the L:D cycle, simulating chronic jet lag, had greater mortality as compared to controls.<sup>123</sup> Thus mortality was increased in animals not allowed to adapt to the external environment.

We found<sup>124</sup> that 22-hour *tau*<sup>+</sup> heterozygote hamsters, when entrained to a rhythm disruptive 24-hour L:D cycle, were normal when young but developed a profound dilated cardiomyopathy and renal pathology over the long-term (Figure 5). They died prematurely from severe heart disease and renal failure. Remarkably, 22-hour *tau*<sup>+</sup> heterozygote animals raised on 22-hour L:D cycles appropriate to their genotype exhibited normal consolidated behavior, including activity and sleep, normal cardiorenal structure and function, and normal survival. Homozygote (20-hour) animals were also normal when in a 24-hour environment as their very short intrinsic circadian period dominated the external environment, allowing consolidated behavior without the conflict seen in the heterozygotes. The 22-hour *tau*<sup>+</sup> heterozygotes also had normal hearts under conditions where they were raised in darkness, or with their SCN removed, again, because no conflict arose between their intrinsic circadian system and the external environment. Indeed, circadian disorganization alone appeared to be a direct and sufficient cause of cardiovascular disease.<sup>112,124</sup> Thus, in the case of the *tau* hamsters, there was cardiac, vascular, and renal damage that developed when there was a conflict between the endogenous tissue clock and the diurnal signals coming from the SCN.

As noted above (in the section The Diurnal Timing of Cardiovascular Disease), Anea et al<sup>29</sup> recently demonstrated the importance of normal clock genes for maintenance of vascular health in mice. Moreover, they showed that circadian mutations were etiologically implicated in the pathogenesis of vascular disease in mice, similar to our observations of cardiorenal disease in *tau* mutant rhythm disturbed hamsters.<sup>124</sup>



**Figure 5.** Histopathology of hamster hearts showing myocyte hypertrophy and myocardial fibrosis in *tau*<sup>+</sup> hamsters compared to wild-type hamsters (+/+) in a 24-hour environment; histology of *tau*<sup>+</sup> hamsters raised in a 22-hour environment was indistinguishable from that of wild type. Top images, Mason's trichrome; bottom images, Picrosirius red. Reprinted from Martino with permission.<sup>124</sup>

Disturbed diurnal rhythms cannot only cause heart disease, but may also exacerbate preexisting or underlying cardiovascular conditions. We demonstrated this using normal C57Bl/6 mice in a transaortic constriction (TAC) model of pressure overload cardiac hypertrophy.<sup>67</sup> TAC mice were exposed to diurnal disruption by housing them in an altered 20-hour L:D environment, as compared to the normal 24-hour diurnal cycle. These 20-hour rhythm-disturbed TAC mice, like *tau*<sup>+</sup> heterozygote hamsters, exhibited a complete disruption of their sleep/wake behavior, unable to consolidate either. Associated with this was marked exacerbation of their cardiovascular disease. This included abnormal histology and increased left ventricular end-systolic and end-diastolic diameters along with reduced cardiac contractility, and increased BP, as compared to “non-rhythm-disturbed” control TAC mice. The degree of myocyte hypertrophy in myocardial and vascular cells was significantly constrained, and accretion of fibrous tissue both in myocardium and in perivascular areas was markedly increased in response to the increased pressure burden; that is cardiovascular remodeling was inappropriate to the rise in blood pressure.

Desynchrony between the external 20-hour environment and the internal 24-hour endogenous circadian system was further manifest even at the molecular level in the heart.<sup>67</sup> Rhythm-disturbed 20-hour TAC mice exhibited abnormal cycling of core circadian clock genes (eg, *bmal1*, *per2*) in the heart as compared to non-rhythm-disturbed 24-hour TAC controls. Furthermore, there was aberrant (decreased) expression of key cardiac hypertrophic/remodeling genes including *ANF*, the RAAS pathway gene *ACE*, matrix remodeling genes (eg, *Col3a1*, collagen), and markers of contractile dysfunction leading to heart failure such as *BNP*. When the animals were moved to a 24-hour day/night environment, that is, the external rhythm now corresponded to the innate



24-hour rhythm of the animal, normal compensatory cardiovascular remodeling processes were resumed (ie, the clock normalized; BP fell to less hypertensive levels; myocyte hypertrophy, previously constrained, now paradoxically increased to levels appropriate to the blood pressure; gene expression returned to normal TAC levels).

The key observation in these studies was that failure to harmonize internal and external rhythms augmented cardiovascular (target) organ damage.

These data hold promise for patients, such as shift-workers, those with sleep disorders (including the elderly), or any individuals subject to adverse cardiovascular health effects associated with the 24/7 demands of society. That is, by maintaining normal diurnal body physiology, treating underlying sleep disorders, and/or restoring the endogenous neuroendocrine hormonal profiles, perhaps by imposing a fixed or regular schedule of zeitgebers such as light/dark, rest/activity, or the timing of meals, we may significantly benefit cardiovascular and renal health or slow the progression of disease.

### Specific Links Between the Circadian Clock and Cardiovascular Physiology

A variety of circadian animal models have been used in cardiovascular rhythms research. Studies using transgenic mice overexpressing rat *per2*, mice bearing a mutation in the highly conserved binding domain of the PER2 protein, *clock* mutant mice, *bmal1*<sup>-/-</sup> and *cry1/2*<sup>-/-</sup> knockouts, and *npas2* mutants have been revealing (for example, see elsewhere<sup>23,32,60,116,117,125–127</sup> and others).

In one such study, *Bmal1*<sup>-/-</sup> mice placed in complete darkness exhibited a complete loss of diurnal variation in MAP, HR, and activity.<sup>32,128</sup> These same mice when placed in L:D exhibited altered vascular stress responses. Also, *bmal1* rhythmic patterns differed in SHR as compared to WKY normal controls.<sup>69</sup> We refer the reader to these references that further detail how the L:D cycle varies and the different physiological effects. Congenic rat studies indicate that the SHR mutation maps near a promoter polymorphism in *bmal1* and that *bmal1*-mediated transcription of the regulatory factor GATA is a mechanism possibly responsible for regulating BP rhythms.<sup>129</sup> Similarly *bmal1* haplotypes in human cohorts have been found and are associated with type 2 diabetes and hypertension.<sup>90</sup>

In other studies, the *per2* mutation was associated with endothelial cell dysfunction. *Per2* mutant mice exhibited impaired endothelium-dependent relaxations to ACh in aortic rings suspended in organ chambers, as well as increased aortic expression of cyclooxygenase-1 and decreased NO release, as compared to wild-type controls.<sup>30</sup> The *per2* mutation was also associated with altered protein kinase *Akt* signaling, cellular senescence, and impaired vascular networking, as compared to wild type.<sup>31</sup>

Cryptochromes appear to be especially important for proper ANS functioning. Cryptochrome knockout mice (*cry1*<sup>-/-</sup>*cry2*<sup>-/-</sup>) exhibited altered autonomic function even under normal diurnal conditions, including increased heart rate and body temperature.<sup>130,131</sup> They also exhibited a loss of

circadian variation in blood pressure in darkness, as compared to wild types.<sup>131–133</sup>

Finally, *Npas2* can substitute as a clock analog or heterodimeric partner for *Bmal1*, and it plays a significant role in maintaining circadian behaviors.<sup>134</sup> In terms of cardiovascular physiology, *Npas2* mutant mice were hypotensive, and exhibited reduced MAP and HR especially around the L:D transition time, and a delayed peak time (acrophase), as compared to control littermates.<sup>32</sup> Comparative diurnal variation of BP and HR in *Npas2* versus *clock* mutants and *Bmal1*<sup>-/-</sup> mice was investigated, as well their relative sympathoadrenal function (diurnal plasma catecholamine profiles and adrenal and cardiac gene expression relevant to catecholamine pathways).<sup>32</sup>

Thus, taken collectively, core elements of the circadian clock mechanism are clearly integrated with cardiovascular health, and the genesis and pathophysiology of cardiovascular disease.

