Circadian misalignment increases cardiovascular disease risk factors in humans

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Shift work is a risk factor for hypertension, inflammation, and cardiovascular disease. This increased risk cannot be fully explained by classic risk factors. One of the key features of shift workers is that their behavioral and environmental cycles are typically misaligned relative to their endogenous circadian system. However, there is little information on the impact of acute circadian misalignment on cardiovascular disease risk in humans. Here we show—by using two 8-d laboratory protocols—that short-term circadian misalignment (12-h inverted behavioral and environmental cycles for three days) adversely affects cardiovascular risk factors in healthy adults. Circadian misalignment increased 24-h systolic blood pressure (SBP) and diastolic blood pressure (DBP) by 3.0 mmHg and 1.5 mmHg, respectively. These results were primarily explained by an increase in blood pressure during sleep opportunities (SBP, +5.6 mmHg; DBP, +1.9 mmHg) and, to a lesser extent, by raised blood pressure during wake periods (SBP, +1.6 mmHg; DBP, +1.4 mmHg). Circadian misalignment decreased wake cardiac vagal modulation by 8–15%, as determined by heart rate variability analysis, and decreased 24-h urinary epinephrine excretion rate by 7%, without a significant effect on 24-h urinary norepinephrine excretion rate. Circadian misalignment increased 24-h serum interleukin-6, C-reactive protein, resistin, and tumor necrosis factor-α levels by 3–29%. We demonstrate that circadian misalignment per se increases blood pressure and inflammatory markers. Our findings may help explain why shift work increases hypertension, inflammation, and cardiovascular disease risk.

circadian misalignment | hypertension | inflammatory markers | night work | shift work

In the United States, almost 15% of the workforce undertakes shift work (1). Epidemiological studies indicate that shift work is a risk factor for elevated blood pressure, hypertension, and cardiovascular disease, even after controlling for traditional risk factors (2–6). These findings raise the question of what the underlying mechanism is. Shift workers frequently undergo circadian misalignment (i.e., misalignment between the endogenous circadian system and 24-h environmental/behavioral cycles). This misalignment has been proposed to explain, in part, why shift work has been reported to be a risk factor for elevated blood pressure, hypertension, and cardiovascular disease (7).

Humans—as well as most other life on Earth—possess an endogenous circadian system that optimally synchronizes physiology and behavior to the solar day (8–10). The mammalian endogenous circadian system consists of the suprachiasmatic nucleus in the hypothalamus and circadian oscillators in virtually all peripheral tissues and organs (8–10). At the molecular level, transcriptional-translation feedback loops are involved in generating intrinsic circadian rhythms (8–10). We have previously shown that circadian misalignment—caused by participants living on a 28-h behavioral cycle under dim light conditions (forced desynchrony protocol)—increases wake blood pressure (11). However, real-life shift workers do not live on 28-h days in dim light (continuous exposure to dim light further slows down the adjustment of the suprachiasmatic nucleus to the new behavioral cycle). Moreover, we did not assess the impact of circadian misalignment on sleep blood pressure, which is a better predictor of adverse cardiovascular events and all-cause mortality than wake blood pressure (12). Inflammatory markers also strongly predict cardiovascular disease risk (13–15) and have been shown to be elevated in shift workers compared with day workers (16–18). However, to our knowledge, only two laboratory studies have assessed the impact of circadian misalignment on inflammatory markers in humans. The first study demonstrated that 8 d of sleep restriction combined with multiple days of circadian misalignment increased C-reactive protein (CRP) levels—a marker of systemic inflammation (19). The second study demonstrated that weeks of gradual changes in circadian misalignment (induced by 24.6-h behavioral cycles in dim light) increased CRP, tumor necrosis factor (TNF)-α (proinflammatory) and interleukin (IL)-10 (antiinflammatory) levels (20). The relevance of the findings of the latter study to shift workers is unclear, considering that shift workers are not kept in dim light conditions (night workers have been reported to be exposed to ∼50–300 lx during night shifts and natural environmental illumination of varying strength) and typically undergo rapid and large shifts of their behavioral/environmental cycles relative to their internal circadian system—that is, due to the inertia of the internal circadian system, the timing of the circadian system cannot quickly adjust to large and rapid shifts in behavioral/environmental cycles (21). The impact of circadian misalignment under simulated night work conditions on blood pressure and inflammatory markers remains poorly understood.

Thus, we tested the impact of circadian misalignment, similar to that experienced by real-life shift workers, on 24-h levels of

Significance

Shift work is a risk factor for hypertension, inflammation, and cardiovascular disease, even after controlling for traditional risk factors. Shift workers frequently undergo circadian misalignment (i.e., misalignment between the endogenous circadian system and 24-h environmental/behavioral cycles). This misalignment has been proposed to explain, in part, why shift work is a risk factor for hypertension, inflammation, and cardiovascular disease. However, the impact of circadian misalignment per se on 24-h blood pressure and inflammatory markers is poorly understood. We show—under highly controlled laboratory conditions—that short-term circadian misalignment increases 24-h blood pressure and inflammatory markers in healthy adults. Our findings may help explain why shift work increases hypertension, inflammation, and cardiovascular disease risk.

