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DOCP-Salt Treatment Leads to Non-dipping Hypertension in *Per1* Knockout Mice

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Abstract

Aim—Increasing evidence demonstrates that circadian clock proteins are important regulators of physiological functions including blood pressure. An established risk factor for developing cardiovascular disease is the absence of a blood pressure dip during the inactive period. The goal of the present study was to determine the effects of a high salt diet plus mineralocorticoid on *PER1*-mediated blood pressure regulation in a salt-resistant, normotensive mouse model, C57BL/6J.

Methods—Blood pressure was measured using radiotelemetry. After control diet, wild type and *Per1* knockout mice were given a high salt diet (4% NaCl) and the long-acting mineralocorticoid deoxycorticosterone pivalate. Blood pressure and activity rhythms were analyzed to evaluate changes over time.

Results—Blood pressure in wild type mice was not affected by a high salt diet plus mineralocorticoid. In contrast, *Per1* knockout mice exhibited significantly increased mean arterial pressure in response to a high salt diet plus mineralocorticoid. The inactive/active phase ratio of mean arterial pressure in wild type mice was unchanged by high salt plus mineralocorticoid treatment. Importantly, this treatment caused *Per1* knockout mice to lose the expected decrease or “dip” in blood pressure during the inactive compared to the active phase.

Conclusion—Loss of *PER1* increased sensitivity to the high salt plus mineralocorticoid treatment. It also resulted in a non-dipper phenotype in this model of salt-sensitive hypertension and provides a unique model of non-dipping. Together these data support an important role for the circadian clock protein *PER1* in the modulation of blood pressure in a high salt/mineralocorticoid model of hypertension.

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Conflicts of Interest

None.

Keywords

blood pressure; circadian rhythm; salt sensitivity; telemetry

Introduction

The circadian (Latin for “around a day”) clock functions to synchronize our daily activities, on a physiological and molecular level, with the time of day and certain behaviors that usually occur around the same time each day (Richards and Gumz, 2012). Many physiological functions exhibit circadian rhythms, including blood pressure (BP). Healthy individuals experience a 10–20% decrease in BP at night. Individuals who do not exhibit this “dip” in resting BP are termed “non-dippers.” Non-dipping is associated with an increased risk of chronic kidney disease (Hermida et al., 2010, Kario and Shimada, 2004), adverse cardiovascular events (Ohkubo et al., 2002), (Liu et al., 2003) and can also be used to predict future cardiovascular events (Fagard et al., 2009). These risks are further increased by chronic hypertension, or high BP, which is also a risk factor for cardiovascular disease (CVD). CVD is the leading cause of death of Americans (Go et al., 2014), accounting for nearly 1 million deaths/year (Mozaffarian et al., 2015). A subset of hypertensive patients exhibit salt-sensitive hypertension in which BP is abnormally elevated after high salt intake, implicating aberrant sodium handling in the pathogenesis of the disease. The underlying mechanisms of these pathologies remain incompletely understood.

At a basic level, the molecular clock is composed of four core genes which encode for BMAL1, CLOCK, CRYPTOCHROME (CRY, homologs 1 and 2) and PERIOD (PER, homologs 1, 2 and 3) proteins (for review of this complex mechanism (Partch et al., 2014)). These four proteins form a Transcription-Translation-Oscillating Loop, with BMAL1:CLOCK heterodimers positively regulating transcription of target genes and thus forming the positive arm of the loop. CRY:PER heterodimers make up the negative arm of the loop due to their antagonism of BMAL1:CLOCK heterodimers bound at target gene E-box response elements and subsequent repression of transcription. A central clock located in the suprachiasmatic nucleus of the brain is entrained by light signals from the retina (Mills and Stanbury, 1952). These signals are then relayed through neuronal and humoral pathways to peripheral clocks located in other tissues and organs of the body.

