This Review is in a thematic series on Inflammation, which includes the following articles:

The Biological Basis for Cardiac Repair After Myocardial Infarction: From Inflammation to Fibrosis
Resolution of Acute Inflammation and the Role of Resolvins in Immunity, Thrombosis, and Vascular Biology
Circadian Influence on Metabolism and Inflammation in Atherosclerosis
Inflammatory Disequilibrium in Stroke
Chronic Heart Failure and Inflammation: What Do We Really Know?

Guest Editors: Nikolaos Frangogiannis and Matthias Nahrendorf

Review

Circadian Influence on Metabolism and Inflammation in Atherosclerosis
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Abstract: Many aspects of human health and disease display daily rhythmicity. The brain’s suprachiasmatic nucleus, which interprets recurring external stimuli, and autonomous molecular networks in peripheral cells together set our biological circadian clock. Disrupted or misaligned circadian rhythms promote multiple pathologies including chronic inflammatory and metabolic diseases such as atherosclerosis. Here, we discuss studies suggesting that circadian fluctuations in the vessel wall and in the circulation contribute to atherogenesis. Data from humans and mice indicate that an impaired molecular clock, disturbed sleep, and shifting light–dark patterns influence leukocyte and lipid supply in the circulation and alter cellular behavior in atherosclerotic lesions. We propose that a better understanding of both local and systemic circadian rhythms in atherosclerosis will enhance clinical management, treatment, and public health policy. (Circ Res. 2016;119:131-141. DOI: 10.1161/CIRCRESAHA.116.308034.)

Key Words: atherosclerosis ■ circadian rhythm ■ inflammation ■ metabolism ■ lifestyle

Rising rates of cardiovascular diseases, metabolic syndromes, and diabetes mellitus have coincided with global industrialization and exponential technological advances. Over the past 100 years, the widespread use of artificial light, increased ease of travel, electronics, and modern communication systems have dramatically altered the timing and quality of our daily rest-and-wake cycles. Increased work and social demands, the use of entertainment platforms, and instant communication platforms encourage us to stay up late using artificial light and luminescent screens. Moreover, frequent long distance travel across multiple time zones and prolonged shift work can chronically disrupt our biological rhythms. Collectively, sleep and disruption of our circadian (derived from circa diem, about a day) patterns can have profound consequences for health and well-being.

Atherosclerosis, the underlying pathology of most cardiovascular diseases (CVDs), is an inflammatory disease characterized by lipid and leukocyte accumulation in the arterial wall.1 The clinical manifestations of atherosclerosis—ischemic heart disease, myocardial infarction (MI), and stroke—arise when lesions occlude the vessel due to abluminal remodeling, plaque erosion, or thrombus formation on rupture. Despite recent advances in treatment and management, atherosclerosis and its complications remain the leading cause of death globally.2,3 Although dyslipidemia, obesity, diabetes mellitus, hypertension, and smoking are well-proven cardiovascular risk factors, accumulating evidence suggests that disruptions in circadian rhythm also promote disease.2 For example, shift workers have a higher risk of MI, stroke, obesity, and diabetes mellitus,4–6 and sleep, or lack thereof, is an emerging cardiometabolic and atherosclerotic risk factor.7–9 A recent analysis found that chronic disruption of normal sleep patterns (7–8 uninterrupted hours per night) was associated with increased risk of developing CVD even after adjusting for other risk factors.7,10,11 Specifically, individuals who sleep <5 hours per night have a 2.20 relative risk (95% confidence interval, 1.78–2.71) of developing CVD.10 Intriguingly, excessive sleep also leads to increased CVD risk (relative risk, 1.41; 95% confidence interval, 1.19–1.68),
suggesting a U-shaped association between CVD risk and sleep duration.\textsuperscript{7} The clinical presentation of CVD also displays daily rhythmicity. Because of increased blood pressure, heart rate, and thrombotic activity, acute MI, cerebral infarction, and pulmonary embolism are most likely to occur in the morning.\textsuperscript{12–14} Recent evidence also suggests that the pathogenesis of atherosclerosis progression may be under circadian control. Here, we review data suggesting that both systemic factors (hematopoiesis and dyslipidemia) and local cellular events (endothelial activation, macrophage behavior, inflammation, and vascular remodeling) in atherosclerosis display circadian patterning.