Much of the physiological variation that occurs in the studies noted above is likely neurohormonal, reflecting day/night changes in the neuroendocrine and autonomic milieu. Glucocorticoid status, in particular, is crucial for maintenance of cardiovascular homeostasis and disease.<sup>135</sup> Rhythmicity is presumably SCN orchestrated and driven down the hypothalamic-pituitary axis by vasoactive intestinal peptide (VIP) (see below), which helps regulate transcriptional activity of glucocorticoid receptors in heart and other organs. CLOCK:BMAL1 heterodimers function as negative regulators of glucocorticoid action.<sup>136</sup> An elegant study by Guo and colleagues<sup>137</sup> helped delineate neural versus endocrine control of molecular rhythms in the heart and other organs, using a parabiotic technique that joined intact mice to SCN-lesioned mice. Nonneural signals were sufficient to maintain circadian clock gene expression in liver and kidney, but inadequate for clocks in heart and muscle. These data demonstrated that SCN pathways, which influence tissue circadian rhythms, differ between organs.

Some physiological variation may be specifically secondary to intrinsic clock rhythms within the cardiomyocyte. Bray and colleagues used mice overexpressing a dominant clock mutation directly within the cardiomyocyte.<sup>23</sup> They showed that the intrinsic myocardial clock contributes to selection of glucose versus fatty acid substrate by the cardiomyocyte and plays a role in the regulation of heart rate and cardiac contractile function including the response of the heart to an increase in workload.

The myocyte sarcomere classically considered only as the motor of contractility has been recently shown also to be integral to myocyte signaling. The protein of the *clock* gene resides within the myofilament Z-disc colocalizing with  $\alpha$ -actinin<sup>138,139</sup> in the myocyte; the subcellular distribution of CLOCK protein can be directly altered by myocyte contractility.<sup>138,139</sup> Positive inotropic conditions appear to stimulate the nuclear translocation of CLOCK; CLOCK has histone acetyltransferase activity; thus, it is implicated in chromatin remodeling.<sup>78</sup> CLOCK in the nucleus also activates the transcription of genes that regulate myocyte metabolism and increase energy supply, coupling it to the increase in contractility.<sup>138,139</sup>

There are many hundreds of genes or their protein products that may be under the direct regulation of the local molecular clockwork. Glycogen synthase kinase (GSK)3 $\beta$  is a recently discovered integral component of the mammalian circadian clock that promotes nuclear translocation of PER2, affects REV-ERB transcriptional activity, and thus can advance or delay the clock phase.<sup>140–142</sup> GSK3 $\beta$  may also be of particular relevance to heart disease *in vivo* because in addition to its role in the clockwork mechanism, it also negatively regulates cardiac hypertrophy via phosphorylation on its serine9 residue. GSK3 $\beta$  can antagonize the cardiac hypertrophic response to stimuli such as pressure overload or catecholamine stimulation.<sup>143</sup> However, interactions between clock disruption, GSK3 $\beta$  expression patterns, and progression of cardiac remodeling are not yet fully elucidated.

Although direct links between the circadian clock and cardiovascular growth, renewal, and remodeling are not yet fully defined, several additional promising areas of investigation have opened recently.

Arginine vasopressin (AVP) is very important for cardiovascular function.<sup>144</sup> Notably, AVP mRNA rhythms in the SCN are abolished in *clock* mutant mice, concurrent with markedly reduced AVP peptide levels.<sup>145</sup>

Another link may be through albumin site D-binding protein (DBP), an output gene/protein of the circadian molecular clockwork mechanism.<sup>146</sup> DBP mRNA rhythms were abolished in all tissues in *clock* mutant mice.<sup>146,147</sup> Also promising is *decl*, a core circadian molecular clock component.<sup>148,149</sup> *Decl* exhibits robust mRNA rhythms in normal mice, and rhythms were disturbed in SCN and heart tissues but not liver in *clock* mutant mice.<sup>150,151</sup> These would seem to be promising new candidates for further study, specifically on the topic of clock related maintenance of heart and vessel homeostasis and in remodeling in cardiovascular disease.

Finally, VIP can exert profound direct effects on the cardiovascular system,<sup>152</sup> and cardiac responses to VIP and/or its receptors (VPAC) are altered in animal models of hypertension.<sup>153</sup> VIP displays diurnal rhythms in blood plasma.<sup>154,155</sup> It is thought to be crucial to SCN functioning as many SCN neurons projecting to the paraventricular nucleus appear to use VIP for signaling.<sup>156</sup> VIP/VPAC animal models used in rhythms research may offer unique new insights into cardiovascular phenotype.

Understanding crosstalk between circadian proteins and myocyte signaling pathways will undoubtedly result in important insights into the pathophysiology of cardiovascular disease and the role of the environment. The circadian system appears to be a profoundly important homeostatic mechanism in cardiovascular health, and circadian dysregulation causes or exacerbates cardiac, vascular, and renal disease. No doubt this applies to other tissues and systems as well.

## Summary and Conclusions for Translational Medicine

### Principles Relevant to Cardiovascular Physiology

This review focuses on 2 important principles relevant to cardiovascular physiology. First, significant aspects of cardiovascular physiology are dynamic, including metabolism,

growth, and remodeling. That is, they do not occur uniformly over the 24-hour diurnal cycle. Diurnal variation is evident at the molecular level, because gene expression in the heart and blood vessels is clearly different in the day as compared to the night. Much of the evidence presented here indicates that growth and renewal (structural remodeling) are highly dependent on processes that occur during the subjective night.

Second, synchrony between our endogenous (internal, intrinsic) circadian rhythms and the exogenous (external, extrinsic) diurnal environment is a fundamentally important aspect of healthy organ growth and renewal. As a corollary, disturbing or disrupting this integral relationship has devastating effects on cardiovascular, renal, and possibly other organ systems.

Taken together, these studies indicate that the day/night schedule is applicable to a broad and important range of clinical issues, far more than just impaired cognitive function or performance caused by fatigue, the foci of contemporary thought.

### Bench to Bedside Applications

These principles highlight several important bench-to-bedside applications. Diurnal molecular variation holds considerable promise for novel discovery of physiologically important biomarkers for aiding in understanding, diagnosing, and/or treating human disease. However, in translating research from bench to bedside, the differences between nocturnal animals and diurnal humans must be considered.