The importance of circadian proteins for BP control is illustrated by knockout (KO) mice. Every clock gene KO mouse that has been tested exhibits a BP phenotype (Richards et al., 2014a). Global *Bmal1* KO mice lose circadian rhythmicity of BP and are hypotensive (Curtis et al., 2007). Loss of *Bmal1* in renin-producing cells in the kidney also leads to a significant decrease in BP compared to controls (Tokonami et al., 2014). Loss of *Clock* causes mice to become hypotensive yet retain the circadian rhythm of BP (Zuber et al., 2009). *Cry1/Cry2* global KO mice exhibit salt-sensitive hypertension (Saifur Rohman et al., 2005), (Doi et al., 2010). Mice lacking all three *Period* isoforms lose their BP dip on a low salt diet (Pati et al., 2016). Thus, the clock is a major regulator of baseline BP and rhythmic BP.

The kidney is a critical regulator of BP and many aspects of renal function oscillate in a circadian manner including sodium excretion, renal blood flow (Pons et al., 1996) and glomerular filtration rate (Koopman et al., 1989). Aldosterone, a critical regulator of renal sodium handling, is also released in a circadian manner (Leliavski et al., 2015). Gumz et al. identified *Per1* as a novel aldosterone target gene in renal collecting ducts cells (Gumz et al., 2003). Circadian clock-mediated regulation of a renal sodium transporter was first reported by Okamura and colleagues who demonstrated that the Na⁺,H⁺ exchanger NHE3 exhibited time-dependent changes in expression (Saifur Rohman et al., 2005). Gumz et al. provided the link between the clock and the epithelial sodium channel ENaC with the finding that PER1 transcriptionally regulates αENaC (Gumz et al., 2009). Subsequent studies provided further evidence that PER1 coordinately regulates several genes encoding proteins that function in renal sodium reabsorption (Solocinski et al., 2015, Richards et al., 2014c, Richards et al., 2014d). Global loss of *Per1* on the hypertensive, salt-sensitive 129/sv mouse strain resulted in significantly reduced BP compared to wild type (WT) controls on a normal diet (Stow et al., 2012).

The goal of the present study was to characterize the role of PER1 in the normotensive C57BL/6J background strain. Small but significant differences in mean arterial pressure (MAP) were observed between WT and PER1 KO mice at baseline. Since *Per1* is an aldosterone target gene and coordinately regulates many renal sodium transport genes, we subjected WT and *Per1* KO mice to a high salt (HS) diet in combination with desoxycorticosterone pivalate (DOCP) injection as a model for evaluating salt-sensitive changes in BP. DOCP is a long-acting aldosterone analog that bypasses the sodium-excreting effects of Renin-Angiotensin-Aldosterone System (RAAS) suppression normally seen with high salt intake (reviewed in (Atlas, 2007)) and leads to increased renal sodium reabsorption. Interestingly, loss of PER1 in C57BL/6 mice subjected to HS/DOCP treatment resulted in significantly elevated BP accompanied by disruption of the normal BP rhythm. Thus, PER1 appears to be an important regulator of baseline and rhythmic BP in C57BL/6J mice.

Methods

Mice

Male WT C57BL/6J mice and C57BL/6J *Per1* KO between the ages of 14–18 weeks were used for these experiments (original heterozygous mice were the kind gift of Dr. Shin Yamazaki, Vanderbilt (Pendergast et al., 2009)). WT and *Per1* KO mice were litter mates derived from mating mice heterozygous for *Per1*. Mice were maintained on a normal 12:12 L:D cycle at the NF/SG VA Healthcare animal facility. Experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with the approval of UF and VA Medical Center IACUCs. Mice were implanted with telemetry devices from Data Sciences International through the carotid artery and extended into the aortic arch following established protocol (Butz and Davissou, 2001) and as described previously (Stow et al., 2012). Mice were allowed at least seven days to recover. Mice were then placed on control diet (0.2% NaCl, Harlan) for at least 3 days, during which time baseline BP recordings were made. Mice were then placed on a high salt (4% NaCl, Harlan)

diet for 3 more days, followed by injection with 75 µg/g bw DOCP. Three days after the injection and after six total days on a high salt diet, telemetry recordings for the HS/DOCP period were made.

