Old and New Roles and Evolving Complexities of Cardiovascular Clocks

Yanyan Xu, Wenhu Pi, and R. D. Rudic*

Department of Pharmacology & Toxicology, Augusta University, Augusta, GA

The cardiovascular (CV†) system has been established to be significantly influenced by the molecular components of circadian rhythm. Oscillations of circadian rhythm occur within the circulation to affect thrombosis and blood pressure and within CV tissues including arteries, heart, and kidney to control function. Physiologic and molecular oscillations of circadian rhythm have been well connected via global, tissue-specific, and transgenic reporter mouse models of key core clock signals such as Bmal1, Period, and Clock, which can produce both pathology and protection with their mutation. With different nuances of CV clock action continuing to emerge in studies of the cardiovascular system, new questions are raised in both new and old mouse model system observations that underscore the importance, complexity, and continued study of the circadian clock mechanism in cardiovascular disease.

THE CIRCADIAN CLOCK

Anticipation and the ability to respond to unexpected stresses is an important aspect of survival at the organismal and cellular level. In biology, the circadian clock acts like a temporal receptor, receiving information regarding physiologic timing and adapting accordingly. This 24-hour sensor has adapted and evolved as a characteristic of the 24-hour earth rotation, which on average provides us with an oscillating light pattern, of 12 hours of light and 12 hours of darkness. The mechanism by which this light information is relayed to the brain is through the melanopsin photopigment [1] contained in retinal ganglion cells [2] and as melanopsin is a non-visual photopigment, it can also relay this information in blindness [3,4] though enucleation (eye removal) abolishes this ability [5], as this opsins is also localized within the eyes. Within the cells of land organisms, a unique set of genes/proteins receive this temporal information via a cascade of signals that are modified by the environmental (zeitge-
CIRCADIAN CLOCKS IN BLOOD VESSELS

Similar to the way SCN can receive input, the peripheral clocks themselves can also be entrained or modified by different molecular, mechanical, or metabolic signals. Peripheral clocks are present and regulate transcription of thousands of genes in different tissues, affecting multiple physiological functions, including the function of the vascular system [17].

It is well known that the fluctuations of blood pressure and heart rate show circadian rhythm. In addition, other cardiovascular outcomes such as acute myocardial infarction, cerebral infarction, stroke, and sudden cardiac death also tend to present a peak onset frequency in the early morning [18]. The evidence is compelling that circadian rhythm dysfunction contributes to many aspects of cardiovascular disease [19,20] and is becoming more appreciated as an important factor in improving both therapeutics [21] and general well-being. Mechanistic studies have proved informative in identifying a clear role of the circadian clock loop and its targets [22-24] in CV function, though gaps in knowledge remain. Indeed, new data has emerged that is uncovering unexpected aspects and complexities of the circadian clock in cardiovascular function and signaling.