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blood pressure and the inflammatory markers CRP, IL-6, TNF-α, and resistin, which are risk factors for cardiovascular disease (14, 15, 22–32). Furthermore, we tested the impact of circadian misalignment on additional cardiovascular-related measures, including autonomic nervous system activity (cardiac vagal modulation and urinary epinephrine and norepinephrine) and factors involved in fibrinolysis [plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (tPA) activity] (33, 34). To do so, we measured—using a randomized, within-participant, cross-over design—blood pressure and inflammatory markers across the 24-h behavioral and light/dark cycles when the behavioral and environmental cycle (including sleep/wake, fasting/feeding, rest/activity, and dark/light cycles) was aligned and misaligned (after a rapid 12-h shift of the behavioral cycle) with the endogenous circadian system (Fig. 1). Moreover, we tested whether the impact of circadian misalignment was dependent on exposure duration to circadian misalignment (acute vs. repeated, test period 1 and 3, respectively) (Fig. 1). Based on previously published 24-h melatonin data collected in test period 1 and 3 of both the circadian alignment and misalignment protocols of the current study, our circadian misalignment protocol resulted in misalignment between the central circadian pacemaker and the 24-h environmental and behavioral cycles (35). Finally, we tested whether the effects of circadian misalignment on our main outcome variables were mediated by the effects of misalignment on sleep duration or were above and beyond any misalignment effects on sleep duration.

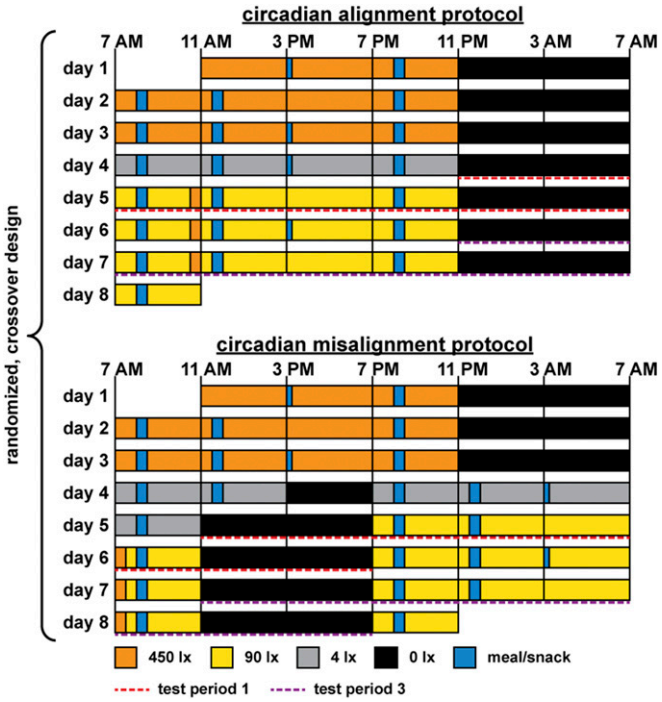

Fig. 1. Circadian alignment protocol (*Top*) and circadian misalignment protocol (*Bottom*). On day 1 of both protocols, participants received an ad libitum lunch at ∼12:00 PM. Light levels indicated are in the horizontal angle of gaze: ∼90 lx, to simulate typical room light intensity, ∼450 lx during the first three baseline wake episodes to enhance circadian entrainment, 30-min periods of ∼450 lx to simulate the morning commute both preceding the day work shift (circadian alignment protocol) and after the night work shift (circadian misalignment protocol), ∼4 lx to permit assessment of the dim-light melatonin onset, and 0 lx during scheduled sleep episodes. Light blue bars represent meals (wide bar) and snacks (narrow bar).

Results

Circadian Misalignment Increased Blood Pressure (Fig. 2). Circadian misalignment, compared with circadian alignment, increased 24-h systolic blood pressure (SBP) by 3.0 mmHg and 24-h diastolic blood pressure (DBP) by 1.5 mmHg (both $P < 0.0001$). Exposure duration to circadian misalignment (test period 1 vs. test period 3) did not significantly modulate the circadian misalignment effect on SBP ($P = 0.90$), but did for DBP ($P = 0.001$). Circadian misalignment increased 24-h DBP to a slightly larger extent during test period 3 (+1.7 mmHg; $P = 0.0002$) compared with test period 1 (+1.4 mmHg; $P = 0.001$). The 24-h BP results seem primarily explained by circadian misalignment increasing SBP during the sleep opportunity by 5.6 mmHg and DBP during the sleep opportunity by 1.9 mmHg, and, to a lesser extent, by circadian misalignment increasing wake-period SBP by 1.6 mmHg and DBP by 1.4 mmHg (all $P \leq 0.0004$). The circadian misalignment effect was not significantly dependent on exposure duration for wake-period or sleep-opportunity SBP or wake-period DBP (all $P \geq 0.11$). However, the effect of circadian misalignment on sleep-opportunity DBP was dependent on circadian misalignment exposure duration ($P = 0.001$), with the increase slightly greater during test period 1 (+2.2 mmHg; $P = 0.002$) compared with test period 3 (+1.6 mmHg; $P = 0.031$).