Tissue dissection and quantitative real time RT-PCR (qPCR)

Tissue dissection and QPCR were performed as described previously in (Solocinski et al., 2015).

Flame Photometry

Flame photometry was used to determine urine and plasma Na and K concentrations.

Aldosterone ELISA

Plasma aldosterone was measured by ELISA as described in (Richards et al., 2013a).

Statistics

Day values are the 3-day averages of MAP between 6am (ZT0) through 5pm (ZT11). Night values are the 3-day averages of MAP recorded between 6pm (ZT12) and 5am (ZT23). Paired t-tests and standard and repeated measures 2-way ANOVAs were performed as appropriate using GraphPad Prism software (version 6.07 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). Cosinor analysis was done using ClockLab software (Actimetrics) and the generous help of Dr. Joshua Speed (University of Alabama Birmingham). $p < 0.05$ was considered significant. JTK Cycle was used as described (Wu et al., 2016). The program cosinor was downloaded from www.Circadian.org (last accessed 09/01/2016). Values are shown as mean $\pm$ SEM.

Results

Given our previous findings that *Per1* regulates a number of genes related to renal sodium reabsorption in vitro and in vivo in hypertensive 129/sv mice, we investigated the expression of candidate genes in WT normotensive C57BL/6 mice compared to *Per1* KO mice. Real time quantitative PCR (QPCR) was used to assess relative differences in gene expression in the renal cortex between WT and *Per1* KO mice at two distinct time points in the circadian cycle. Kidneys were collected at noon and midnight in order to capture time points at the midpoint of the rest phase and the active phase, respectively. We investigated time and *PER1*-dependent changes in steady state mRNA expression for genes known to contribute to renal sodium reabsorption or the regulation of this process. The Angiotensin II receptor (AT_1R) leads to increased sodium reabsorption and BP through the RAAS and has positive effects on ENaC (Crowley and Coffman, 2012) and expression displays a significant change over time due to the interaction of time and genotype ($p = 0.0075$) (Figure 1A). UBE2E3, a ubiquitin E3 ligase, has been previously shown to regulate the alpha subunit of the epithelial sodium channel ($\alpha ENaC$) (Debonneville and Staub, 2004) and is regulated by *PER1* (Richards et al., 2014c). As shown in Figure 1B, there was a significant effect of time on UBE2E3 expression ($p = 0.015$) with a trend towards a genotype effect as well ($p = 0.065$). Nitric oxide synthase 1 (NOS1) also regulates $\alpha ENaC$ (Hyndman et al., 2015) (Figure 1C) and trended towards an interaction between genotype and time. With-no-lysine kinase 1

(WNK1) is a regulator of the sodium chloride cotransporter (Chavez-Canales et al., 2014) and trended towards a genotype-time interaction as well (Figure 1D). Figure 1E shows that α ENaC expression changed significantly with time ($p=0.024$) and a possible trend for an interaction between time and genotype was observed ($p=0.12$) (Figure 1E). In contrast to these changes, PCSK9, an ENaC-regulating protease, did not undergo any changes in expression relative to time or *Per1* KO (Supplemental Figure 1A). Likewise, mRNA levels for the Endothelin A Receptor, a regulator of renal sodium balance and blood pressure, did not change with time or *Per1* KO (Supplemental Figure 1B). Therefore, *Per1* appears to selectively regulate only certain genes which govern sodium transport and reabsorption.