The risk/benefit ratio of some therapeutic strategies is not the same across the 24-hour diurnal cycle. For example, one practical target is ACE; this enzyme is involved in tissue remodeling and blood pressure control and is upregulated in our TAC hypertrophy model.<sup>67</sup> ACE inhibitors (ACEis) are the first line agents in the clinical management of hypertension, heart failure and after myocardial infarction in humans. Captopril is a short-acting ACEi, and our pilot experiments revealed that it is ideal for chronopharmacologic investigation, comparing efficacy in cardiac reverse remodeling if administered in the day versus night.<sup>157</sup> A chronotherapeutic approach enhancing efficacy of ACEi has also been reported in the 1-clip renal hypertensive rat<sup>158</sup> and when comparing brief versus continuous infusion of angiotensin to rats.<sup>159</sup> Other established blood pressure medications also exhibit chronotherapeutic promise, including the calcium channel blockers verapamil and diltiazem and the  $\beta$ -blocker propranolol.<sup>160–162</sup> The ACEis enalapril, quinapril, and ramipril may also beneficially impact on nocturnal blood pressure profiles.<sup>161</sup> Clinical applications of chronotherapy promise to improve treatment of a wide range of health concerns in addition to hypertension, as has been well reviewed.<sup>163–171</sup>

Disregard for diurnal rhythms may contribute to differences in therapeutic efficacy, which may be observed, between nocturnal animal models and human patients. Clinical trials should routinely take into account the differing safety and efficacy profiles over 24-hour daily cycles. An avoidable bench to bedside variable is added when a drug is tested in rodents during the laboratory day (nocturnal rodent sleep-time), for administration to humans during human wake-time. Chronotherapeutics offers an approach, which may enhance

drug efficacy, reduce side effects, attain better patient compliance and perhaps reduce costs even for long established drugs.

These studies also show that sleep disruption or an inappropriately synchronized wake/sleep schedule may be an important environmental determinant affecting the expression of a disease phenotype. Above we showed an interaction between environment and the casein kinase 1 $\epsilon$  (*tau*) gene<sup>124</sup> resulted in heart disease. Also, our TAC studies<sup>67</sup> suggested chronic disruption of diurnal rhythm in the setting of hypertension could exacerbate both hypertension and target organ damage. The clinical observations that an abnormal sleep profile is a recognized cause of resistant hypertension, heart attack, heart failure, and stroke are consistent with this hypothesis. One would also anticipate that chronic diurnal rhythm disruption might exacerbate the phenotype of familial hypertrophic or dilated cardiomyopathy or perhaps impair quality of tissue repair following myocardial infarction.

Intensive and cardiac care units often use multibedded rooms, subjecting critically ill patients to preventable light and sleep disturbances, when the patient in the neighboring bed needs medical attention.<sup>172,173</sup> Save for possible inquiry regarding OSA, clinicians and society largely disregard regular day:night schedules or sleep profile as a risk factor for disease; it rarely forms part of the functional inquiry of a typical physician and is not usually in surveys of healthy lifestyle choices.<sup>174,175</sup> Drugs that promise to reduce the need for sleep may reap “the law of unintended consequences.”

Day:night rhythms, including sleep, have been viewed mainly from a neuroscience perspective; an article in *Nature* as recently as 2005<sup>176</sup> was entitled “Sleep Is of the Brain, by the Brain and for the Brain.” Sleep may be “of” the brain, but biological rhythms are found in all organs (“by” all organs), and the above studies show that the integrity of biological rhythms, including sleep, are likely “for” all organs, certainly for the health and integrity of the cardiovascular and renal systems.

In conclusion, synchrony between external and internal diurnal rhythms and harmony among the molecular rhythms within the cell is essential for normal organ biology. The substrate and enzymatic components of a given metabolic pathway must be present not only in the right compartmental space within the cell but also at the right time. Cell physiology is 4 dimensional. Harmony between our biology and our environment is a key to good health.

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### Disclosures

None.

### References

- Dardente H, Cermakian N. Molecular circadian rhythms in central and peripheral clocks in mammals. *Chronobiol Int.* 2007;24:195–213.
- Roenneberg T, Merrow M. Timeline: circadian clocks - the fall and rise of physiology. *Nat Rev Mol Cell Biol.* 2005;6:965–971.
- Young ME. The circadian clock within the heart: potential influence on myocardial gene expression, metabolism, and function. *Am J Physiol Heart Circ Physiol.* 2006;290:H1–H16.
- Reilly DF, Westgate EJ, FitzGerald GA. Peripheral circadian clocks in the vasculature. *Arterioscler Thromb Vasc Biol.* 2007;27:1694–1705.
- Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, Sothorn RB, Hrushesky WJ, Ben-David Y. Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. *Am J Pathol.* 2001;158:1793–1801.
- Boivin DB, James FO, Wu A, Cho-Park PF, Xiong H, Sun ZS. Circadian clock genes oscillate in human peripheral blood mononuclear cells. *Blood.* 2003;102:4143–4145.
- Leibetseder V, Humpeler S, Svoboda M, Schmid D, Thalhammer T, Zuckermann A, Markt W, Ekmekcioglu C. Clock genes display rhythmic expression in human hearts. *Chronobiol Int.* 2009;26:621–636.
- Martino T, Arab S, Straume M, Belsham DD, Tata N, Cai F, Liu P, Trivieri M, Ralph M, Sole MJ. Day/night rhythms in gene expression of the normal murine heart. *J Mol Med.* 2004;82:256–264.
- Rudic RD, McNamara P, Reilly D, Grosser T, Curtis AM, Price TS, Panda S, Hogenesch JB, FitzGerald GA. Bioinformatic analysis of circadian gene oscillation in mouse aorta. *Circulation.* 2005;112:2716–2724.
- Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ. Extensive and divergent circadian gene expression in liver and heart. *Nature.* 2002;417:78–83.
- Young ME, Razeghi P, Taegtmeier H. Clock genes in the heart: characterization and attenuation with hypertrophy. *Circ Res.* 2001;88:1142–1150.
- Brzezinski A. Melatonin in humans. *N Engl J Med.* 1997;336:186–195.
- Charloux A, Gronfier C, Lonsdorfer-Wolf E, Piquard F, Brandenberger G. Aldosterone release during the sleep-wake cycle in humans. *Am J Physiol.* 1999;276(1 pt 1):E43–E49.
- Haus E, Nicolau G, Lakatua DJ, Sackett-Lundeen L, Petrescu E. Circadian rhythm parameters of endocrine functions in elderly subjects during the seventh to the ninth decade of life. *Chronobiologia.* 1989;16:331–352.
- Lightman SL, James VH, Linsell C, Mullen PE, Peart WS, Sever PS. Studies of diurnal changes in plasma renin activity, and plasma noradrenaline, aldosterone and cortisol concentrations in man. *Clin Endocrinol (Oxf).* 1981;14:213–223.
- Obal F Jr, Krueger JM. GHRH and sleep. *Sleep Med Rev.* 2004;8:367–377.
- Arendt J. Melatonin and human rhythms. *Chronobiol Int.* 2006;23:21–37.
- Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C, Reichardt HM, Schutz G, Schibler U. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science.* 2000;289:2344–2347.
- Chalmers JA, Lin SY, Martino TA, Arab S, Liu P, Husain M, Sole MJ, Belsham DD. Diurnal profiling of neuroendocrine genes in murine heart, and shift in proopiomelanocortin gene expression with pressure-overload cardiac hypertrophy. *J Mol Endocrinol.* 2008;41:117–124.
- Dickmeis T. Glucocorticoids and the circadian clock. *J Endocrinol.* 2009;200:3–22.
- Nonaka H, Emoto N, Ikeda K, Fukuya H, Rohman MS, Raharjo SB, Yagita K, Okamura H, Yokoyama M. Angiotensin II induces circadian gene expression of clock genes in cultured vascular smooth muscle cells. *Circulation.* 2001;104:1746–1748.
- Tanaka K, Ashizawa N, Kawano H, Sato O, Seto S, Nishihara E, Terazono H, Isomoto S, Shinohara K, Yano K. Aldosterone induces circadian gene expression of clock genes in H9c2 cardiomyoblasts. *Heart Vessels.* 2007;22:254–260.
- Bray MS, Shaw CA, Moore MW, Garcia RA, Zanquetta MM, Durgan DJ, Jeong WJ, Tsai JY, Bugger H, Zhang D, Rohrwasser A, Rennison JH, Dyck JR, Litwin SE, Hardin PE, Chow CW, Chandler MP, Abel ED, Young ME. Disruption of the circadian clock within the cardiomyocyte influences myocardial contractile function, metabolism, and gene expression. *Am J Physiol Heart Circ Physiol.* 2008;294:H1036–H1047.