Circadian Misalignment Decreased Heart Rate During Wake Periods and Increased Heart Rate During Sleep Opportunities (Fig. 2). There was no significant effect of condition on 24-h heart rate ($P = 0.20$). However, there was an effect of circadian misalignment exposure duration ($P < 0.0001$). Twenty-four-hour heart rate was 1.6 beats per minute higher in the circadian misalignment than alignment condition during test period 1 ($P = 0.021$), without significant difference during test period 3 ($P = 0.61$). Circadian misalignment decreased wake-period heart rate by 0.9 beats per minute and increased sleep-period heart rate by 3.6 beats per minute (both $P \leq 0.008$). These effects were dependent on exposure duration to circadian misalignment (both $P \leq 0.019$). Wake-period heart rate was 1.5 beats per minute lower in the circadian misalignment than alignment condition during test period 3 ($P = 0.011$), without significant difference during test period 1 ($P = 0.23$). Circadian misalignment increased sleep-opportunity heart rate to a greater extent during test period 1 (+5.3 beats per minute; $P < 0.0001$) compared with test period 3 (+2.0 beats per minute; $P = 0.014$).

Circadian Misalignment Reduced the Sleep Opportunity-Associated Dipping in Blood Pressure and Heart Rate (Fig. 3). Circadian misalignment reduced SBP dipping during the sleep opportunity by 21% ($P = 0.012$), but had no significant impact on DBP dipping ($P = 0.19$). Circadian misalignment also reduced heart rate dipping during the sleep opportunity by 33% ($P < 0.0001$). These findings were not significantly affected by circadian misalignment exposure duration (all $P \geq 0.18$).

Effect of Circadian Misalignment on 24-h Urinary Epinephrine and Norepinephrine Excretion Rates (Fig. 4). Circadian misalignment decreased 24-h urinary epinephrine excretion by 7% ($P = 0.005$) but had no significant effect on 24-h urinary norepinephrine excretion ($P = 0.25$). The epinephrine profile was dependent on alignment condition (interaction of the factor alignment/misalignment with time since wake; $P < 0.0001$); epinephrine was higher during the sleep opportunity, no different a few hours after scheduled wake, but lower for the remainder of scheduled wake in the circadian misalignment than alignment condition, causing a flattening of the rhythm. The norepinephrine profile was not significantly different between alignment conditions ($P = 0.26$). None of the above effects were significantly dependent on circadian misalignment exposure duration (all $P \geq 0.32$). Of note, circadian misalignment increased urinary epinephrine by

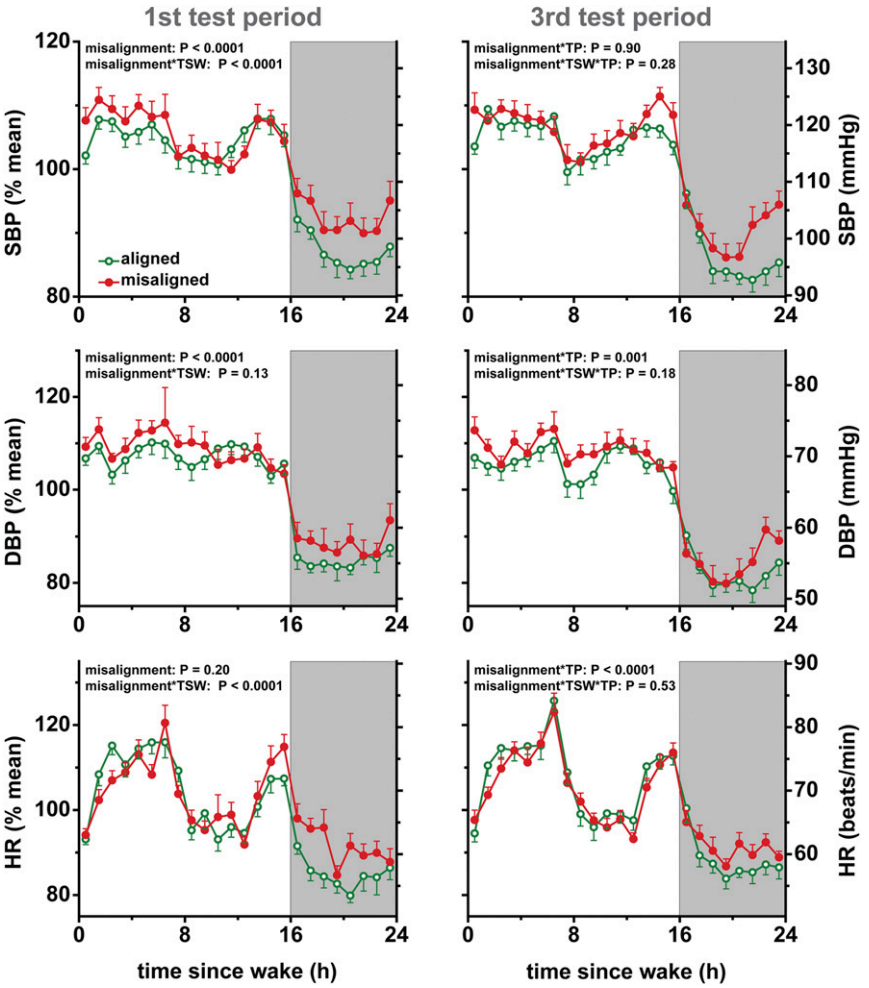

Fig. 2. Effects of circadian misalignment on blood pressure and heart rate levels. DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; TP, test period; TSW, time since wake. Gray bar, sleep opportunity. Probability values are based on 24-h analyses. Data are represented as mean ± SEM.

82% during the sleep opportunities in which blood pressure and heart rate were measured ($P = 0.004$).