Given our previous findings that *PER1* acts as a coordinate regulator of several genes in the kidney that participate in the regulation of renal sodium reabsorption, we hypothesized that *Per1* KO mice would exhibit altered BP. Due to the altered gene expression patterns observed in Figure 1, we compared MAP between WT and KO mice under baseline conditions. The mice were implanted with telemetry devices and after recovery MAP was monitored over a 3 day period. During the day when mice are inactive, WT mice have a MAP of 102.1 ± 1.7 mmHg which increases to 112.5 ± 1.08 mmHg during the night when they are active, an increase of 10.4 ± 1.5 mmHg (Figure 2A). *Per1* KO mice exhibit an inactive phase MAP of 103 ± 1.4 mmHg which rises to $119.8 \pm .9$ during the active phase. This is a change of 16.8 ± 1.7 mmHg which is significantly higher than the inactive/active phase difference observed in WT mice ($p < 0.05$). In Figure 2B, hourly MAP data are shown. The 12 hour period of the inactive phase is indicated by the white areas between Zeitgeber Time 0 (ZT0, “lights on”) and ZT12. The active phase is indicated by the shaded areas between ZT12 and ZT 0. The dip in MAP during the inactive phase is easily visualized, as well as the slightly higher MAP in the *Per1* KO mice seen during the dark/active period. Under these baseline conditions, significant differences between WT and *Per1* KO mice in terms of plasma aldosterone levels, plasma Na or K, or body weight were not observed (Table 1).

Given the subtle but significant differences in MAP between WT and *Per1* KO mice under control conditions, we examined the response of both groups to an established model of salt-sensitivity: high salt diet plus mineralocorticoid treatment. Following the baseline recordings presented in Figure 2, the same mice were given a high salt diet (4% NaCl) in addition to injection of DOCP. Figure 3A shows day and night averages taken the same way as described in Figure 2. WT mice exhibit a significant inactive/active phase difference in BP with an increase from 101.5 ± 1.92 mmHg during the inactive phase to 114.5 ± 1.1 mmHg during the active phase. This is a 13 ± 2.2 mmHg increase which is not different from the inactive/active difference in BP of WT mice observed under control conditions. In contrast, *Per1* KO mice exhibited an inactive phase average MAP of 118.1 ± 1 mmHg that increased to 125.3 ± 1.5 mmHg during the active phase. This is a difference of only 7.2 ± 1.8 mmHg, which is significantly different from the WT and, importantly, classifies these mice as non-dippers. Panel B shows the hourly BP measurements, clearly illustrating the attenuated dip of the KO mice during the inactive phase.

In order to analyze the dipping pattern of the mice, we compared inactive/active phase differences in MAP of WT and *Per1* KO mice under control and HS/DOCP conditions (Figure 4). It is clear that *Per1* KO mice have a greater decrease in MAP from the active to

inactive phase compared to WT on control diet (Figure 4A). The *Per1* KO dip of $14 \pm 1.4\%$ is significantly larger than the WT $9.2 \pm 1.3\%$ dip ($p < 0.05$) under baseline conditions. On HS/DOCP, WT mice maintain their dip in BP whereas *Per1* KO mice exhibit an attenuated inactive/active phase difference in BP (panel B). WT MAP decreases by $11.4 \pm 1.9\%$ from night to day while *Per1* KO mice decrease only $5.7 \pm 1.4\%$ ($P < 0.05$). Diastolic and systolic BP changed in parallel to MAP (Supplemental Figure 2). In contrast to these differences in BP, inactive/active phase differences in activity patterns were higher in WT mice compared to *Per1* KO mice under control and HS/DOCP conditions (Supplemental Figure 3). Thus, activity levels do not account for the higher MAP observed in *Per1* KO mice.

Finally, in order to determine if there was a change in the 24hr rhythm of BP, we subjected the hourly telemetry data to cosinor analysis. This allowed us to examine changes in circadian parameters that may have occurred in response to HS/DOCP and loss of PER1. Table 2 lists the values for amplitude (difference between the highest and lowest BP values), mesor (mean value for MAP across all time points), and phase (Zeitgeber time at which MAP peaks) (Refinetti et al., 2007). Consistent with our findings in Figure 3, there was a significant change in mesor between WT (107.6 ± 5) and *Per1* KO (121.2 ± 5.3) on HS/DOCP. *Per1* KO mice on HS/DOCP also had a significant phase shift (22.3 ± 1.3) compared to WT mice on HS/DOCP (19 ± 0.3 , $p = 0.018$) as well as KO mice on control diet (18.2 ± 0.1 , $p = 0.013$). Use of JTK cycle and cosinor analysis to evaluate circadian rhythms demonstrated significant rhythms in MAP (Supplemental Tables 1 and 2) ($p < 0.0001$ for each mouse in every group).