The circadian clock is found in all layers of the vas-
The circadian clock is known to influence blood pressure. This has been shown from global Bmal1 disruption [41], tamoxifen induced disruption of Bmal1 [42], smooth muscle cell disruption of Bmal1 [43], Cry [44], and Per disruption in mice [45]. Given the importance of the heart and cardiac output in blood pressure regulation, it should also be stated numerous studies have demonstrated the key importance of the heart and more specifically the cardiomyocyte clock in heart metabolism and function [46-55]. Recent work now implicates Dec (deleted in esophageal cancer) transcription factors in blood pressure regulation. The positive limb of the core clock, Dec1 and Dec2 are bHLH transcription factors and also play a role in controlling circadian rhythms [56], albeit less well studied. Recent data has revealed that Dec1 KO mice exhibited decreased blood pressure, and mechanistically it was found that Dec1 suppressed expression of ATP1B1 which encodes the beta subunit Na+/K+-ATPase. This effect was through a heterodimeric partnership between Dec and Clock that bound the ATP1B1 promoter to inhibit ATP1B1 transcription. While Dec1/Clock was a repressor, the Bmal1/Clock heterodimer was an activator of ATP1B1 promoter activity. Such ascribed alterations in the dynamics of circadian clock heterodimerization with Dec1 that occur in the kidney likely also contribute to the altered ATP1B1 expression observed in the heart and vasculature in Dec1 KO mice [57]. Other work demonstrated the importance of other transporters/channels such as eNAC in blood pressure regulation [58] and alterations in clock oscillations in the hypertensive, high salt-challenged kidney [59]. There is also evidence that the renin-angiotensin pathway also contributes to circadian clock mediated blood pressure regulation [60], although the site of action (central or peripheral receptors) of AngII signaling in circadian blood pressure regulation has not been fully elucidated. One recent study approached this by administering angiotensin receptor blockers infused either in the brain or in the periphery in a hypertensive strain of mice at different times of day. In these studies, the ARBs exhibited greater effects to lower blood pressure when given at night in the BPH/2J mice, and this hypotensive effect was compara-
ble when ARBs were administered either via intracerebroventricular or subcutaneous routes of delivery, with the authors concluding that central angiotensin II receptor type I inhibition was not contributing to the hypertension in this strain of mice [61]. The db/db obese mouse has also been a valuable model to examine circadian rhythm in blood pressure in conditions of a genetically intact clock. In the obese and diabetic db/db mouse, blood pressure dipping is impaired [62], and recent studies identify numerous disruptions in peripheral clock oscillations. In these studies, the db/db mouse was crossed to the reporter PerLuc mice to generate an obese non-dipping BP mice with this circadian luciferase readout; the results showed that there were impaired rhythms in liver, kidney, and submandibular glands, but without interrupting SCN Per mutation, that there were impaired rhythms in liver, kidney, and submandibular glands, but without interrupting SCN Per rhythms [63].

Some interesting complexities remain with regard to blood pressure and the circadian clock. Like Bmal1-KO mice [64], the Dec1-KO [57] and Perl-KO mice [65] were also shown to have a lower blood pressure than WT mice, though Clock mutant mice in the Dec1 studies were shown to have a higher blood pressure than their WT counterparts [57]. That blood pressure is lower with circadian clock gene mutation is counter-intuitive. However, this has now been shown in multiple models. Most recently, disruption of Bmal1 in perivascular adipose tissue (PVAT) caused a super-dipper phenotype, via a lower blood pressure dip during the rest period, and mechanistically by a Bmal1 induced regulation of angiotensinogen specific to the PVAT [66]. Thus, circadian clock gene mutation can cause endothelial dysfunction [25,28], accelerated thrombosis [67,68], and vascular pathology [33,69-73] despite modest lowering of blood pressure. Studies in SMC Bmal1-KO mice may have shed some insight on this quandary. SMC Bmal1-KO mice were shown to exhibit lower blood pressure like the global knockout of Bmal1. The investigators parsed systolic and diastolic blood pressure data and found that the increment of diastolic pressure reduction was greater than that of systolic blood pressure resulting in an increase in the derived pulse pressure [43]. Thus, it may be that increased pulse pressure (which can be a predictor of vessel stiffening, and has been shown to occur in Bmal1-KO and Per-KO mice [31]) could explain the susceptibility to vascular disease in face of lowered blood pressure in the circadian clock knockout models. There are other mechanisms also likely at play in this paradox. Formation of Dec/Clock heterodimers to influence the core clock mechanism may effect kidney-controlled blood pressure and vascular function controlled remodeling differently. Moreover, there could be additional tissue specific bHLH’s that can modify signaling and CV responses. Additionally, there are bi-directional signals like Akt that have exhibited complex up or down regulation depending on tissue and circadian clock mutation, with data showing Akt is upregulated by Clock mutation [50], downregulated by Bmal1 mutation [27,74,75], and upregulated by Per mutation, while Akt also can act to feedback and regulate Bmal1 [76] and Clock phosphorylation [77], which could condition their ability to transactivate target genes. Indeed, more evidence is emerging regarding the good and bad of broken clocks.

Can a Broken Clock be a Good Thing?