Circadian Misalignment Decreased Markers of Cardiac Vagal Modulation (Fig. 5). Circadian misalignment decreased the root mean square differences of consecutive heartbeat intervals (RMSSD) by 11% and the percentage of consecutive heartbeat intervals differing by >20 ms (pNN20) by 8% (both $P \leq 0.037$), reflecting a decrease in cardiac vagal modulation. There was no significant effect of circadian misalignment on pNN50 ($P = 0.10$) (Fig. S1). The circadian misalignment effects on RMSSD, pNN20, and pNN50 were dependent on circadian misalignment exposure duration (all $P \leq 0.027$). RMSSD was 15% lower in the circadian misalignment than alignment condition during test period 1 ($P = 0.025$) and without difference during test period 3 ($P = 0.49$). There were statistical trends for pNN20 ($P = 0.053$) and pNN50 ($P = 0.054$) to be lower in the circadian misalignment than alignment condition during test period 1 and to be without significant difference during test period 3 (both $P \geq 0.29$).

Circadian Misalignment Increased Inflammatory Markers (Figs. 6 and 7). Circadian misalignment increased 24-h IL-6 by 15% ($P = 0.014$). This effect was dependent on circadian misalignment exposure duration ($P < 0.0001$), with levels being 29% higher in the circadian misalignment than alignment condition during test period 1 ($P = 0.001$) and without a difference during test period 3 ($P = 0.92$). The 24-h IL-6 profile was dependent on alignment condition ($P = 0.028$), with levels being higher in the early part of the wake period in the circadian misalignment condition, compared with the circadian alignment condition; this difference was not significantly dependent on circadian misalignment exposure duration ($P = 0.99$). Circadian misalignment increased 24-h CRP, resistin, and TNF-α by 7%, 5%, and 3%, respectively (all $P \leq 0.030$). There was no significant effect of circadian misalignment on the 24-h profiles of CRP, resistin, or TNF-α (all $P \geq 0.050$). None of the circadian misalignment effects on CRP, resistin, or TNF-α were significantly dependent on circadian misalignment exposure duration (all $P \geq 0.11$).

Circadian Misalignment Decreased PAI-1 Levels (Fig. 8). Circadian misalignment decreased 24-h PAI-1 by 11% ($P = 0.014$). This effect was dependent on circadian misalignment exposure duration ($P = 0.037$), with no effect of alignment condition during test period 1 ($P = 0.77$), but with circadian misalignment decreasing 24-h PAI-1 levels by 21% on test period 3 ($P = 0.002$). The 24-h PAI-1 profile was dependent on alignment condition ($P < 0.0001$), with lower levels at the end of the sleep opportunity and the beginning of the wake period, and higher levels during the latter half of the wake period and the start of the sleep opportunity, causing a flattening of the rhythm. The profile differences were dependent

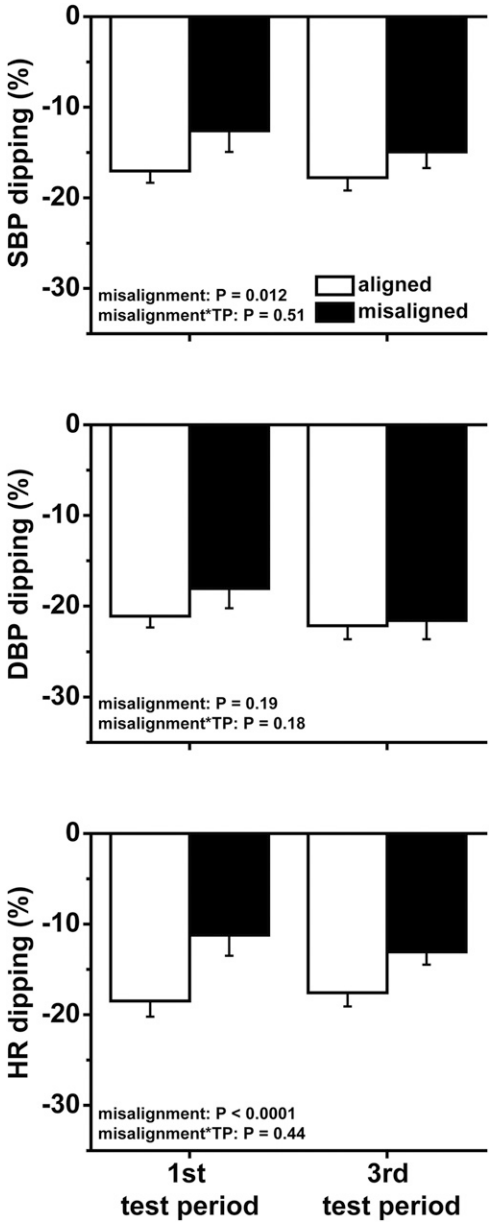

Fig. 3. Effects of circadian misalignment on sleep opportunity-associated dipping in blood pressure and heart rate. DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; TP, test period. Data are represented as mean ± SEM.

on circadian misalignment exposure duration ($P = 0.025$), reflecting that PAI-1 levels were higher at the end of the wake period during test period 1 but not during test period 3. Circadian misalignment had no significant effect on 24-h tPA activity or its profile, regardless of exposure duration (all $P \geq 0.069$; $n = 6$).