Discussion

The most striking finding of this study was that loss of a single gene, *Per1*, in combination with high salt and mineralocorticoid treatment resulted in a non-dipping phenotype. Importantly, these significant results were observed in a relatively mild HS/DOCP model without surgical nephrectomy. This is also accompanied by a significant phase shift in the peak of MAP in *Per1* KO compared to WT mice. To the best of our knowledge, this is the first demonstration of a non-dipping, phase-shifted BP phenotype in a single clock gene KO mouse in response to a model of salt-sensitive hypertension.

We have previously demonstrated a role for PER1 in the regulation of renal sodium handling with accompanying effects on BP (Solocinski et al., 2015, Richards et al., 2014c, Richards et al., 2014b, Richards et al., 2013a, Richards et al., 2013b). The results of the present study further implicate PER1 as an important regulator of BP. When challenged with HS/DOCP, *Per1* KO mice undergo increased BP as well as a disruption of the normal 24 hr rhythm of BP. Our previous work in the hypertensive 129/sv mouse strain demonstrated a protective effect of *Per1* KO on BP. This is not surprising given that strain-dependent differences in BP regulation are an established phenomenon, with wide-ranging baseline BP (Korshunov, 2016), sodium sensitivity (Cechova et al., 2013), (Hartner et al., 2003) and susceptibility to renal damage (Ma and Fogo, 2003) and diabetic nephropathy (Brosius et al., 2009). Thus, the differences in baseline BP between *Per1* KO mice on 129/sv and C57BL/6J backgrounds are likely due to strain-dependent differences in background genetics.

Even though non-dipping is a recognized problem with estimates of non-dipping prevalence as high as 45% (Viera et al., 2011), the need for better treatments and thus better animal models remains. Other animal models of non-dipping hypertension have been identified using chronic models (Ivy et al., 2016) or ablation of multiple genes (Pati et al., 2016) (Masuki et al., 2005). In depth testing of Period isoform KOs in 129/sv mice by Rudic and colleagues further supports the importance of the PER proteins in blood pressure regulation (Pati et al., 2016). In this study, Angiotensin II infusion into *Per2* KO mice in total darkness for 3 weeks elicited a non-dipping phenotype. Interestingly, a low salt diet resulted in non-dipping in *Per1/2/3* KO mice in normal light conditions as well. It is difficult to directly compare these results due to the differences in background strain and treatment conditions. However, it is clear that the PER proteins are important regulators of BP. Thus the novel model presented in this study is the first single gene knockout model to produce a hypertensive non-dipping phenotype which develops quickly under normal light/dark conditions. In addition, WT C57Bl/6J mice are quite resistant to the current study regimen and usually require some additional insult to see a phenotype (Hartner et al., 2003, Mohammed-Ali et al., 2015), making the non-dipping exhibited by *Per1* KO mice even more remarkable. Moreover, C57Bl/6J *Per1* KO mice are slightly hypertensive at baseline, which is a common characteristic of many non-dippers (Friedman and Logan, 2009), (Cakici et al., 2014).