### The Physiological Clock

All organisms, from unicellular bacteria to plants and mammals, have intrinsic body clocks that respond to environmental cues and control major components of their physiology. In mammals, photic cues from light–dark cycles signal the suprachiasmatic nucleus (SCN) in the hypothalamus via the retinohypothalamic tract. Known as the central clock, the SCN responds to light–dark patterns and regulates peripheral tissue functions via sympathetic nervous system (SNS) signaling and hormone release. Through SNS innervation, the SCN coordinates the pace and phase of circadian oscillations in peripheral tissues. However, peripheral mammalian cells also contain autonomous clocks able to sustain circadian timing independently of the SCN and SNS. These peripheral clocks were identified after observations that isolated mammalian cells in weeks-long culture retain circadian rhythms, and the phase of their rhythms can be set by serum shock.\textsuperscript{15} The peripheral clocks can regulate circadian gene transcription, protein synthesis, and cellular behavior.

The molecular clock machinery, found in both peripheral and SCN cells, is comprised of a positive arm and negative feedback loops (simplistically outlined in Figure 1). The positive arm includes 2 transcription factors: circadian locomotor output cycles kaput (Clk) and brain and muscle aryl-hydrocarbon receptor nuclear translocator-like (Bmal)1/2. The Clock and Bmal1/2 proteins form a heterodimer and promote the transcription of genes that contain E-box cis-regulatory enhancer sequences known as Clock-controlled genes which make up \(-5\%\) to \(10\%\) of the transcriptome in most mammalian cells and up to \(16\%\) in liver tissue.\textsuperscript{16–18} The components of the negative feedback loop are also upregulated by Clock/Bmal1/2 which include the Period (Per)1/2/3 and cryptochrome (Cry)1/2 proteins, as well as the retinoic acid–related orphan receptor, Rev-erbα/β. The Per and Cry proteins dimerize and inhibit Clock/Bmal1/2 activity, whereas Rev-erbα binds to the retinoic acid–related orphan receptor response element located in the Bmal1 promoter, thereby inhibiting its transcription. The molecular clock is also controlled post-translationally. For example, the Per and Cry proteins can be phosphorylated by the serine-threonine kinases casein kinase 1 (CK1) \(\epsilon\) and \(\delta\), marking them for proteasomal degradation by E3 ubiquitin ligases. In addition, AMP-activated protein kinase is activated during periods of low nutrient availability and phosphorylates Cry1, marking it for degradation.\textsuperscript{19} Recent studies have also suggested that other kinase networks including PI3K/Akt, glycogen synthase kinase (GSK)-3, mTor, and mitogen-activated protein kinases help regulate circadian cycles.\textsuperscript{20–23} Regulation of circadian gene expression is also accomplished by chromatin remodeling. Histone deacetylase 3 is rhythmically recruited to the genome where it complexes with Rev-erbα promoting its binding to target genes.\textsuperscript{24,25} Moreover, histone methylation is circadian, and the methyltransferase MLL1 associates with Clock:Bmal1 promoting the dimer’s transcriptional activity.\textsuperscript{26} Finally, Clock itself has acetyltrasferase activity and acetylates histones as well as its partner Bmal1.\textsuperscript{27}

Genetic variants of the core molecular clock machinery have been associated with metabolic disease in humans. Single nucleotide polymorphisms in Clock, Bmal1, and Cry2 alter an individual’s risk of developing type 2 diabetes mellitus, obesity, and dyslipidemia in addition to disturbing sleep/wake patterns.\textsuperscript{28–34} Importantly, a recent analysis demonstrated that the Clock s4580704 single nucleotide polymorphism is significantly associated with CVD incidence in type 2 diabetes mellitus patients.\textsuperscript{35} We guide readers to reviews\textsuperscript{34,36} outlining the relationship between genetic variants of the core molecular clock machinery and disease risk in humans.

To probe the underlying mechanisms linking circadian rhythm and disease, many transgenic mouse models with targeted disruptions in the molecular clock have been developed. When maintained in complete darkness, both Bmal1 knock-out mice and mice with a dominant negative Clock mutation (ClnkA19/A19) lose circadian rhythmicity in multiple tissues.\textsuperscript{37} In addition, Clock mutant mice sleep up to 2 hours less per day than control mice.\textsuperscript{38} Mice deficient in negative feedback loop components including Per, Cry, or Rev-erbα display a robust arrhythmic phenotype even during regular light–dark cycles.\textsuperscript{39–42} These animal models have provided great insight into the role of circadian timing in physiology. Experiments relying on these models, however, must be interpreted with caution because the core molecular clock may have noncircadian functionality\textsuperscript{43,44} and the use of global transgenic mice impedes assessing the role of the SCN versus peripheral clocks. To overcome some of these hurdles, tissue-specific models have been developed\textsuperscript{45–48} although they require further study, particularly in the context of inflammation and atherosclerosis.