Within-Participant Correlations and Mediation Analyses. We have previously reported that circadian misalignment decreased polysomnography (PSG)-assessed total sleep duration by 56 min in the current protocol (35). To investigate to what degree the observed effects of circadian misalignment on cardiovascular risk markers were related to changes in total sleep duration, we used PSG sleep recordings and actigraphic sleep recordings. We assessed whether PSG sleep recordings (on sleep periods 4 and 6 for the alignment protocol and sleep periods 5 and 7 for the misalignment protocol) were correlated with cardiovascular risk markers during the 24-h assessments (inflammatory makers) or measured during the subsequent wake periods (blood pressure and heart rate). We also tested whether actigraphic estimates of sleep (on sleep opportunities 5 and 7 for the alignment/misalignment protocol and sleep opportunities 6 and 8 for the alignment/misalignment protocol) were correlated with blood pressure and heart rate measured during the same sleep opportunities (PSG was not assessed simultaneously with the blood pressure assessments). There was no correlation between PSG-assessed total sleep duration and SBP, DBP, or heart rate during the subsequent wake period (all $P \geq 0.50$). Actigraphy-assessed total sleep duration was negatively correlated with SBP during the same sleep opportunity ($r = -0.27$; $P = 0.035$), but not correlated with DBP or heart rate during the same sleep opportunity (both $P \geq 0.16$). Mediation analysis indicated that the circadian misalignment-mediated increase in SBP during the sleep opportunity was partially mediated by the circadian misalignment-mediated decrease in total sleep duration as assessed by actigraphy, and partially independent of changes in total sleep duration. That is, misalignment increased sleep-opportunity SBP (standardized $\beta = 0.37$, $P < 0.0001$) even when including actigraphy-assessed total sleep duration (standardized $\beta = -0.25$, $P < 0.0002$) in the model. PSG-assessed total sleep duration was negatively correlated with 24-h TNF-α levels ($r = -0.24$, $P = 0.038$), but not correlated with 24-h IL-6, CRP, or resistin levels (all $P \geq 0.17$). In subsequent mediation analysis for TNF-α, the effects of circadian misalignment and PSG-assessed total sleep duration were both nonsignificant (both $P \geq 0.21$), possibly because of insufficient statistical power.

Sleep-opportunity urinary epinephrine excretion rate—measured across the same sleep periods in which blood pressure was assessed—was positively correlated with sleep-opportunity SBP ($r = 0.37$, $P = 0.002$), but not sleep-opportunity DBP ($P = 0.069$). Mediation analysis indicated that the circadian misalignment-mediated increase in sleep-opportunity SBP was partially mediated by the circadian misalignment-mediated increase in sleep-opportunity urinary epinephrine excretion rate (standardized $\beta = 0.19$, $P < 0.0001$) and partially independently by circadian misalignment (standardized $\beta = 0.24$, $P = 0.0002$). There was no within-participant correlation between wake-period SBP or DBP and cardiac vagal markers RMSSD and pNN20 (all $P \geq 0.11$).

Discussion

We found that short-term circadian misalignment, resulting from a rapid 12-h inversion of the behavioral cycle (including the sleep/wake and fasting/feeding cycle) and which is typical in shift workers, increased 24-h blood pressure. The magnitude of the effect of circadian misalignment on 24-h blood pressure was similar to that reported in a Dietary Approaches to Stop Hypertension (DASH) study and the individual impact of some antihypertensive drugs (36, 37). The circadian misalignment-mediated increase in blood pressure may be clinically important considering that there is an increased risk of cardiovascular disease with progressive elevations in blood pressure, beginning at normal blood pressure levels (32). The adverse effect of circadian misalignment on 24-h blood pressure was primarily a result of an increase in blood pressure during sleep opportunities rather than wake periods. Sleep blood pressure is a better predictor of adverse cardiovascular events and all-cause mortality than wake blood pressure (12). Circadian misalignment also reduced SBP dipping during sleep opportunities. Reduced blood pressure dipping during sleep is also an independent predictor of adverse cardiovascular events and all-cause mortality (12). We also found that circadian misalignment increased the inflammatory markers CRP, TNF-α, resistin, and IL-6. CRP is a marker of systemic inflammation whereas TNF-α and resistin have proinflammatory functions, and IL-6 has both pro- and

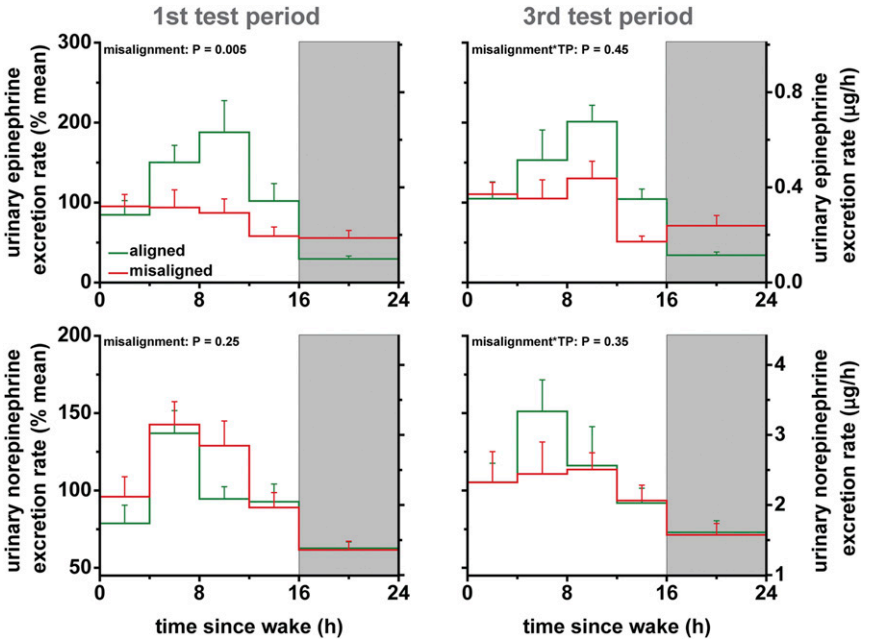

Fig. 4. Effects of circadian misalignment on urinary epinephrine and norepinephrine excretion rates. TP, test period. Gray bar, sleep opportunity. Probability values are based on 24-h analyses. Data are represented as mean ± SEM.