More evidence has emerged that the absence of a functional clock may not always induce a bad outcome for CV health (in mice). Disruption of Bmal1 in vascular smooth muscle cells was protective in one study assessing experimentally induced aortic aneurysm. In those studies aneurysm induced by either an aldosterone infusion combined with a high salt diet or AngII infusion combined with hypercholesterolemia model robustly caused aneurysm in wild type mice, but Bmal1-KO mice did not develop robust aneurysms, potentially through an increase in TIMP-4 to suppress MMP activation and elastin breaks [78]. In a different set of studies using another model of cardiovascular insult, induced hypertension via high salt and mineralocorticoids, induced hypertension in wild type mice, but blood pressure in mice with Perl disruption was actually lower [65]. These studies were done in mice where clock genes were disrupted prenatally, but what about clock disruption postnatally? Recently, studies in which Bmal1 was disrupted in adult mice (tamoxifen-induced Bmal1 disruption) also demonstrated that mice with broken clocks are protected from jet-lag. In these studies, induced Bmal1 disruption in 3-month-old mice, facilitated adaptation to a range of light cycle perturbation models, resulting in improved adaptation with regard to central locomotor activity and peripheral metabolic homeostasis [79]. These observations in clock knockout mice suggest that lacking an oscillator may be protective by rendering the mice impervious to environmental disturbances of circadian rhythm. There may be additional complexities that involve differences between clock disruption or dysfunction pre-natally versus post-natally. In utero, clock genes are thought to not oscillate in the fetus [80,81]. That said, there are still “non-clock” fetal rhythms persisting in the fetus coordinated through the mother [82,83] (perhaps reminiscent of the way single cells pass clock time to daughter cells [84]). Work by Yang et al. identified phenotypic disparities between pre-natal and post-natal clock gene disruption in mice challenging “rhythm” roles in the progression of some pathologies [42]. In these studies, some phenotypes previously reported in global embryonic Bmal1 knockout mice were not recapitulated by inducible disruption of Bmal1 at 3 months (tamoxifen treatment in 3 month-old floxed Bmal1 mice) leading to Fitzgerald and colleagues to propose a circadian modification of the Barker hy-
pothesis [42,85], the latter hypothesis being that the fetal environment can impact future adult disease including hypertension [86]. That compensatory signaling occurs in knockout mice has long been a consideration and a challenge in identifying direct causation in genetic mouse models perhaps reflecting a molecular flow chart whose primary order is sustenance and life [87]. In the current context of clocks, it may be that embryonic Bmal1 disruption can: one, cause injury due to the inability to anticipate time appropriately to experimentally-induced circadian stresses; two, (the opposite) cause protection due to the ambivalence (via loss of a time sensor) to experimentally-induced circadian stresses; or three, cause injury or protection that is induced by compensatory changes in utero that persist through life and are independent of circadian rhythmicity. It is also interesting that the embryo and its clock, though not oscillating, can still be sensitive to circadian disturbances experienced maternally, sensitivities that become more evident with advancing age. In this recent work, a pregnant mother (gestational mouse) exposed to circadian stress produced offspring that developed heart and bone disease at 10 months of age, while the livers of younger 4-week old pups that were so-exposed while in utero exhibited a correlative change in epigenetic memory at a CV-related microRNA cluster that was hypermethylated [88]. These data demonstrated that pregnant mice exposed to experimental jet lag (“a fly East, lose sleep model”) have offspring (aged to 10 months) that exhibit increased cardiac disease as quantified by left ventricular hypertrophy. Despite this peripheral clock defect in the cardiovascular system, these offspring mice born and developing in conditions of prenatal stress were better able to adapt their central clock circadian timing in locomotor rhythms to jet-lag as adults (or advances), while (as expected) “a fly West, gain extra hours” was less hurtful to health and life as has also been shown in the mortality of aged mice [89].