### Circadian Influences on Hematopoiesis

As early as the 1960s, researchers noted that mouse susceptibility to infection depended on the time of day of inoculation.\textsuperscript{49} This observation suggested that aspects of an organism’s immune system may fluctuate over the course of a day, thus enabling an individual to anticipate persistent and recurring stresses or environmental challenges. Recently, coined to represent anticipatory inflammation, this phenomenon profoundly impacts immunobiology.\textsuperscript{45}

### Nonstandard Abbreviations and Acronyms

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Ccl</td>
<td>chemokine C-C motif ligand</td>
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<td>HSC</td>
<td>hematopoietic stem cell</td>
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<td>IL</td>
<td>interleukin</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<td>SCN</td>
<td>suprachiasmatic nucleus</td>
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<td>SNS</td>
<td>sympathetic nervous system</td>
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In healthy adults, the bone marrow is the primary site of hematopoiesis. The hematopoietic cascade results in the production of increasingly differentiated cells that eventually give rise to all cellular components of blood including leukocytes, platelets, and erythrocytes. This highly regulated process can be influenced by changes to the bone marrow environment, such as the presence of toll-like receptor ligands or dyslipidemia. Hematopoiesis is also influenced by circadian rhythms. In mice, the number of circulating inflammatory Ly-6Chigh monocytes is 2-fold higher during the resting period (zeitgeber time [ZT] 4–8; ZT 0: lights on, ZT12: lights off) than during the active phase (ZT12-20). A similar pattern occurs in the spleen where the number of Ly-6C<sup>high</sup> monocytes peaks at ZT8. Ly-6C<sup>low</sup> monocytes, meanwhile, do not display circadian rhythm in blood. Similarly, in humans, circulating monocyte numbers are highest during our rest period. The molecular mechanisms mediating monocyte oscillations are not fully understood. In mice, monocytic Bmal1 expression is rhythmic, and myeloid Bmal1 deletion attenuates circadian monocyte fluctuations. Importantly, chemokine C-C motif ligand (Ccl) 2 (also known as MCP-1), a chemokine critical to monocyte egress from the bone marrow, is also under circadian control. Ccl2 levels peak during the resting period (when monocyte levels are also high), and myeloid Bmal1 deletion enhances Ccl2 levels in the blood and blunts its fluctuation. These observations suggest that circadian monocyte fluctuations are at least partly mediated by the peripheral clock located within myeloid cells, independent of the SCN. Circadian influence on circulating and splenic monocyte levels has functional impact. For example, mice infected with *Listeria monocytogenes* or injected with LPS at the beginning of the rest phase, when Ly-6C<sup>high</sup> monocyte levels are low, survive longer, and clear pathogen more effectively than mice inoculated at later times in the day, when monocyte levels are high.

Circulating monocytes derive from pluripotent hematopoietic stem cell (HSC) precursors. In the steady state, the majority of HSCs reside in the bone marrow; however, small populations can be found in the circulation and peripheral tissues. Despite their low abundance in the circulation, HSC numbers in mouse blood oscillate throughout the day with a 3-fold change between peak and trough levels. Interestingly, in mice, HSC fluctuation in the blood accords with monocyte levels, peaking during the rest phase (ZT 5). In humans, the number of circulating HSCs peaks in the afternoon. The number of HSCs in the bone marrow also undergoes circadian changes; 1 study found that the number of CD34<sup>+</sup> HSCs in human bone marrow vacillate 6-fold over 24 hours, with an acrophase in the morning. Bone marrow HSC populations renew by proliferation. In human bone marrow, cell proliferation, determined by the number of cells in S-phase as a measure of DNA synthesis, peaks in the middle of the day. Mice, on the contrary, seem to have 2 acrophases of bone marrow cell proliferation, one at the start of the rest period and another in the middle of the active period. In the bone marrow, HSCs associate with stromal cells, including endothelial cells, osteoblasts, and mesenchymal cells, to comprise the hematopoietic niche. Intriguingly, the number of stromal cells also fluctuates during a 24-hour period, which suggests that the bone marrow niche’s size and capacity may be under circadian control.