antiinflammatory properties (38–40). It is now well-accepted that inflammation has an important role in the development of cardiovascular disease and that each of the inflammatory markers we measured predicts cardiovascular disease (14, 15, 22–31). Epidemiologic studies convincingly show an increased prevalence of cardiovascular disease in night workers versus day workers, and field studies in shift workers have shown increased blood pressure and inflammatory markers during or after night work compared with day work or days off (41–45). However, such studies can not definitively distinguish a possible causal role of circadian misalignment in the observed adverse health effects of shift work versus that of differences in other factors, such as work stressors, dietary habits, and physical activity, as well as family, financial, genetic, health, and social factors, etc. In the current highly controlled, experimental, within-participant protocol, we could determine the influence of circadian misalignment per se on cardiovascular and inflammatory risk factors, while controlling these other factors. Our finding that circadian misalignment per se increases blood pressure and inflammatory markers provides evidence for circadian misalignment as an underlying mechanism to explain why shift work is a risk factor for hypertension and cardiovascular disease (2–6).

Possible Mechanisms Involved in Circadian Misalignment-Mediated Increases in Blood Pressure and Inflammatory Markers. Experimental studies show that short-term sleep restriction per se increases daytime and nighttime blood pressure in normotensive and hypertensive humans (46–49). Thus, we assessed whether sleep duration in our study was associated with blood pressure. Although PSG-assessed total sleep duration, measured immediately before the wake periods during which blood pressure was recorded, was reduced by our circadian misalignment protocol, we found no correlation with blood pressure. Because no PSG recordings were performed concurrently with blood pressure recordings during sleep opportunities, we also estimated total sleep duration via actigraphy during the sleep opportunities in which blood pressure was measured. Actigraphy-estimated total sleep duration was reduced during circadian misalignment and was negatively correlated with sleep-opportunity SBP, but not with sleep-opportunity DBP. Furthermore, mediation analysis indicated that the circadian misalignment-mediated increase in sleep-opportunity SBP was partially mediated by circadian misalignment decreasing actigraphy-estimated total sleep duration and partially independent of total sleep duration.

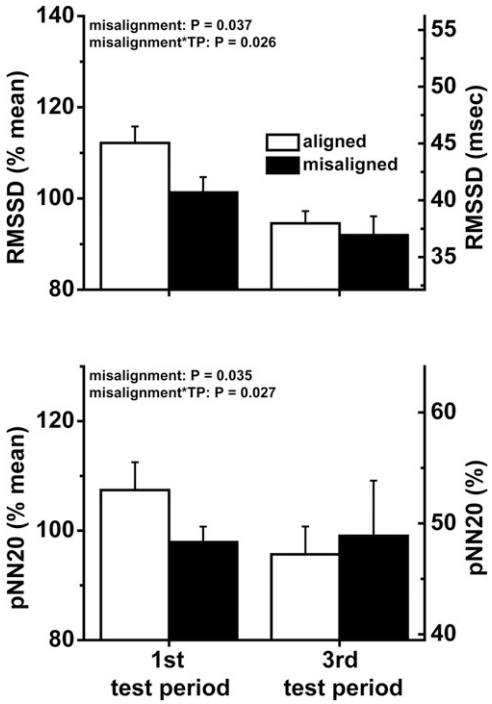

Fig. 5. Effects of circadian misalignment on wake period cardiac vagal modulation. pNN20, percentage of consecutive heartbeat intervals differing by >20 ms; RMSSD, root mean square differences of consecutive heartbeat intervals; TP, test period. Data are represented as mean ± SEM.

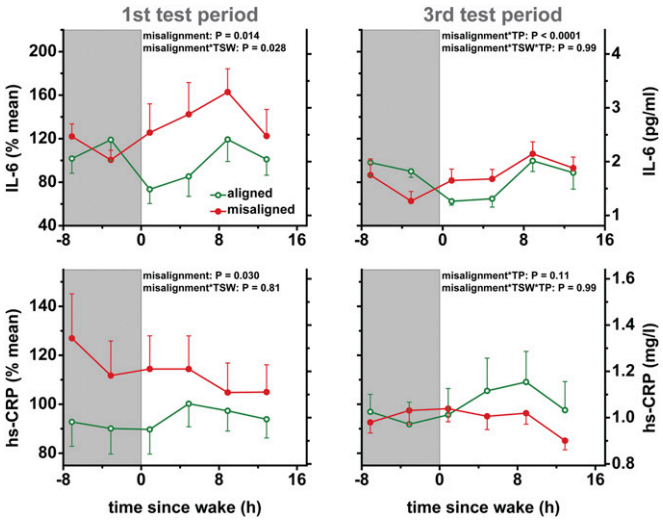

Fig. 6. Effects of circadian misalignment on interleukin-6 and high-sensitivity C-reactive protein levels. hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TP, test period; TSW, time since wake. Gray bar, sleep opportunity. Probability values are based on 24-h analyses. Data are represented as mean ± SEM.