24. de la Sierra A, Redon J, Banegas JR, Segura J, Parati G, Gorostidi M, de la Cruz JJ, Sobrino J, Llisterri JL, Alonso J, Vinyoles E, Pallares V, Sarria A, Aranda P, Ruilope LM. Prevalence and factors associated with circadian blood pressure patterns in hypertensive patients. *Hypertension*. 2009;53:466–472.
25. Eguchi K, Pickering TG, Schwartz JE, Hoshide S, Ishikawa J, Ishikawa S, Shimada K, Kario K. Short sleep duration as an independent predictor of cardiovascular events in Japanese patients with hypertension. *Arch Intern Med*. 2008;168:2225–2231.
26. Grassi G, Seravalle G, Quarti-Trevano F, Dell'Oro R, Bombelli M, Cuspidi C, Facchetti R, Bolla G, Mancia G. Adrenergic, metabolic, and reflex abnormalities in reverse and extreme dipper hypertensives. *Hypertension*. 2008;52:925–931.
27. Eguchi K, Ishikawa J, Hoshide S, Pickering TG, Schwartz JE, Shimada K, Kario K. Night time blood pressure variability is a strong predictor for cardiovascular events in patients with type 2 diabetes. *Am J Hypertens*. 2009;22:46–51.
28. Hansen TW, Li Y, Staessen JA. Blood pressure variability remains an elusive predictor of cardiovascular outcome. *Am J Hypertens*. 2009;22:3–4.
29. Anea CB, Zhang M, Stepp DW, Simkins GB, Reed G, Fulton DJ, Rudic RD. Vascular disease in mice with a dysfunctional circadian clock. *Circulation*. 2009;119:1510–1517.
30. Viswambharan H, Carvas JM, Antic V, Marecic A, Jud C, Zaugg CE, Ming XF, Montani JP, Albrecht U, Yang Z. Mutation of the circadian clock gene *Per2* alters vascular endothelial function. *Circulation*. 2007;115:2188–2195.
31. Wang CY, Wen MS, Wang HW, Hsieh IC, Li Y, Liu PY, Lin FC, Liao JK. Increased vascular senescence and impaired endothelial progenitor cell function mediated by mutation of circadian gene *Per2*. *Circulation*. 2008;118:2166–2173.
32. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitzgerald GA. Circadian variation of blood pressure and the vascular response to asynchronous stress. *Proc Natl Acad Sci U S A*. 2007;104:3450–3455.
33. Cohen MC, Rohlta KM, Lavery CE, Muller JE, Mittleman MA. Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. *Am J Cardiol*. 1997;79:1512–1516.
34. Muller JE, Stone PH, Turi ZG, Rutherford JD, Zeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T, et al. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med*. 1985;313:1315–1322.
35. Mukamal KJ, Muller JE, Maclure M, Sherwood JB, Mittleman MA. Increased risk of congestive heart failure among infarctions with nighttime onset. *Am Heart J*. 2000;140:438–442.
36. Kuniyoshi FH, Garcia-Touchard A, Gami AS, Romero-Corral A, van der Walt C, Pusalavidyasagar S, Kara T, Caples SM, Pressman GS, Vasquez EC, Lopez-Jimenez F, Somers VK. Day-night variation of acute myocardial infarction in obstructive sleep apnea. *J Am Coll Cardiol*. 2008;52:343–346.
37. Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, Stone PH. Circadian variation in the frequency of sudden cardiac death. *Circulation*. 1987;75:131–138.
38. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. *Am J Cardiol*. 1987;60:801–806.
39. Kong TQ Jr, Goldberger JJ, Parker M, Wang T, Kadish AH. Circadian variation in human ventricular refractoriness. *Circulation*. 1995;92:1507–1516.
40. Venditti FJ Jr, John RM, Hull M, Tofler GH, Shahian DM, Martin DT. Circadian variation in defibrillation energy requirements. *Circulation*. 1996;94:1607–1612.
41. Eksik A, Akyol A, Norgaz T, Aksu H, Erdinler I, Cakmak N, Alper AT, Cinar B, Yildirim A, Gurkan K. Circadian pattern of spontaneous ventricular tachyarrhythmias in patients with implantable cardioverter defibrillators. *Med Sci Monit*. 2007;13:CR412–CR416.
42. Tofler GH, Gebara OC, Mittleman MA, Taylor P, Siegel W, Venditti FJ Jr, Rasmussen CA, Muller JE. Morning peak in ventricular tachyarrhythmias detected by time of implantable cardioverter/defibrillator therapy. The CPI Investigators. *Circulation*. 1995;92:1203–1208.
43. Manfredini R, Boari B, Gallerani M, Salmi R, Bossone E, Distante A, Eagle KA, Mehta RH. Chronobiology of rupture and dissection of aortic aneurysms. *J Vasc Surg*. 2004;40:382–388.
44. Mehta RH, Manfredini R, Hassan F, Sechtem U, Bossone E, Oh JK, Cooper JV, Smith DE, Portaluppi F, Penn M, Hutchison S, Nienaber CA, Isselbacher EM, Eagle KA. Chronobiological patterns of acute aortic dissection. *Circulation*. 2002;106:1110–1115.