The mechanisms regulating circadian HSC proliferation and release from the bone marrow are not fully understood. Although bone marrow cells, including HSCs, express components of the core molecular clock, the bulk of the data suggest that the central clock in the hypothalamus is the primary regulator of circadian bone marrow function. This theory is supported by the observation that the number of circulating HSCs do not fluctuate when mice are maintained in constant light. This suggests that the SNS transmits signals from the SCN, which is receptive to photic cues, to the periphery, including the bone marrow and spleen, where it mediates hematopoiesis. The SNS neurotransmitters, epinephrine and norepinephrine, display circadian rhythmicity and promote HSC migration and proliferation by signaling through β-adrenergic receptors located on HSCs. Indeed, sympathectomy abolishes HSC fluctuations in the circulation. Mechanistically, SNS innervation of HSCs in the bone marrow lowers levels of Ccl12, a key HSC retention factor. The SNS’s effect on Ccl12 expression in HSCs seems to be independent of the peripheral molecular clock within these cells as β-adrenergic receptor stimulation attenuates Ccl12 even in Bmal1<sup>−/−</sup>, Per1<sup>−/−</sup> or Per2mut HSCs. Moreover, the minor
fluctuations in Bmal1, Clock, Per1, Per2, Cry1, and Rev-erbα expression in bone marrow cells depend on consistent light–dark cycles.⁴⁴ Taken together, these data suggest that light signals interpreted by the SCN mediate the daily oscillations in hematopoiesis and HSC proliferation independent of the peripheral molecular clock found in HSCs.

Circadian Control of Peripheral Lipid Supply
Circulating lipids, including cholesterol, triglyceride, and ApoB-containing low-density lipoproteins, display circadian fluctuations in ad libitum–fed animals.⁶⁹,⁷¹ In mice, most of these lipids rise during the active period (ZT 18), when nutritional and energy demand is high, and fall during the rest period (ZT 5).⁶⁹,⁷⁰ Plasma high-density lipoprotein, however, peaks early in the rest phase (ZT 1–2) and remains relatively low during the active phase.⁶⁹ The role of circadian rhythms in lipid metabolism is complicated by observations that both plasma lipid levels and the phase of peripheral molecular clocks, particularly in the liver, are food and light entrainable.⁷²,⁷³ Although plasma lipid levels rise postprandially, moderate oscillations, particularly in triglyceride levels, still occur in feeding-restricted mice.⁶⁹,⁷⁴ Photic cues also affect peripheral lipid levels, as oscillations are abolished in mice fed ad libitum but kept in constant light suggesting an important role for the SCN.⁶⁹ Although Clock mutant, Bmal1−/− and Rev-erbα−/− mice are hyperlipidemic, these models cannot discern whether this phenomenon depends on deletion of these genes in the SCN or in peripheral tissues.⁴⁶,⁷⁵ Importantly, liver-specific Bmal1 or Rev-erbα deletion elevates circulating levels of triglycerides, cholesterol, and free fatty acids.⁴⁶,⁷⁶ Together, these studies indicate that both independent peripheral clocks in the liver and external stimuli interpreted by the SCN maintain circulating lipid supply.

Peripheral lipid supply is delicately balanced between absorption from diet and biosynthesis in the liver. After being absorbed by enterocytes that line the gut, lipids are packaged into chylomicrons for transportation to the liver. Once in the liver, dietary lipids are broken down and rearranged into various apolipoprotein-containing particles. Mouse enterocytes rhythmically express molecular clock genes, and lipid absorption efficiency by enterocytes is high during the active phase and low during the rest phase.⁷⁶ This dynamic is food entrainable, as restricted feeding immediately increases both the rate of enterocyte lipid absorption and the expression of regulatory genes, including ApoB, MTP, and ApoAIV.⁷⁷,⁷⁸ Clock mutant mice, however, lack the circadian pattern of enterocyte gene expression and lipid absorption.⁷⁹,⁸⁰ Instead, these mice display an overall increase in lipid absorption as Clk1A19/A19 mice gavaged with radiolabeled cholesterol have 3-fold more circulating labeled cholesterol than wild-type mice.⁷⁹