Non-dipping in humans can likely be attributed to many factors including kidney damage and salt-sensitive hypertension (reviewed in (Birkenhager and van den Meiracker, 2007)). The model presented here is pertinent to human disease as it has been demonstrated that high salt intake can blunt the nocturnal dip in humans (Higashi et al., 1997) and conversely, restricted salt intake can help increase this dip (Uzu et al., 1997). The changes observed in these mice likely involve the contribution of multiple organ systems since the current model is a global KO. It is evident that the vasculature and heart contribute to rhythms in cardiovascular function (reviewed in (Martino and Young, 2015)) and the contribution of these systems to the regulation of BP rhythms is an active area of investigation. For example, Db/db mice displayed a nondipping phenotype along with altered expression of clock gene mRNA levels in vascular smooth muscle tissue, which likely contributed to the nondipping phenotype observed in this mouse model of diabetes (Su et al., 2012). While the actions of PER1 in the vasculature remain largely unknown, the vasculature is an important mediator of BP regulation through changes in vessel compliance. Decreased compliance is accompanied by an increase in SBP and no change or a decrease in DBP (Gavish and Izzo, 2016). The parallel changes in DBP and SBP observed in these mice suggest a largely volume-dependent mechanism of hypertension which likely involves the action of the kidneys. Diuretics have been shown to reverse non-dipping in humans (Uzu and Kimura, 1999), suggesting a volume-dependent mechanism as well. The kidney is further implicated in the etiology of non-dipping through the association of renal transplantation with an improvement in dipping status in humans (Gatzka et al., 1995). While certain SNPs in *Bmal1* have been associated with hypertension (Woon et al., 2007), to the best of our knowledge there have been no studies large enough to identify SNPs in *Per1* which could be attributed to salt-sensitivity or non-dipping in humans. Taken together with the results of the present study, these findings support a role for the circadian clock and the kidney in the

maintenance of baseline BP and rhythmic BP. In conclusion, *Per1* KO C57BL/6J mice on HS/DOCP regimen may represent an important new model for understanding mechanisms underlying non-dipping hypertension.

Limitations of this study include the fact that the mice used in this study are global KO. Thus, tissue-specific contributions to the non-dipping phenotype cannot be assessed. These studies were also limited to male mice. The experiments presented here were performed in a normal 12:12 light/dark cycle. Since a truly circadian process is defined as one that persists in the absence of light cues, determination of a true circadian defect would require the use of total darkness, an experimental maneuver that is beyond the scope of the present report.

To place these findings in a clinical perspective, accumulating evidence suggests that chronotherapy, or dosing of medication at a specific time of day, can be helpful in treating non-dipping in certain patients (Portaluppi et al., 1995), (Hermida et al., 2008). While chronotherapy or lifestyle changes can decrease non-dipping prevalence in some patients, not all are helped by these interventions. Since non-dipping increases the risk of cardiovascular events, there is a need for better models and treatments. Our novel model of *Per1* KO mice treated with a HS/DOCP regimen models a diminished dipping phenotype similar to that seen in humans and may provide a new way to test interventions. *PER1* is again implicated in BP control and thus presents as a putative target for modulation of dipping status.

The correlation between non-dipping and cardiovascular risk highlights not only the importance of normal circadian rhythms to overall health but also the way in which BP measurements are taken. 24 hour ambulatory BP monitoring (ABPM) is a much more accurate predictor of dipping status and therefore future risk as it markedly reduces white-coat hypertension and placebo effects (Coats, 1996), (Syrseoudis et al., 2011), (de la Sierra et al., 2012). Somewhat similar to white-coat hypertension experienced by humans, mice can become stressed by tail-cuff BP measurements (Batchu et al., 2015), (Whitesall et al., 2004). ABPM is a powerful method for BP studies since recordings are taken continuously as opposed to the snapshots generated from tail-cuff or office measurements.

In summary, here we present a new model of non-dipping hypertension: *Per1* KO mice on a HS/DOCP regimen. This is the first report of a single clock gene KO resulting in a non-dipping phenotype in response to high salt plus mineralocorticoid. The data implicate *PER1* in the control of a normal dip in BP during the inactive period. This is especially significant given that C57BL/6J mice are often resistant to perturbations in BP. The new model of non-dipping proposed here (*Per1* KO mice on HS/DOCP) can also serve as a means of discovering new ways to treat non-dipping. In contrast to WT mice, global C57BL/6J *Per1* KO mice are relatively hypertensive at baseline and are sensitive to a high salt/mineralocorticoid model of hypertension. These *Per1* KO mice display attenuated dipping of BP which is similar to non-dipping hypertension in humans that is associated with increased risk of adverse cardiovascular events.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Differences in renal gene expression between WT and *Per1* KO mice at noon and midnight on control diet

Kidney cortex from WT (black bars) and *Per1* KO (checked bars) mice collected at noon (inactive phase) and midnight (active phase) time points was analyzed by qPCR for differences in genes related to renal sodium transport. (A) *AT₁R*, (B) *UBE2E3*, (C) *NOS1*, (D) *WNK1* and (E) α ENaC. Significance was calculated by two-way ANOVA. $n=3$ for KO at noon, $n=4$ for all others. $p < 0.05$ was considered significant.