45. Sumiyoshi M, Kojima S, Arima M, Suwa S, Nakazato Y, Sakurai H, Kanoh T, Nakata Y, Daida H. Circadian, weekly, and seasonal variation at the onset of acute aortic dissection. *Am J Cardiol*. 2002;89:619–623.
46. Couch RD. Travel, time zones, and sudden cardiac death. Emporiatic pathology. *Am J Forensic Med Pathol*. 1990;11:106–111.
47. Krantz DS, Kop WJ, Gabbay FH, Rozanski A, Barnard M, Klein J, Pardo Y, Gottdiener JS. Circadian variation of ambulatory myocardial ischemia. Triggering by daily activities and evidence for an endogenous circadian component. *Circulation*. 1996;93:1364–1371.
48. Hu K, Ivanov P, Hilton MF, Chen Z, Ayers RT, Stanley HE, Shea SA. Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. *Proc Natl Acad Sci U S A*. 2004;101:18223–18227.
49. Andrews NP, Gralnick HR, Merryman P, Vail M, Quyyumi AA. Mechanisms underlying the morning increase in platelet aggregation: a flow cytometry study. *J Am Coll Cardiol*. 1996;28:1789–1795.
50. Maemura K, de la Monte SM, Chin MT, Layne MD, Hsieh CM, Yet SF, Perrella MA, Lee ME. CLIF, a novel cycle-like factor, regulates the circadian oscillation of plasminogen activator inhibitor-1 gene expression. *J Biol Chem*. 2000;275:36847–36851.
51. Otto ME, Svatikova A, Barretto RB, Santos S, Hoffmann M, Khandheria B, Somers V. Early morning attenuation of endothelial function in healthy humans. *Circulation*. 2004;109:2507–2510.
52. Decousus HA, Croze M, Levi FA, Jaubert JG, Perpoint BM, De Bonadona JF, Reinberg A, Queneau PM. Circadian changes in anticoagulant effect of heparin infused at a constant rate. *BMJ (Clin Res Ed)*. 1985;290:341–344.
53. Angleton P, Chandler WL, Schmer G. Diurnal variation of tissue-type plasminogen activator and its rapid inhibitor (PAI-1). *Circulation*. 1989;79:101–106.
54. Schoenhard JA, Smith LH, Painter CA, Eren M, Johnson CH, Vaughan DE. Regulation of the PAI-1 promoter by circadian clock components: differential activation by BMAL1 and BMAL2. *J Mol Cell Cardiol*. 2003;35:473–481.
55. Oishi K, Miyazaki K, Uchida D, Ohkura N, Wakabayashi M, Doi R, Matsuda J, Ishida N. PERIOD2 is a circadian negative regulator of PAI-1 gene expression in mice. *J Mol Cell Cardiol*. 2009;46:545–552.
56. Minami Y, Horikawa K, Akiyama M, Shibata S. Restricted feeding induces daily expression of clock genes and *Pai-1* mRNA in the heart of Clock mutant mice. *FEBS Lett*. 2002;526:115–118.
57. Zhang X, Zhao F, Xu C, Lu C, Jin H, Chen S, Qian R. Circadian rhythm disorder of thrombosis and thrombolysis-related gene expression in apolipoprotein E knock-out mice. *Int J Mol Med*. 2008;22:149–153.
58. Kudo T, Nakayama E, Suzuki S, Akiyama M, Shibata S. Cholesterol diet enhances daily rhythm of *Pai-1* mRNA in the mouse liver. *Am J Physiol Endocrinol Metab*. 2004;287:E644–E651.
59. Tsujino T, Naito Y, Kawasaki D, Okuda S, Sakoda T, Fujioka Y, Sugaya T, Ohyanagi M. Circadian expression of plasminogen activator inhibitor-1 in angiotensin II type 1a receptor knockout mice. *Clin Exp Hypertens*. 2005;27:159–168.
60. Takeda N, Maemura K, Horie S, Oishi K, Imai Y, Harada T, Saito T, Shiga T, Amiya E, Manabe I, Ishida N, Nagai R. Thrombomodulin is a clock-controlled gene in vascular endothelial cells. *J Biol Chem*. 2007;282:32561–32567.
61. Westgate EJ, Cheng Y, Reilly DF, Price TS, Walisser JA, Bradfield CA, FitzGerald GA. Genetic components of the circadian clock regulate thrombogenesis in vivo. *Circulation*. 2008;117:2087–2095.
62. Parmacek MS, Epstein JA. Cardiomyocyte renewal. *N Engl J Med*. 2009;361:86–88.
63. Buja LM, Vela D. Cardiomyocyte death and renewal in the normal and diseased heart. *Cardiovasc Pathol*. 2008;17:349–374.
64. Papageorgopoulos C, Caldwell K, Schweingrubber H, Neese RA, Shackleton CH, Hellerstein M. Measuring synthesis rates of muscle creatine kinase and myosin with stable isotopes and mass spectrometry. *Anal Biochem*. 2002;309:1–10.
65. Young ME. Circadian rhythms in cardiac gene expression. *Curr Hypertens Rep*. 2003;5:445–453.
66. Davidson AJ, London B, Block GD, Menaker M. Cardiovascular tissues contain independent circadian clocks. *Clin Exp Hypertens*. 2005;27:307–311.
67. Martino TA, Tata N, Belsham DD, Chalmers J, Straume M, Lee P, Pribiag H, Khaper N, Liu PP, Dawood F, Backx PH, Ralph MR, Sole MJ. Disturbed diurnal rhythm alters gene expression and exacerbates