Lipid biosynthesis in the liver is also under circadian influence. Genes mediating triglyceride and cholesterol synthesis including sterol regulatory element-binding protein-1c, fatty acid synthase, acetyl co-A carboxylase, acetyl-CoA synthase, glycerol-3-phosphate acyltranferase, and 3-hydroxy-3-methyl-glutaryl-coenzyme A show circadian expression patterns in the livers of ad libitum–fed animals.⁷⁰,⁸¹–⁸⁴ Furthermore, deletion or knockdown of Clock or Bmal1 abolishes these genes’ rhythmic shifts.⁴⁶,⁸⁴,⁸⁵ Rev-erbα mediates the rhythmic transcription of insulin-induced gene 2 (Insig2), a protein that dampens sterol regulatory element-binding protein activity.⁸⁷ As a result, Rev-erbα knockout mice have elevated Insig2 expression, reduced hepatic triglyceride and cholesterol levels, and increased plasma lipid levels.⁸⁷ Studies show that 17% of lipid species in liver tissue oscillate in a circadian manner.⁸⁸ The bulk of these (33%) are triglycerides that display an acrophase in the middle of the rest period and a nadir during the active period.⁷⁰ It appears, therefore, that lipid synthesis—or at least accumulation—in the liver is antiphasic to enterocyte lipid absorption in the gut. Balance between these 2 processes is presumably critical in maintaining appropriate circulating lipid levels.

Circadian Rhythm in Atherosclerosis
Emerging evidence suggests that circadian rhythms play an important role in vascular function and health. Circadian rhythms not only influence systemic atherosclerosis mediators, including leukocytes and lipids, but also locally control cells within the vessel wall. Studies conducted >15 years ago demonstrated the existence of a functional circadian clock in the vasculature.⁸⁸–⁹⁰ Gene profiling found that 330 genes, 5% to 10% of the transcriptome, exhibit circadian expression patterns in mouse aortae.⁹¹ Circadian patterned genes include those related to the core molecular clock, lipid and glucose metabolism, protein folding, and vascular integrity. The central SCN’s role in mediating the circadian rhythm of the vasculature is not clear. In the healthy mouse aorta, Per1 and Per2 expressions peak during the rest phase, while Bmal1 expression peaks during the dark phase, which is in alignment with the SCN.⁹² However, circadian oscillations in vascular cells are retained in mice devoid of light/dark cues.⁹² This suggests that, at least in part, peripheral clocks in vascular cells mediate cell function independent of the SCN.

Alterations to the molecular clock influence atherosclerosis in mice. For example, global Clock mutation in Apoε−/− and Ldlr−/− mice fed either a Western or chow diet accelerates atherosclerosis throughout the aorta.⁹³ Furthermore, augmented Cry1 expression or Rev-erbβ agonist delivery suppresses atherogenesis in Apoε−/− and Ldlr−/− mice, respectively.⁹³,⁹⁴ As discussed, these mouse models have significantly altered lipid metabolism, hematopoiesis, and inflammatory state all of which likely contribute to altered atherogenesis. Importantly however, a bone marrow cell–specific Clock mutation accelerates atherosclerosis in Apoε−/− mice.⁹⁷ Moreover, knockdown of Rev-erbβ in bone marrow cells does not alter systemic lipid levels but promotes atherosclerosis in Ldlr−/− mice, potentially by shifting macrophages toward a more inflammatory phenotype.⁹⁵ Most strikingly, aortae excised from wild-type mice do not develop transplant atherosclerosis when inserted into Bmal1−/− or Per1/2−/− mice.⁹⁶ Yet, when aortae from Bmal1−/− or Per1/2−/− mice are transplanted into WT mice, significant atherosclerosis develops in the transplanted graft.⁹⁶ These observations show that cell-intrinsic molecular clocks function locally in the vessel wall independently from SCN signaling or systemic factor rhythms.

Endothelial Cells
Dysfunction in the vascular endothelium initiates atherogenesis. Endothelial cells can be damaged and consequent-
hyperlipidemia. Endothelial cell activation leads to the expression of adhesion molecules, loss of barrier function, leukocyte migration into the vessel wall, and enhanced inflammatory responses. In mice, loss of Bmal1 in endothelial cells increases the expression of the chemokines Cxcl5, Ccl20, and Ccl8 and impairs endothelial integrity and barrier function, culminating in increased leukocyte trafficking across the endothelial layer.97 Moreover, endothelial cell expression of the adhesion molecules intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 is under circadian control.98,99 By binding to its enhancer element, Clock promotes intercellular adhesion molecule-1 expression in endothelial cells leading to increased adhesion and diapedesis of monocytes.98