CONCLUSION

Generally speaking, circadian rhythm disruption is likely not good for human or mouse health. That said, the current discussion underscores the complexity of clocks; there are circadian clock effects that appear to occur even independent of oscillation; there are clock perturbations in the fetus that can influence biology and disease into adulthood; a mutated genetic clock can be in certain models of disease protective and in other models of disease injurious. Another possibility is that these complexities reflect some divergence of the mouse models from the human condition. Perhaps subtle lowering of blood pressure in a mouse is not such a significant influence to disease in a mouse, or another possibility is that blood pressure and coincident endothelial dysfunction are disconnected in the mouse models or even disconnected altogether. Many of the disparities are observed in models of mice where the clock is genetically mutated. And, while there are human mutations in clocks that may relate to the mouse models [90], non-genomic dysfunctions of circadian clocks and rhythms that arise in the timing of drug dosing, social jet lag, shift work, and sleep disorders are important to be studied in both the genetic mouse models of mutation and in conditions of sleep deprivation and light cycle alteration that may yield more insight into the complexities of the circadian clock in cardiovascular disease. Given these complexities there are certain things that are well established:

- Circadian clocks oscillate after birth.
- Circadian clocks regulate downstream signals, (whether those target signals oscillate or not).
- Circadian clocks can sense changes in timing.

Given the significance of temporal routines (or lack thereof in human life), the science of clock signaling remains a very important and complicated biology to understand, but one that is certain to provide new clues into cardiovascular health.

REFERENCES

1. Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM, et al. Melanopsin is required for non-image-forming photic responses in blind mice. Science. 2003;301(5632):525-7.
2. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. Science. 2002;295(5557):1070-3.
3. Brown RL, Robinson PR. Melanopsin--shedding light on the elusive circadian photopigment. Chronobiology International. 2004;21(2):189-204.
4. Freedman MS, Lucas RJ, Soni B, von Schantz M, Munoz M, David-Gray Z, et al. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science. 1999;284(5413):502-4.
5. Freedman MS, Lucas RJ, Soni B, von Schantz M, Muñoz M, David-Gray Z, et al. Regulation of Mammalian Circadian Behavior by Non-rod, Non-cone, Ocular Photoreceptors. Science. 1999;284(5413):502-4.
6. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogeness JB. A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A. 2014;111(45):16219-24.
7. Duffy JF, Czeisler CA. Effect of light on human circadian physiology. Sleep Medicine Clinics. 2009;4(2):165-77.
8. Ko ML., Shi L, Tsai JY, Young ME, Neuendorff N, Earnest DJ, et al. Cardiac-specific mutation of Clock alters the quantitative measurements of physical activities without changing behavioral circadian rhythms. J Biol Rhythms. 2011;26(5):412-22.
9. Westgate Elizabeth J, Cheng Y, Reilly Dermot F, Price Tom...
S, Walisser Jacqueline A, Bradfield Christopher A, et al. Genetic components of the circadian clock regulate thrombogenesis in vivo. Circulation. 2008;117(16):2087-95.

10. Duong ATH, Reitz CJ, Louth EL, Creighton SD, Rasouli M, Zwaian A, et al. The Clock Mechanism Influences Neurobiology and Adaptations to Heart Failure in Clock(19/19) Mice With Implications for Circadian Medicne. 2019;9(1):4994.

11. Rudic RD. Time is of the essence: vascular implications of the circadian clock. Circulation. 2009;120(17):1714-21.

12. McNamara P, Seo SB, 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(7):877-89.

13. Callaway E, Ledford H. Medicine Nobel awarded for work on circadian clocks. Nature. 2017;550(7674):18.

14. Klarsfeld A, Birman S, Rouyer F. [Nobel time for the circa
dian clock - Nobel Prize in Medicine 2017: Jeffrey C. Hall, Michael Rosbash and Michael W. Young]. Med Sci (Paris). 2018;34(5):480-4.

15. Dunlap JC. Molecular bases for circadian clocks. Cell. 1999;99(2):271-90.

16. Aryal RP, Kwat PB, Tamayo AG, Gebert M, Chiu PL, Walz T, et al. Macromolecular Assemblies of the Mamma
dian Circadian Clock. Molecular Cell. 2017;67(5):770-9.