- cardiovascular disease with rescue by resynchronization. *Hypertension*. 2007;49:1104–1113.
68. McNamara P, Seo SP, Rudic RD, Sehgal A, Chakravarti D, FitzGerald GA. Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. *Cell*. 2001;105:877–889.
  69. Naito Y, Tsujino T, Kawasaki D, Okumura T, Morimoto S, Masai M, Sakoda T, Fujioka Y, Ohyanagi M, Iwasaki T. Circadian gene expression of clock genes and plasminogen activator inhibitor-1 in heart and aorta of spontaneously hypertensive and Wistar-Kyoto rats. *J Hypertens*. 2003;21:1107–1115.
  70. Chalmers JA, Martino TA, Tata N, Ralph MR, Sole MJ, Belsham DD. Vascular circadian rhythms in a mouse vascular smooth muscle cell line (Movas-1). *Am J Physiol Regul Integr Comp Physiol*. 2008;295:R1529–R1538.
  71. Curtis AM, Seo SB, Westgate EJ, Rudic RD, Smyth EM, Chakravarti D, FitzGerald GA, McNamara P. Histone acetyltransferase-dependent chromatin remodeling and the vascular clock. *J Biol Chem*. 2004;279:7091–7097.
  72. Sothorn RB, Cornelissen G, Yamamoto T, Takumi T, Halberg F. Time microscopy of circadian expression of cardiac clock gene mRNA transcription: chronodiagnostic and chrono-therapeutic implications. *Clin Ter*. 2009;160:ep25–ep34.
  73. Wang N, Yang G, Jia Z, Zhang H, Aoyagi T, Soodvilai S, Symons JD, Schnermann JB, Gonzalez FJ, Litwin SE, Yang T. Vascular PPARgamma controls circadian variation in blood pressure and heart rate through Bmal1. *Cell Metab*. 2008;8:482–491.
  74. Mohri T, Emoto N, Nonaka H, Fukuya H, Yagita K, Okamura H, Yokoyama M. Alterations of circadian expressions of clock genes in Dahl salt-sensitive rats fed a high-salt diet. *Hypertension*. 2003;42:189–194.
  75. Young ME, Wilson CR, Razeghi P, Guthrie PH, Taegtmeier H. Alterations of the circadian clock in the heart by streptozotocin-induced diabetes. *J Mol Cell Cardiol*. 2002;34:223–231.
  76. Naito Y, Tsujino T, Fujioka Y, Ohyanagi M, Iwasaki T. Augmented diurnal variations of the cardiac renin-angiotensin system in hypertensive rats. *Hypertension*. 2002;40:827–833.
  77. Etchegaray JP, Lee C, Wade PA, Reppert SM. Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. *Nature*. 2003;421:177–182.
  78. Doi M, Hirayama J, Sassone-Corsi P. Circadian regulator CLOCK is a histone acetyltransferase. *Cell*. 2006;125:497–508.
  79. Grimaldi B, Nakahata Y, Kaluzova M, Masubuchi S, Sassone-Corsi P. Chromatin remodeling, metabolism and circadian clocks: the interplay of CLOCK and SIRT1. *Int J Biochem Cell Biol*. 2009;41:81–86.
  80. Backs J, Olson EN. Control of cardiac growth by histone acetylation/deacetylation. *Circ Res*. 2006;98:15–24.
  81. Ueda HR, Chen W, Minami Y, Honma S, Honma K, Iino M, Hashimoto S. Molecular-timetable methods for detection of body time and rhythm disorders from single-time-point genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2004;101:11227–11232.
  82. Reddy AB, Karp NA, Maywood ES, Sage EA, Deery M, O'Neill JS, Wong GK, Chesham J, Odell M, Lilley KS, Kyriacou CP, Hastings MH. Circadian orchestration of the hepatic proteome. *Curr Biol*. 2006;16:1107–1115.
  83. Martino TA, Tata N, Bjarnason GA, Straume M, Sole MJ. Diurnal protein expression in blood revealed by high throughput mass spectrometry proteomics and implications for translational medicine and body time of day. *Am J Physiol Regul Integr Comp Physiol*. 2007;293:R1430–R1437.
  84. Minami Y, Kasukawa T, Kakazu Y, Iigo M, Sugimoto M, Ikeda S, Yasui A, van der Horst GT, Soga T, Ueda HR. Measurement of internal body time by blood metabolomics. *Proc Natl Acad Sci U S A*. 2009;106:9890–9895.
  85. Tsuji T, Hirota T, Takemori N, Komori N, Yoshitane H, Fukuda M, Matsumoto H, Fukada Y. Circadian proteomics of the mouse retina. *Proteomics*. 2007;7:3500–3508.
  86. Hatcher NG, Atkins N Jr, Annangudi SP, Forbes AJ, Kelleher NL, Gillette MU, Sweedler JV. Mass spectrometry-based discovery of circadian peptides. *Proc Natl Acad Sci U S A*. 2008;105:12527–12532.
  87. Moller M, Sparre T, Bache N, Roepstorff P, Vorum H. Proteomic analysis of day-night variations in protein levels in the rat pineal gland. *Proteomics*. 2007;7:2009–2018.
  88. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Sneliotes EK, Taskiran MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Tewhey R, Blumensiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science*. 2007;316:1331–1336.
  89. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS, McCarthy MI, Hattersley AT. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science*. 2007;316:1336–1341.
  90. Woon PY, Kaisaki PJ, Braganca J, Bihoreau MT, Levy JC, Farrall M, Gauguier D. Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proc Natl Acad Sci U S A*. 2007;104:14412–14417.
  91. de Lange M, Spector TD, Andrew T. Genome-wide scan for blood pressure suggests linkage to chromosome 11, and replication of loci on 16, 17, and 22. *Hypertension*. 2004;44:872–877.
  92. Englund A, Kovanen L, Saarikoski ST, Haukka J, Reunanen A, Aromaa A, Lonnqvist J, Partonen T. NPAS2 and PER2 are linked to risk factors of the metabolic syndrome. *J Circadian Rhythms*. 2009;7:5.
  93. Gottlieb DJ, O'Connor GT, Wilk JB. Genome-wide association of sleep and circadian phenotypes. *BMC Med Genet*. 2007;8(suppl 1):S9.
  94. Rau E, Meyer DK. A diurnal rhythm of incorporation of L-[3H] leucine in myocardium of the rat. *Recent Adv Stud Cardiac Struct Metab*. 1975;7:105–110.
  95. Young ME, Razeghi P, Cedars AM, Guthrie PH, Taegtmeier H. Intrinsic diurnal variations in cardiac metabolism and contractile function. *Circ Res*. 2001;89:1199–1208.
  96. Verdecchia P, Schillaci G, Guerrieri M, Gatteschi C, Benemio G, Boldrini F, Porcellati C. Circadian blood pressure changes and left ventricular hypertrophy in essential hypertension. *Circulation*. 1990;81:528–536.
  97. Hermida RC. Ambulatory blood pressure monitoring in the prediction of cardiovascular events and effects of chronotherapy: rationale and design of the MAPEC study. *Chronobiol Int*. 2007;24:749–775.
  98. Glaser R, Naidu SS, Selzer F, Jacobs AK, Laskey WK, Srinivas VS, Slater JN, Wilensky RL. Factors associated with poorer prognosis for patients undergoing primary percutaneous coronary intervention during off-hours: biology or systems failure? *J Am Coll Cardiol Cardiovasc Interv*. 2008;1:681–688.
  99. Henriques JP, Haasdijk AP, Zijlstra F. Outcome of primary angioplasty for acute myocardial infarction during routine duty hours versus during off-hours. *J Am Coll Cardiol*. 2003;41:2138–2142.
  100. Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T. Sleep apnea and cardiovascular disease: an American Heart Association/American College of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council on Cardiovascular Nursing. *J Am Coll Cardiol*. 2008;52:686–717.
  101. Bradley TD, Floras JS. Obstructive sleep apnoea and its cardiovascular consequences. *Lancet*. 2009;373:82–93.
  102. Mansfield DR, Gollogly NC, Kaye DM, Richardson M, Bergin P, Naughton MT. Controlled trial of continuous positive airway pressure in obstructive sleep apnea and heart failure. *Am J Respir Crit Care Med*. 2004;169:361–366.
  103. Ryan CM, Usui K, Floras JS, Bradley TD. Effect of continuous positive airway pressure on ventricular ectopy in heart failure patients with obstructive sleep apnoea. *Thorax*. 2005;60:781–785.
  104. Usui K, Bradley TD, Spaak J, Ryan CM, Kubo T, Kaneko Y, Floras JS. Inhibition of awake sympathetic nerve activity of heart failure patients with obstructive sleep apnea by nocturnal continuous positive airway pressure. *J Am Coll Cardiol*. 2005;45:2008–2011.
  105. Kawachi I, Colditz GA, Stampfer MJ, Willett WC, Manson JE, Speizer FE, Hennekens CH. Prospective study of shift work and risk of coronary heart disease in women. *Circulation*. 1995;92:3178–3182.