Proper vasodynamics are essential to vascular health, and a loss of vascular tone can result in hypertension.100–102 Human endothelial function, as measured by flow-mediated dilation, is lower in the morning coinciding with increased risk of MI at this time.103–105 In cultured aortic rings, Clock mutation, Per1 mutation, or Bmal1 deletion attenuates endothelium-dependent vascular relaxation.103–106 These observations have been attributed to imbalanced production of the vasodilator nitric oxide and the vasoconstrictor coxyclooxygenase.106–108 The endothelial cells of Bmal1−/− mice have significantly blunted endothelial nitric oxide synthase activation and consequently reduced nitric oxide production and increased superoxides.104,105 Conversely, Per2 mutant mice have enhanced vasoconstriction caused by increased coxyclooxygenase expression.103 The regulation of blood pressure and blood flow dynamics by circadian and sleep/wake rhythms is complex. We guide readers to recent reviews discussing this topic at length.108–110

Coagulation cascade components are also under circadian influence in endothelial cells. For example, Bmal1 mediates the expression of the prothrombotic factors plasminogen activator inhibitor-1, fibrinogen, and von Willebrand factor in aortic endothelial cells.110–113 In addition, by binding to its enhancer element, the Clock:Bmal2 heterodimer promotes the expression of thrombomodulin, an endothelial membrane protein that activates protein C and inhibits coagulation.114 In agreement with these findings, blood’s coagulatory capacity is under circadian control and is influenced by the Clock:Bmal1 heterodimer.115,116 In mice, whole blood’s coagulability peaks early in the rest period (ZT 2) and early in the active period (ZT 14), whereas Bmal1 deletion abolishes this fluctuation and promotes a thrombogenic state.116 The circadian fluctuations in coagulation may contribute to the observation that thrombus formation in humans is most likely to occur in the morning.117,118

Vascular Smooth Muscle Cells

Vascular smooth muscle cells (VSMC) make up arteries’ medial layer and play a critical role in vascular tone. Deleting Bmal1 in VSMCs impairs vessel contractility, increases arterial lumen diameter and consequently, reduces mean arterial pressure.119 During atherosclerosis progression, VSMC proliferate and migrate from the media to the lumenal edge of the developing lesion to form a part of the fibrous cap. By secreting cellular products, VSMC mediate collagen production and extracellular matrix breakdown that enables cell migration. In addition to the molecular clock machinery, VSMCs display circadian expression patterns of tissue inhibitor of metalloproteinase 1 and 3 (timp1/3) and collagen 3α1 (coll3α1).118,119 Intriguingly, core molecular clock genes in VSMC isolated from human atherosclerotic lesions have dramatically impaired circadian rhythm compared with cells isolated from healthy regions of the artery.120 Specifically, VSMCs from disease-free arteries display robust peaks and troughs in Clock, Bmal1, Per2, Cry1, and Rev-erβb expression, whereas VSMCs derived from lesioned areas of the artery show flat, blunted expression patterns.120 Similarly, hyperglycemia and hyperlipidemia attenuate the circadian expression of core molecular clock genes in VSMC.121,122

Macrophages

Macrophages make up the cellular bulk of atherosclerotic lesions. As with endothelial cells and VSMC, circadian rhythms mediate macrophage function and up to 8% of the macrophage transcriptome fluctuates over 24 hours.123 This includes not only classic components of the core molecular clock but also key mediators of macrophage function.

In the diseased vessel wall, macrophages perpetuate atherosclerosis by secreting cytokines, chemokines, and other inflammatory mediators. Studies completed 50 years ago demonstrated that the magnitude of the inflammatory response to a pathogen depends on the time of day the animal is infected.49 Specifically, the cytokine storm in response to infection is highest if mice are infected at the beginning of their active period.53 Macrophage production of cytokines and chemokines, including interleukin (IL)-6, IL-12, IL-1β, tumor necrosis factor-α, GM-CSF (granulocyte-macrophage colony-stimulating factor), Cxcl11, and Ccl5, fluctuates in a circadian pattern and generally peaks at the end of the rest period (ZT 8–12).51,123,124 The molecular mechanisms regulating macrophages’ cytokine production remains unclear. Deleting Rev-erβb or Bmal1 in macrophages enhances cytokine production and ablates their rhythmic expression.51 Likewise, Clock mutant mice have higher circulating IL-12, IL-17, and G-CSF levels and altered macrophage nuclear factor-kB activation compared with wild-type control mice.79,125 Moreover, in macrophages, Rev-erβb mediates enhancer-derived RNAs that influence the expression of nearby genes including Mmp9 and Cx3cr1.126 In addition to core molecular clock machinery mediating inflammation directly, macrophages’ ability to sense danger-associated molecular patterns is also circadian. For example, the Clock:Bmal1 heterodimer binds to toll-like receptor-9’s promoter sequence, thereby mediating its rhythmic expression in leukocytes.127 Furthermore, several components of macrophage toll-like receptor-4 signaling are circadian, including toll-like receptor-4 dimerization and the activity and expression of its downstream targets Erk, Akt, and Mek1.91,123