17. Takeda N, Maemura K. Circadian clock - Nobel Prize in Medicine 2017: Jeffrey C. Hall, Michael Rosbash and Michael W. Young. [Nobel time for the circa
dian clock - Nobel Prize in Medicine 2017: Jeffrey C. Hall, Michael Rosbash and Michael W. Young]. Med Sci (Paris). 2017;34(5):473-81.

18. White WB. The risk of waking-up: impact of the morning
surge in blood pressure. Hypertension. 2010;55(4):835-7.

19. Cheng B, Anea CB, Yao L, Chen F, Patel V, Merloiu A, et al. Tissue-intrinsic dysfunction of circadian clock confers transplant arteriosclerosis. Proc Natl Acad Sci U S A. 2011;108(41):17147-52.

20. Crnko S, Du Pre BC, Sluijer JPG, Van Laake LW. Circadi
yan rhythms and the molecular clock in cardiovascular biology and disease. Nat Rev Cardiology. 2019.

21. Mistry P, Duong A, Kirshenbaum L, Martino TA. Car
diac Clocks and Preclinical Translation. Heart Fail Clin. 2017;13(4):657-72.

22. Paschos GK, Fitzgerald GA. Circadian clocks and vascular function. Circ Res. 2010;106(5):833-41.

23. Crnko S, Cour M, Van Laake LW, Lecour S. Vasculature on the clock: Circadian rhythm and vascular dysfunction. Vascular Pharmacol. 2018;108:1-7.

24. Rudic RD. Time Is of the Essence Vascular Implications of the Circadian Clock. Circulation. 2009;120(17):1714-21.

25. Anea CB, Morin AM, Fulton DJR, Patel V, Rudic RD. Immunohistochemistry of the circadian clock in mouse and human vascular tissues. Vessel Plus. 2018;2.

26. 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(5):473-81.

27. Anea CB, Zhang M, Stepp DW, Simkins GB, Reed G, Fulton DJ, et al. Vascular disease in mice with a dysfunctional circadian clock. Circulation. 2009;119(11):1510-7.

28. Viswambharan H, Carvas JM, Antic V, Marecic A, Jud C, Zaugg CE, et al. Mutation of the circadian clock gene Per2 alters vascular endothelial function. Circulation. 2007;115(16):2188-95.

29. Anea CB, Cheng B, Sharma S, Kumar S, Caldwell RW, Yao L, et al. Increased Superoxide and Endothelial NO Synthase Uncoupling in Blood Vessels of Bmal1-Knockout Mice. Circulation Res. 2012;111(9):1157-65.

30. Shang X, Pari P, Anea CB, Fulton DJ, Rudic RD. Differential Regulation of Bmal1, Clock, and Endothelial Signaling in the Aortic Arch and Ligated Common Carotid Artery. J Vasc Res. 2016;53(5-6):269-78.

31. Anea CB, Ali MI, Osmond JM, Sullivan JC, Stepp DW, Merloiu AM, et al. Matrix metalloproteinase 2 and 9 dysfunction underlie vascular stiffness in circa
dian clock mutant mice. Arterioscler Thromb Vasc Biol. 2010;30(12):2535-43.

32. Hemmeryckx B, Van Hove CE, Fransen P, Emmerechts J, Kauskot A, Bult H, et al. Progression of the prothrombotic state in aging Bmal1-deficient mice. Arterioscler Thromb Vasc Biol. 2011;31(11):2552-9.

33. Bhawadakar AD, Beli E, Diao Y, Chen J, Luo Q, Alex A, et al. Conditional Deletion of Bmal1 Accentuates Microvascular and Macrovascular Injury. Am J Pathol. 2017;186(6):1426-35.

34. Nakazato R, Kawabe K, Yamada D, Ikeno S, Mieda M. Disruption of Bmal1 Impairs Blood-Brain Barrier Integrity via Pericyte Dysfunction. J Neurosci. 2017;37(42):10052-62.

35. Zhang SL, Yue Z, Arnold DM, Artiushin G, Sehgal A. A Circadian Clock in the Blood-Brain Barrier Regulates Xenobiotic Efflux. Cell. 2018;173(1):130-9.e10.