Control Diet

Figure 2. Inactive/active averages and 72 hour telemetry recordings of MAP in WT and *Per1* KO mice on control diet

A. Day and night MAP averages. Day values are the 3-day averages of MAP between 6am ZT0-ZT11. Night values are the 3-day averages of MAP recorded between ZT12-ZT23. **B.** Telemetry recording of MAP with lights on from ZT0-ZT12 depicted with light areas and ZT12-ZT0, the time when lights are off, shaded gray. n=8 for WT, n=6 for *Per1* KO.

Significance was calculated by two-way ANOVA. * $p < 0.01$, ** $p < 0.001$ compared to same genotype, ψ $p < 0.05$ compared to same time.

HS/DOCP Diet

Figure 3. Inactive/active averages and 72 hour telemetry recordings of MAP in WT and *Per1* KO mice on HS/DOCP diet

MAP recordings from day 3 to day 6 on HS/DOCP. **A.** Day and night MAP averages. Values were calculated as in Figure 2. **B.** Telemetry recording of MAP depicted the same as in Figure 2. $n=8$ for WT, $n=6$ for *Per1* KO. Significance was calculated by two-way ANOVA. * $p < 0.01$, ** $p < 0.001$ compared to same genotype, ψ $p < 0.05$ compared to same time.

Figure 4. *Per1* KO mice lose the inactive/active difference in MAP on HS/DOCP diet

A. The change in MAP from night to day is shown for WT mice and *Per1* KO mice on control diet. **B.** The change in MAP from night to day is shown for WT and *Per1* KO mice on HS/DOCP diet for 6 days. Percent dip is given for each group. Significance was calculated using a t-test. *p<0.05. n=8 for WT, n=6 for *Per1* KO.

Table 1Baseline characteristics of WT and *Per1* KO mice.

	WT mice		<i>Per1</i> KO mice		Significance	
	Noon	Midnight	Noon	Midnight	Time	Genotype
Plasma Aldosterone (pg/mL)	551.6±193	292.1±47.9	384.2±29.2	260.894±76.5	*	NS
Plasma Na ⁺ (mM)	150.7±1.1	152.5±0.62	149.9±1.38	153±0.72	*	NS
Plasma K ⁺ (mM)	4.54±0.14	4.71±0.07	4.98±0.21	4.69±0.07	NS	NS
Body weight (g)	27.6±0.56		27.3±0.35		NS	

Plasma concentrations of aldosterone, sodium (Na) and potassium (K) were determined from samples taken at noon and midnight from WT and *Per1* KO mice. Significance was determined by two-way ANOVA (excluding body weight). Data are presented as mean ± SEM.

* p<0.05, n=4 mice per group.

Table 2

Cosinor analysis of HS/DOCP effects on MAP in WT and *Per1* KO mice.

	WT Control	<i>Per1</i> KO Control	WT HS/DOCP	<i>Per1</i> KO HS/DOCP
Amplitude	15.8 ± 1.8	22.4 ± 3.4	21.1 ± 2.7	23.3 ± 4.5
Mesor	106.9 ± 4	111.4 ± 1.3	107.6 ± 5	121.2 ± 5.3 [‡]
Phase	18.9 ± 0.56	18.2 ± 0.1	19 ± 0.3 [‡]	22.3 ± 1.3 [*]

Using ClockLab software, we determined amplitude, mesor and phase data from MAP measurements. All values were calculated separately for each animal and the mean is given ± SEM. Significance was calculated by t-test.

*
p<0.05 compared to same genotype

[‡]
p<0.05 compared to same treatment. n=6 for KO, n=8 for WT.