The chemokine Ccl2 is critical to atherosclerosis. Upon binding to its receptor, Ccl2 promotes Ly-6Cε/ε monocyte mobilization and recruitment from the bone marrow to inflamed tissues in the periphery including atherosclerotic lesions.128–131 Deletion of Ccl2 in Apoe−/− mice abolishes Ly-6Cε/εCCR2+ monocyte recruitment to the vessel wall and attenuates atherosclerosis.132 Ccl2 expression and production in monocytes and macrophages have significant circadian rhythmicity that peaks early in the active phase.45,53,123 Deleting either Rev-erβb or Bmal1 in myeloid cells augments Ccl2 production, blunts its
rhythmic expression, and increases monocyte recruitment and accumulation in peripheral tissues. Mechanistically, Rev-erba seems to mediate Ccl2 expression by binding to and inhibiting its promoter. It is therefore conceivable that monocyte flux into the vessel wall fluctuates throughout the day and peaks with Ccl2 levels.

Once in the vessel wall, macrophages proliferate locally and phagocytose lipid particles, particularly modified ApoB-containing low-density lipoprotein. As disease worsens, macrophages fail to remove lipids and become lipid-engorged foam cells. Because several lipid types are cytotoxic, some lipid engorged macrophages undergo apoptosis resulting in an acellular and highly thrombotic necrotic core within the lesion. Macrophages isolated from Clock mutant mice have higher intracellular levels of total, free, and esterified cholesterol than macrophages from wild-type mice. This increased lipid load in Clock mutant macrophages could be due to increased lipid uptake or decreased lipid transport out of the cell. With regard to the first possibility, Clock mutant macrophages have higher expression of the scavenger receptors SRA and CD36 and phagocytose 2-fold more low-density lipoprotein than wild-type cells. Furthermore, Clock mutant Apoe−/− mice injected with photo-labeled acetylated low-density lipoprotein showed higher uptake into the aorta than Apoe−/− control mice. Reverse cholesterol transport is the mechanism by which macrophages expel esterified cholesterol for transport to the liver and excretion from the body. Clock mutant macrophages have reduced expression of the transporters ABCA1 and ABCG1 and a blunted ability to efflux cholesterol to ApoA1.79 Reverse cholesterol transport is the mechanism by which macrophages expel esterified cholesterol for transport to the liver and excretion from the body. Clock mutant macrophages have reduced expression of the transporters ABCA1 and ABCG1 and a blunted ability to efflux cholesterol to ApoA1.79

It is clear that circadian patterns play an important role in cardiovascular health (Figure 2). By influencing leukocyte and lipid supply, circadian rhythms affect systemic drivers of atherosclerosis. In addition, the molecular clock in endothelial cells, smooth muscle cells, and macrophages found in atherosclerotic lesions directs cell function and behavior. In this way, circadian patterns command both peripheral and local factors during lesion progression.

**Impact of Sleep on Inflammation and Metabolism**

Sleep is essential to our well-being and survival, yet many of us remain chronically sleep deprived. In the United States, 35% of adults get fewer than 7 hours of sleep per night, the minimum amount recommended by the National Sleep Foundation. Although temporary sleep deficiency impairs cognition, alertness, and performance, chronic sleep loss contributes to numerous adverse health outcomes including elevated blood pressure, atherosclerosis, stroke, and heart failure. Despite spending much of our lives asleep, the fundamental biological role of sleep and how it contributes to or protects from disease is not yet fully understood. Most studies analyzing the impact of sleep on disease have focused on individuals with sleep apnea. Sleep apnea affects 2% to 5% of the population and is characterized by repeated collapses on the upper airway during sleep resulting in stops and starts of breathing and frequent arousals. The complications associated with sleep apnea are often attributed to loss of blood oxygen and therefore the role of fragmented sleep is difficult to assess. In this review, we focus on studies conducted on individuals without clinically diagnosed sleep apnea.