106. Knutsson A, Boggild H. Shiftwork and cardiovascular disease: review of disease mechanisms. *Rev Environ Health*. 2000;15:359–372.
107. Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci U S A*. 2009;106:4453–4458.
108. Chan CT, Floras JS, Miller JA, Richardson RM, Pierratos A. Regression of left ventricular hypertrophy after conversion to nocturnal hemodialysis. *Kidney Int*. 2002;61:2235–2239.
109. Lawton G. Get Up and Go. *New Sci*. 2006;189(34–38).
110. Webb IC, Pollock MS, Mistlberger RE. Modafinil [2-[(diphenylmethyl) sulfinyl]acetamide] and circadian rhythms in syrian hamsters: assessment of the chronobiotic potential of a novel alerting compound. *J Pharmacol Exp Ther*. 2006;317:882–889.
111. Bass J, Turek FW. Sleepless in America: a pathway to obesity and the metabolic syndrome? *Arch Intern Med*. 2005;165:15–16.
112. Turek FW. Staying off the dance floor: when no rhythm is better than bad rhythm. *Am J Physiol Regul Integr Comp Physiol*. 2008;294:R1672–R1674.
113. Bradley TD, Floras JS. Sleep apnea and heart failure: Part I: obstructive sleep apnea. *Circulation*. 2003;107:1671–1678.
114. Bradley TD, Floras JS. Sleep apnea and heart failure: Part II: central sleep apnea. *Circulation*. 2003;107:1822–1826.
115. Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet*. 2005;365:1046–1053.
116. Turek FW, Joshi C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*. 2005;308:1043–1045.
117. Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, Hogenesch JB, Fitzgerald GA. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biol*. 2004;2:e377.
118. Yang S, Liu A, Weidenhammer A, Cooksey RC, McClain D, Kim MK, Aguilera G, Abel ED, Chung JH. The role of mPer2 clock gene in glucocorticoid and feeding rhythms. *Endocrinology*. 2009;150:2153–2160.
119. Ralph MR, Menaker M. A mutation of the circadian system in golden hamsters. *Science*. 1988;241:1225–1227.
120. Lowrey PL, Shimomura K, Antoch MP, Yamazaki S, Zemenides PD, Ralph MR, Menaker M, Takahashi JS. Positional synteny cloning and functional characterization of the mammalian circadian mutation tau. *Science*. 2000;288:483–492.
121. Ralph MR, Foster RG, Davis FC, Menaker M. Transplanted suprachiasmatic nucleus determines circadian period. *Science*. 1990;247:975–978.
122. Penev PD, Kolker DE, Zee PC, Turek FW. Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic heart disease. *Am J Physiol*. 1998;275(6 pt 2):H2334–H2337.
123. Davidson AJ, Sellix MT, Daniel J, Yamazaki S, Menaker M, Block GD. Chronic jet-lag increases mortality in aged mice. *Curr Biol*. 2006;16:R914–R916.
124. Martino TA, Oudit GY, Herzenberg AM, Tata N, Koletar MM, Kabir GM, Belsham DD, Backx PH, Ralph MR, Sole MJ. Circadian rhythm disorganization produces profound cardiovascular and renal disease in hamsters. *Am J Physiol Regul Integr Comp Physiol*. 2008;294:R1675–R1683.
125. Durgan DJ, Trexler NA, Egbejimi O, McElfresh TA, Suk HY, Petterson LE, Shaw CA, Hardin PE, Bray MS, Chandler MP, Chow CW, Young ME. The circadian clock within the cardiomyocyte is essential for responsiveness of the heart to fatty acids. *J Biol Chem*. 2006;281:24254–24269.
126. Zheng B, Larkin DW, Albrecht U, Sun ZS, Sage M, Eichele G, Lee CC, Bradley A. The mPer2 gene encodes a functional component of the mammalian circadian clock. *Nature*. 1999;400:169–173.
127. Sei H, Oishi K, Chikahisa S, Kitaoka K, Takeda E, Ishida N. Diurnal amplitudes of arterial pressure and heart rate are dampened in Clock mutant mice and adrenalectomized mice. *Endocrinology*. 2008;149:3576–3580.
128. Bunker MK, Wilsbacher LD, Moran SM, Clendenen C, Radcliffe LA, Hogenesch JB, Simon MC, Takahashi JS, Bradfield CA. Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell*. 2000;103:1009–1017.
129. Clemitson JR, Dixon RJ, Haines S, Bingham AJ, Patel BR, Hall L, Lo M, Sassard J, Charchar FJ, Samani NJ. Genetic dissection of a blood pressure quantitative trait locus on rat chromosome 1 and gene expression analysis identifies SPON1 as a novel candidate hypertension gene. *Circ Res*. 2007;100:992–999.
130. Ikeda H, Yong Q, Kurose T, Todo T, Mizunoya W, Fushiki T, Seino Y, Yamada Y. Clock gene defect disrupts light-dependency of autonomic nerve activity. *Biochem Biophys Res Commun*. 2007;364:457–463.
131. Masuki S, Todo T, Nakano Y, Okamura H, Nose H. Reduced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in Cry-deficient mice lacking a biological clock. *J Physiol*. 2005;566(pt 1):213–224.
132. van der Horst GT, Muijtens M, Kobayashi K, Takano R, Kanno S, Takao M, de Wit J, Verkerk A, Eker AP, van Leenen D, Buijs R, Bootsma D, Hoeijmakers JH, Yasui A. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature*. 1999;398:627–630.
133. Vitaterna MH, Selby CP, Todo T, Niwa H, Thompson C, Fruechte EM, Hitomi K, Thresher RJ, Ishikawa T, Miyazaki J, Takahashi JS, Sancar A. Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. *Proc Natl Acad Sci U S A*. 1999;96:12114–12119.
134. Dudley CA, Erbel-Sieler C, Estill SJ, Reick M, Franken P, Pitts S, McKnight SL. Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. *Science*. 2003;301:379–383.
135. Walker BR. Glucocorticoids and cardiovascular disease. *Eur J Endocrinol*. 2007;157:545–559.
136. Nader N, Chrousos GP, Kino T. Circadian rhythm transcription factor CLOCK regulates the transcriptional activity of the glucocorticoid receptor by acetylating its hinge region lysine cluster: potential physiological implications. *FASEB J*. 2009;23:1572–1583.
137. Guo H, Brewer JM, Champhekar A, Harris RB, Bittman EL. Differential control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. *Proc Natl Acad Sci U S A*. 2005;102:3111–3116.
138. Boateng SY, Goldspink PH. Assembly and maintenance of the sarcomere night and day. *Cardiovasc Res*. 2008;77:667–675.
139. Qi L, Boateng SY. The circadian protein Clock localizes to the sarcomeric Z-disk and is a sensor of myofilament cross-bridge activity in cardiac myocytes. *Biochem Biophys Res Commun*. 2006;351:1054–1059.
140. Itaka C, Miyazaki K, Akaiki T, Ishida N. A role for glycogen synthase kinase-3beta in the mammalian circadian clock. *J Biol Chem*. 2005;280:29397–29402.
141. Yin L, Wang J, Klein PS, Lazar MA. Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. *Science*. 2006;311:1002–1005.
142. Hirota T, Lewis WG, Liu AC, Lee JW, Schultz PG, Kay SA. A chemical biology approach reveals period shortening of the mammalian circadian clock by specific inhibition of GSK-3beta. *Proc Natl Acad Sci U S A*. 2008;105:20746–20751.
143. Hardt SE, Sadoshima J. Glycogen synthase kinase-3beta: a novel regulator of cardiac hypertrophy and development. *Circ Res*. 2002;90:1055–1063.
144. Oghlaktion G, Klapholz M. Vasopressin and vasopressin receptor antagonists in heart failure. *Cardiol Rev*. 2009;17:10–15.
145. Jin X, Shearman LP, Weaver DR, Zylka MJ, de Vries GJ, Reppert SM. A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. *Cell*. 1999;96:57–68.
146. Ripperger JA, Shearman LP, Reppert SM, Schibler U. CLOCK, an essential pacemaker component, controls expression of the circadian transcription factor DBP. *Genes Dev*. 2000;14:679–689.
147. Oishi K, Fukui H, Ishida N. Rhythmic expression of BMAL1 mRNA is altered in Clock mutant mice: differential regulation in the suprachiasmatic nucleus and peripheral tissues. *Biochem Biophys Res Commun*. 2000;268:164–171.
148. Hamaguchi H, Fujimoto K, Kawamoto T, Noshiro M, Maemura K, Takeda N, Nagai R, Furukawa M, Honma S, Honma K, Kurihara H, Kato Y. Expression of the gene for Dec2, a basic helix-loop-helix transcription factor, is regulated by a molecular clock system. *Biochem J*. 2004;382(pt 1):43–50.
149. Honma S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, Kato Y, Honma K. Dec1 and Dec2 are regulators of the mammalian molecular clock. *Nature*. 2002;419:841–844.
150. Butler MP, Honma S, Fukumoto T, Kawamoto T, Fujimoto K, Noshiro M, Kato Y, Honma K. Dec1 and Dec2 expression is disrupted in the