36. Phelan P, Bacon JP, A Davies J, Stebbings LA, Todman MG. Innexins: a family of invertebrate gap-junction pro
tems. Trends in Genetics. 1998;14(9):348-9.

37. Rudic RD, McNamara P, Reilly D, Grosser T, Curtis A-M, Price TS, et al. Bioinformatic Analysis of Cir
cadian Gene Oscillation in Mouse Aorta. Circulation. 2005;112(17):2716-24.

38. Yang L, Zhang Y, Ma Y, Du J, Gu L, Zheng L, et al. Effect of Melatonin on EGF- and VEGF-induced monolayer permeability of HUVECs. Am J Physiol Heart Circ Physiol. 2018.

39. Baker J, Kimpinski K. Role of melatonin in blood pressure regulation: An adjunct anti-hypertensive agent. Clin Exp Pharmacol Physiol. 2018;45(8):755-66.

40. Zeman M, Herichova I. Melatonin and clock genes expres
sion in the cardiovascular system. Front Biosci (Schol Ed). 2013;5:743-53.

41. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitz
gerald GA. Circadian variation of blood pressure and the vascular response to asynchronous stress. Proc Natl Acad Sci U S A. 2007;104(9):3450-5.

42. Walisser Jacqueline A, Bradfield Christopher A, et al. Genetic components of the circadian clock regulate thrombogenesis in vivo. Circulation. 2008;117(16):2087-95.
duced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in Cry-deficient mice lacking a biological clock. J Physiol. 2005;566(Pt 1):213-24.

45. Stow LR, Richards J, Cheng KY, Lynch IJ, Jeffers LA, Greenlee MM, et al. The circadian protein period 1 contributes to blood pressure control and coordinately regulates renal sodium transport genes. Hypertension. 2012;59(6):1151-6.

46. Young ME, Razeghi P, Taegtmeyer H. Clock genes in the heart: characterization and attenuation with hypertrophy. Circulation Res. 2001;88(11):1142-50. Epub 2001/06/09. PubMed PMID: 11397780.

47. Chatham JC, Young ME. Regulation of myocardial metabolism by the cardiomyocyte circadian clock. J Mol Cell Cardiol. 2013;55:139-46.

48. He L, Hamm JA, Reddy A, Sams D, Peliciari-Garcia RA, McGinnis GR, et al. Biotinylation: a novel posttranslational modification linking cell autonomous circadian clocks with metabolism. Am J Physiol Heart Circ Physiol. 2016;310(11):H1520-32.

49. Martino TA, Young ME. Influence of the cardiomyocyte circadian clock on cardiac physiology and pathophysiology. J Biol Rhythms. 2015;30(3):183-205.

50. McGinnis GR, Tang Y, Brewer RA, Brahma MK, Stanley HL, Shannugam G, et al. Genetic disruption of the cardiomyocyte circadian clock differentially influences insulin-mediated processes in the heart. J Mol Cell Cardiol. 2017;110:80-95.

51. McGinnis GR, Young ME. Circadian regulation of metabolic homeostasis: causes and consequences. Nat Sci Sleep. 2016;8:163-80.

52. Peliciari-Garcia RA, Previde RM, Nunes MT, Young ME. Interrelationship between 3,5,3'-triiodothyronine and the circadian clock in the rodent heart. Chronobiol Int. 2016;33(10):1444-54.

53. Young ME. Circadian Control of Cardiac Metabolism: Physiologic Roles and Pathologic Implications. Methodist Debakey Cardiovasc J. 2017;13(1):15-9.

54. Young ME, Brewer RA, Peliciari-Garcia RA, Collins HE, He L, Birky TL, et al. Cardiomyocyte-specific Bmal1 plays critical roles in metabolism, signaling, and maintenance of contractile function of the heart. J Biol Rhythms. 2014;29(4):257-76.

55. Young ME, Peliciari-Garcia RA, Goel M, Aristorenas JA, Shah K, He L, et al. Altered myocardial metabolic adaptation to increased fatty acid availability in cardiomyocyte-specific Clock mutant mice. Am J Physiol Heart Circ Physiol. 2016;1861(10):1579-95.