Our knowledge of the relationship between sleep and lipid metabolism is incomplete. Sleeping <6 hours per night is associated with increased body mass index, impaired glucose metabolism, and diabetes mellitus, yet the association between sleep duration and circulating lipid levels is less clear. A recent study analyzed 263 lipid species in the plasma of healthy people undergoing 40 hours of sleep deprivation and observed that numerous lipid types (17.8%) increased with sleep deprivation, whereas many others (9.3%) decreased. Furthermore, although 1 study concluded that frequent sleep disruptions are associated with increased circulating triglyceride and cholesterol levels, other studies have found that these lipids are reduced in sleep-deprived individuals. In mice, 8 weeks of sleep fragmentation increase body weight because of elevated food intake and increased visceral and subcutaneous fat deposition. More work is needed to elucidate the relationship between sleep and peripheral lipid levels. For example, does sleep deprivation and fragmentation alter lipid absorption by enterocytes or biosynthesis by hepatocytes? If so, how does this alter levels in plasma?

Like disruptions in the molecular machinery of the central clock, sleep deprivation affects leukocytosis. Yet, research on this topic has produced inconsistent results, likely because of

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**Figure 2. Circadian influence on atherosclerosis.** Circadian rhythms influence the supply of leukocytes and lipids in the circulation and the behavior of endothelial cells, smooth muscle cells, and macrophages in the vessel wall. Together, these systemic factors and local cellular events mediate atherosclerosis. HSC indicates hematopoietic stem cell.
differences in methodologies, timing, and subjects. For example, human sleep deprivation has been shown to increase, decrease, or have no effect on circulating T cells, B cells, natural killer cells, and neutrophils. The data on sleep deprivation and monocytecytosis in humans are more consistent with most studies concluding that monocyte levels rise after partial or total sleep deprivation. The mechanisms by which sleep influences monocyte supply are unclear. Given that sleep deprivation increases SNS activity, does the SNS subsequently stimulate HSC expansion and CC2 production? Further research will have to probe the relationship between sleep and hematopoiesis.

The connection between cytokine levels and sleep is complex. Although sleep deprivation increases circulating inflammatory cytokine levels including IL-1β, IL-6, and tumor necrosis factor-α, increased cytokine levels may contribute to sleep initiation. For example, in healthy subjects, IL-1β and tumor necrosis factor-α levels rise immediately before sleep onset, and tumor necrosis factor-α-deficient mice have impaired and fragmented sleep patterns. Recent data suggest that when humans sleep, monocytes increase IL-12 production and decrease IL-10 production, suggesting a shift from an inflammatory to an anti-inflammatory state. Functionally, macrophages’ phagocytic potential and HSCs’ motility and homing capacity is limited by sleep curtailment. Sleep fragmentation in mice leads to monocyte infiltration and accumulation in peripheral tissues including adipose. Increased macrophage numbers are observed in the aortic wall of normolipemic mice after 20 weeks of sleep fragmentation. How sleep alters leukocyte behavior in atherogenesis has not been explored.

Conclusions

Sleep and circadian misalignment are understudied atherosclerosis risk factors. Despite recent advances, our understanding of how daily rhythms and sleep patterns influence cardiovascular pathology remains limited. The data reviewed here suggest that desynchronized circadian rhythms affect inflammation, metabolism, and atherosclerosis. Future studies will need to explore the biological mechanisms linking circadian oscillations to disease in order to identify novel processes. For example, if disturbing circadian fluctuations promote inflammation and dyslipidemia, does returning to a proper diurnal cycle and sleep pattern resolve these pathologies? What are the underlying mechanisms? Cell tracing studies will need to be performed to determine whether cellular events during lesion progression, such as macrophage proliferation, occur at specific times of the day. These studies may identify new pathways that could be targeted therapeutically to reduce the global burden of atherosclerosis.

Lack of sleep is an emerging public health concern. Because of increasing competitiveness in advanced societies, many people feel pressure to forgo sleep and continue to work or study late into the evening. More than 20% of Americans work ≥10 hours/wk at home outside of their regular work hours. This not only leads to lack of sleep but also has increased rates of mental illness. Organizations and governments are beginning to address this issue by promoting proper sleep and allowing employees to set their own schedules. Although we still have much to learn about the underlying biology, promoting a healthy lifestyle that includes sufficient, regular sleep should be an important consideration for public health policy.

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None.

References

19. Lamia KA, Sachdeva UM, D’Iacchio L, Williams EC, Alvarez IG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ, Thompson CB,


Smaldaas R, Laerum OD, Sothern RB, Sletvold O, Bjerknes L, Rote K. Colony-forming unit-granulocyte-macrophage and DNA synthesis of...


100. Smolensky MH. Circadian rhythms in blood pressure regulation and optimization of hypertension treatment with ACE inhibitor and ARB medications.