To test the role of the autonomic nervous system in the increase in blood pressure due to circadian misalignment, we determined the effect of circadian misalignment on urinary epinephrine and norepinephrine excretion rates as markers of sympathetic activity and on heart rate variability as estimates of parasympathetic cardiac modulation. Overall, circadian misalignment decreased epinephrine excretion rate and markers of parasympathetic tone but had no effect on norepinephrine excretion rate. The decrease in parasympathetic measures could help explain why circadian misalignment increased blood pressure although we found no within-participant correlation between wake-period blood pressure and the cardiac vagal markers. Of note, circadian misalignment increased epinephrine excretion rate measured across the sleep opportunities in which blood pressure was assessed, which was positively correlated with sleep-opportunity SBP. Moreover, mediation analysis indicated that the circadian misalignment-mediated increase in sleep-opportunity SBP is partially explained by circadian misalignment increasing sleep-opportunity epinephrine excretion rate.

In humans, melatonin and cortisol administration decrease and increase blood pressure, respectively (50–52). Melatonin and cortisol data from the current study have been previously reported (35). We found that circadian misalignment decreased mean 24-h melatonin levels but had no effect on mean 24-h cortisol levels. Thus, the decrease in 24-h melatonin levels during circadian misalignment may have contributed to the increased blood pressure.

The endogenous circadian system causes blood pressure to gradually rise from the biological morning to the evening and fall across the biological night (53, 54). This circadian rhythm may contribute to the fall in blood pressure observed during normally timed sleep (at night). However, during our circadian misalignment protocol, participants attempted to sleep across the biological morning, afternoon, and evening, when the circadian system is promoting an increase in blood pressure. This misalignment between the circadian rhythm in blood pressure and timing of the sleep opportunity may help explain the increased sleep-opportunity blood pressure during circadian misalignment.

Experimental studies show that short-term sleep restriction per se increases inflammatory markers, including IL-6, CRP, and TNF-α (49, 55–57). We found a negative correlation between PSG-assessed total sleep duration and 24-h TNF-α levels, but not with 24-h IL-6, CRP, or resistin levels. Future studies are required to further test the relative contribution of changes in sleep versus sleep-independent factors in the effects of misalignment on inflammatory markers. We have no evidence that stress was a causal factor in the effects of circadian misalignment on inflammatory markers or the other outcome variables. Indeed,

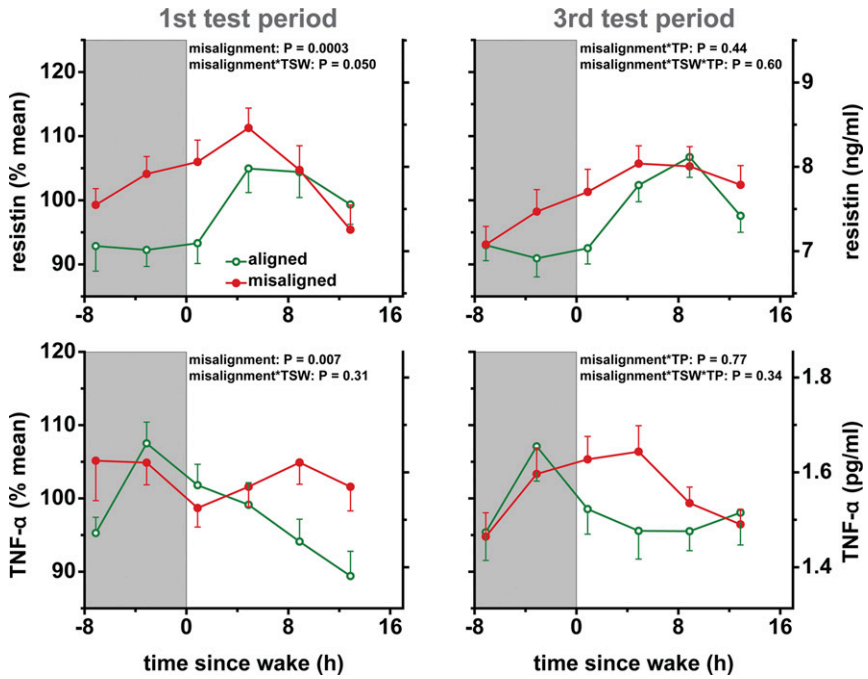

Fig. 7. Effects of circadian misalignment on resistin and tumor necrosis factor-alpha levels. TNF-α, tumor necrosis factor-α; TP, test period; TSW, time since wake. Gray bar, sleep opportunity. Probability values are based on 24-h analyses. Data are represented as mean ± SEM.