56. Homna S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, et al. Dec1 and Dec2 are regulators of the mammalian molecular clock. Nature. 2002;419(6909):841-4.

57. Nakashima A, Kawamoto T, Noshiro M, Ueno T, Doi S, Honda K, et al. Dec1 and CLOCK Regulate Na(+)/K(+)-ATPase beta1 Subunit Expression and Blood Pressure. Hypertension. 2018;72(3):746-54.

58. Gumz ML, Stow LR, Lynch IJ, Greenlee MM, Rudin A, Cain BD, et al. The circadian clock protein Period 1 regulates expression of the renal epithelial sodium channel in mice. The Journal of clinical investigation. 2009;119(8):2423-34.

59. Speed JS, Hyndman KA. High dietary sodium causes dysynchrony of the renal molecular clock in rats. Am J Physiol Renal Physiol. 2018;314(1):F89-F98.

60. Pati P, Fulton DJ, Bagi Z, Chen F, Wang Y, Kitchens J, et al. Low-Salt Diet and Circadian Dysfunction Synergize to Induce Angiotensin II-Dependent Hypertension in Mice. Hypertension. 2016;67(3):661-8.

61. Jackson KL, Marques FZ, Lim K, Davern PJ, Head GA. Circadian Differences in the Contribution of the Brain Renin-Angiotensin System in Genetically Hypertensive Mice. Front Physiol. 2018;9:231.

62. Su W, Guo Z, Randall DC, Cassis L, Brown DR, Gong MC. Hypertension and disrupted blood pressure circadian rhythm in type 2 diabetic db/db mice. Am J Physiol Heart Circ Physiol. 2008;295(4):H1634-41.

63. Hou T, Su W, Guo Z, Gong MC. A Novel Diabetic Mouse Model for Real-Time Monitoring of Clock Gene Oscillation and Blood Pressure Circadian Rhythm. J Biol Rhythms. 2019;34(1):51-68.

64. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitzgerald GA. Circadian variation of blood pressure and the vascular response to asynchronous stress. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(9):3450-5.

65. Alli AA, Yu L, Holzworth MR, Richards J, Cheng KY, Lynch IJ, et al. Direct and Indirect Inhibition of the Circadian Clock Protein PER1: Effects on ENaC and Blood Pressure. Am J Physiol Renal Physiol. 2019.

66. Chang L, Xiong W, Zhao X, Fan Y, Guo Y, Garcia-Barrio M, et al. Bmal1 in Perivascular Adipose Tissue Regulates Resting-Phase Blood Pressure Through Transcriptional Regulation of Angiotensinogen. Circulation. 2018;138(1):67-79.

67. Hemmeryckx B, Frederix L, Lijnen HR. Deficiency of Bmal1 disrupts the diurnal rhythm of haemostasis. Exp Gerontol. 2019;118:1-8.

68. Westgate EJ, Cheng Y, Reilly DF, Price TS, Walisser JA, Bradford CA, et al. Genetic components of the circadian clock regulate thrombogenesis in vivo. Circulation. 2008;117(16):2087-95.

69. Hao M, Huang Y, Qu D, Zhang H, Wong WT, Chawla A, et al. Myeloid Bmal1 deletion increases monocyte recruitment and worsens atherosclerosis. Faseb J. 2017;31(3):1097-106.

70. McAlpine CS, Swirski FK. Circadian Influence on Metabolism and Inflammation in Atherosclerosis. Circ Res. 2016;119(1):131-41.

71. Pan X, Jiang XC, Hussain MM. Impaired cholesterol metabolism and enhanced atherosclerosis in clock mutant mice. Circulation. 2013;128(16):1758-69.

72. Yang L, Chu Y, Wang L, Wang Y, Zhao X, He W, et al. Overexpression of CRY1 protects against the development of atherosclerosis via the TLR/NF-kappaB pathway. Int Immunopharmacol. 2015;28(1):525-30.

73. Somnath PR, Podrez EA, Chen J, Ma Y, Marchant K, Antoch M, et al. Deficiency in core circadian protein Bmal1 is associated with a prothrombotic and vascular phenotype. J Cell Physiol. 2011;226(1):132-40.