- suprachiasmatic nuclei of Clock mutant mice. *J Biol Rhythms*. 2004;19:126–134.
151. Noshiro M, Furukawa M, Honma S, Kawamoto T, Hamada T, Honma K, Kato Y. Tissue-specific disruption of rhythmic expression of Dec1 and Dec2 in clock mutant mice. *J Biol Rhythms*. 2005;20:404–418.
  152. Henning RJ, Sawmiller DR. Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc Res*. 2001;49:27–37.
  153. Eilam R, Malach R, Bergmann F, Segal M. Hypertension induced by hypothalamic transplantation from genetically hypertensive to normotensive rats. *J Neurosci*. 1991;11:401–411.
  154. Cugini P, Lucia P, Di Palma L, Re M, Leone G, Battisti P, Canova R, Gasbarrone L, Cianetti A. Vasoactive intestinal peptide fluctuates in human blood with a circadian rhythm. *Regul Pept*. 1991;34:141–148.
  155. Lockinger A, Koberle D, Konig PS, Saria A, Herold M, Cornelissen G, Halberg F. Neuropeptide chronomics in clinically healthy young adults: circadian and circadian patterns. *Peptides*. 2004;25:533–542.
  156. Teclemariam-Mesbah R, Kalsbeek A, Pevet P, Buijs RM. Direct vasoactive intestinal polypeptide-containing projection from the suprachiasmatic nucleus to spinal projecting hypothalamic paraventricular neurons. *Brain Res*. 1997;748:71–76.
  157. Tata N, Martino TA, Vanderlaan R, Dawood F, Kabir GM, Khaper N, Liu PP, Husain M, Backx PH, Sole MJ. Chronotherapy: diurnal efficacy of captopril. *J Card Fail*. 2005;11:S99. Abstract.
  158. Morgan TO, Brunner HR, Aubert JF, Wang Q, Griffiths C, Delbridge L. Cardiac hypertrophy depends upon sleep blood pressure: a study in rats. *J Hypertens*. 2000;18:445–451.
  159. Morgan TO, Aubert JF, Wang Q. Sodium, angiotensin II, blood pressure, and cardiac hypertrophy. *Kidney Int Suppl*. 1998;67:S213–S215.
  160. Prisant LM. Chronotherapeutics: a surge of ideas. *Clin Cornerstone*. 2004;6:7–17.
  161. Hermida RC, Ayala DE, Calvo C. Administration-time-dependent effects of antihypertensive treatment on the circadian pattern of blood pressure. *Curr Opin Nephrol Hypertens*. 2005;14:453–459.
  162. Guo YF, Stein PK. Circadian rhythm in the cardiovascular system: chronocardiology. *Am Heart J*. 2003;145:779–786.
  163. Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. *Nat Rev Cancer*. 2003;3:350–361.
  164. Gorbacheva VY, Kondratov RV, Zhang R, Cherukuri S, Gudkov AV, Takahashi JS, Antoch MP. Circadian sensitivity to the chemotherapeutic agent cyclophosphamide depends on the functional status of the CLOCK/BMAL1 transactivation complex. *Proc Natl Acad Sci U S A*. 2005;102:3407–3412.
  165. Green CB. Time for chronotherapy? Clock genes dictate sensitivity to cyclophosphamide. *Proc Natl Acad Sci U S A*. 2005;102:3529–3530.
  166. Klerman EB. Clinical aspects of human circadian rhythms. *J Biol Rhythms*. 2005;20:375–386.
  167. Smolensky MH, Haus E. Circadian rhythms and clinical medicine with applications to hypertension. *Am J Hypertens*. 2001;14(9 pt 2):280S–290S.
  168. Hermida RC, Ayala DE, Calvo C, Lopez JE, Fernandez JR, Mojon A, Dominguez MJ, Covelo M. Administration time-dependent effects of aspirin on blood pressure in untreated hypertensive patients. *Hypertension*. 2003;41:1259–1267.
  169. Smolensky MH, Hermida RC, Haus E, Portaluppi F, Reinberg A. Biological rhythms, medication safety, and women's health. *J Womens Health (Larchmt)*. 2005;14:38–46.
  170. Storms WW. Pharmacologic approaches to daytime and nighttime symptoms of allergic rhinitis. *J Allergy Clin Immunol*. 2004;114(5 suppl):S146–S153.
  171. White WB. Cardiovascular risk and therapeutic intervention for the early morning surge in blood pressure and heart rate. *Blood Press Monit*. 2001;6:63–72.
  172. Drouot X, Cabello B, d'Ortho MP, Brochard L. Sleep in the intensive care unit. *Sleep Med Rev*. 2008;12:391–403.
  173. Paul T, Lemmer B. Disturbance of circadian rhythms in analgesia-intensive care unit patients with and without craniocerebral injury. *Chronobiol Int*. 2007;24:45–61.
  174. King DP, Takahashi JS. Molecular genetics of circadian rhythms in mammals. *Annu Rev Neurosci*. 2000;23:713–742.
  175. Djousse L, Driver JA, Gaziano JM. Relation between modifiable lifestyle factors and lifetime risk of heart failure. *JAMA*. 2009;302:394–400.
  176. Hobson JA. Sleep is of the brain, by the brain and for the brain. *Nature*. 2005;437:1254–1256.

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