74. Zhang D, Tong X, Arthurs B, Guha A, Rui L, Kamath A,
et al. Liver clock protein BMAL1 promotes de novo lipo-
genesis through insulin-mTORC2-AKT signaling. J Biol
Chem. 2014;289(37):25925-35.
75. Chen Y, Zhu D, Yuan J, Han Z, Wang Y, Qian Z, et al. 
CLOCK-BMAL1 regulate the cardiac L-type calcium 
channel subunit CACNA1C through PI3K-Akt signaling 
pathway. Can J Physiol Pharmacol. 2016;94(9):1023-32.
76. Dang F, Sun X, Ma X, Wu R, Zhang D, Chen Y, et al. Insu-
lin post-transcriptionally modulates Bmal1 protein to affect 
the hepatic circadian clock. Nat Commun. 2016;7:12696.
77. Luciano AK, Zhou W, Santana JM, Kyriakides C, 
Velazquez H, Sessa WC. CLOCK phosphorylation by 
AKT regulates its nuclear accumulation and circadi-
an gene expression in peripheral tissues. J Biol Chem. 
2018;293(23):9126-36.
78. Lutshumba J, Liu S, Zhong Y, Hou T, Daugherty A, Lu H, 
et al. Deletion of BMAL1 in Smooth Muscle Cells Protects 
Mice From Abdominal Aortic Aneurysms. Arterioscler 
Thromb Vasc Biol. 2018;38(5):1063-75.
79. Yang G, Chen L, Zhang J, Ren B, FitzGerald GA. Bmal1 
deletion in mice facilitates adaptation to disrupted light/ 
dark conditions. JCI Insight. 2019;5.
80. Li X, Davis FC. Developmental expression of clock genes 
in the Syrian hamster. Developmental Brain Research. 
2005;158(1):31-40.
81. Sládek M, Jindráková Z, Bendová Z, Sumová A. Postna-
tal ontogenesis of the circadian clock within the rat liver. 
American Journal of Physiology-Regulatory, Integrative 
and Comparative Physiology. 2007;292(3):R1224-R9.
82. Reppert SM, Schwartz WJ. Maternal coordina-
tion of the fetal biological clock in utero. Science. 
1983;220(4600):969-71.
83. Seron-Ferre M, Valenzuela GJ, Torres-Farfan C. Circa-
dian clocks during embryonic and fetal development. 
Birth Defects Research Part C: Embryo Today: Reviews. 
2007;81(3):204-14.
84. Nagoshi E, Saini C, Bauer C, Laroche T, Naef F, Schibler 
U. Circadian gene expression in individual fibroblasts: 
cell-autonomous and self-sustained oscillators pass time to 
daughter cells. Cell. 2004;119(5):693-705.
85. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and 
placental size and risk of hypertension in adult life. BMJ. 
1990;301(6746):259-62.
86. Barker DJ. The fetal and infant origins of adult disease. 
BMJ (Clinical research ed.). 1990;301(6761):1111-
87. Augustine K, Liu ET, Sadler TW. Antisense attenuation of 
Wnt-1 and Wnt-3a expression in whole embryo culture reveals roles for these genes in craniofacial, spinal cord, and 
cardiac morphogenesis. Dev Genet. 1993;14(6):500-20.
88. Chaves I, van der Eerden B, Boers R, Boers J, Streng AA, 
Ridwan Y, et al. Gestational jet lag predisposes to later-life skeletal and cardiac disease. Chronobiology International. 
2019;36(5):657-71.
89. Davidson AJ, Sellix MT, Daniel J, Yamazaki S, Menaker 
M, Block GD. Chronic jet-lag increases mortality in aged 
mice. Current Biology. 2006;16(21):R914-R6.
90. Patke A, Murphy PJ, Onat OE, Krieger AC, Ozcelik T, 
Campbell SS, et al. Mutation of the Human Circadian 
Clock Gene CRY1 in Familial Delayed Sleep Phase Disor-
der. Cell. 2017;169(2):203-15.e13.