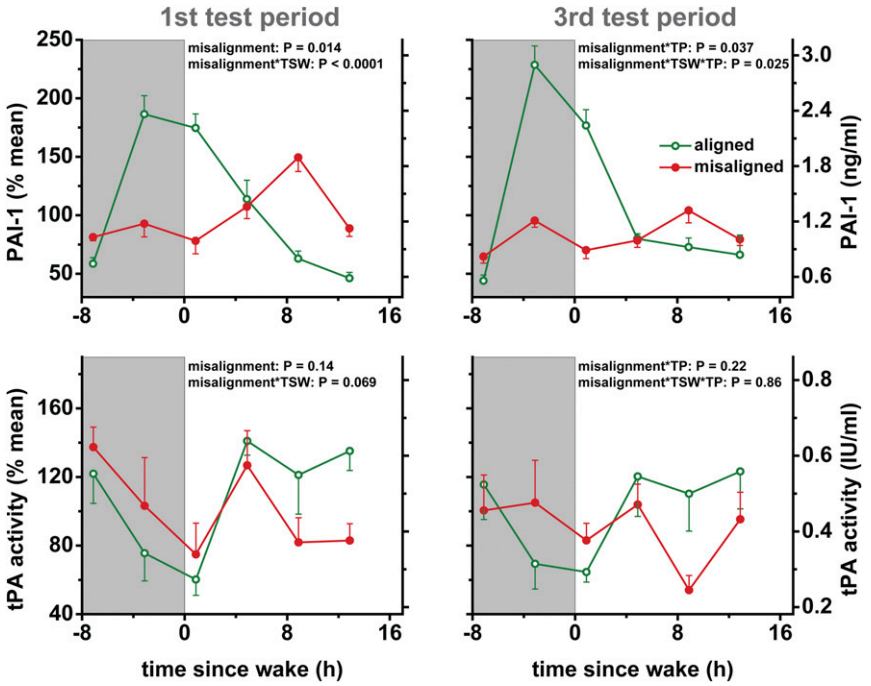

Fig. 8. Effects of circadian misalignment on plasminogen activator inhibitor-1 and tissue plasminogen activator activity levels. PAI-1, plasminogen activator inhibitor-1; TP, test period; tPA, tissue plasminogen activator; TSW, time since wake. Gray bar, sleep opportunity. For tPA data, $n = 6$. Probability values are based on 24-h analyses. Data are represented as mean ± SEM.

average 24-h serum cortisol levels were not affected by circadian misalignment in our study (35). Animal studies have shown that circadian disruption by jet lag simulation exaggerates inflammatory responses that were also not explained by sleep loss or stress measures and was suggested to be related to altered clock gene expression in the central clock and peripheral clocks (58).

Comparisons with Previous Research. In chronic shift workers, blood pressure is higher, and the day/night blood pressure rhythm is blunted while working night shifts compared with day shifts (41–45). Such study designs can not isolate the independent effect of circadian misalignment on blood pressure versus shift work-induced changes in factors such as diet, physical activity, light exposure, etc. We have previously shown that circadian misalignment, resulting from a forced desynchrony protocol, increases blood pressure measured during wakefulness (11). Here, we show that circadian misalignment resulting from a rapid inversion of the behavioral and light/dark cycle—typical in shift workers—increases 24-h blood pressure, both by increasing blood pressure during the wake episode but primarily by increasing blood pressure during the sleep opportunity. Furthermore, we demonstrate that circadian misalignment decreased the SBP drop during the sleep opportunity, reflecting a blunted 24-h SBP rhythm (i.e., "non-dipping"). Manipulating the light/dark cycle and circadian clock genes have also been reported to affect blood pressure in rodents (59–61).

It has been reported in chronic shift workers that cardiac sympathetic modulation is decreased during night shifts compared with day shifts (62). However, such a study can not determine the independent effect of circadian misalignment on autonomic nervous system activity versus shift work-induced changes in factors such as work load, diet, physical activity, etc. Here, we showed that circadian misalignment per se decreases wake cardiac vagal modulation and increases epinephrine excretion rate during sleep opportunities.

Epidemiological studies have shown inflammatory markers to be higher in shift workers than day workers (16–18). Moreover, inflammatory markers have been shown to be higher after a night shift than a day shift (63). Such epidemiological and field studies can not determine the independent effect of circadian misalignment on inflammation. Recently, it was shown that 8 d of sleep restriction, including multiple days of circadian misalignment, increased CRP levels in humans (19). This finding is in accordance with the increase in CRP levels we observed as a result of 3 d of circadian misalignment. Wright et al. (20) reported that weeks of gradual changes in circadian alignment (induced by 24.6-h behavioral cycles under dim light) increased CRP as well as TNF-α and IL-10 (antiinflammatory) levels in humans. However, shift workers typically undergo rapid, large shifts of their behavioral and light/dark cycles relative to their internal body clock, as occurred in our study. We show that this type of circadian misalignment not only increases CRP levels [as shown previously (19)] but also increases TNF-α, IL-6, and resistin levels. Different forms of circadian disruption (e.g., nighttime light exposure, shifting the light/dark cycle) have also been shown to alter inflammatory markers in rodents (58, 64).

PAI-1 is a prothrombotic protein. PAI-1 is the primary inhibitor of tPA and urokinase and is thus an inhibitor of fibrinolysis (breakdown of blood clots). PAI-1 levels are increased in mice that undergo four consecutive weekly 6-h phase advances of the light/dark cycle (65). We found that short-term circadian misalignment decreases 24-h PAI-1 levels in humans. Circadian misalignment caused an inversion of the 24-h PAI-1 rhythm relative to the behavioral cycle, suggesting that PAI-1 is more importantly controlled by the circadian cycle than by the behavioral/environmental cycle. This interpretation is consistent with an important role for the circadian system in the regulation of PAI-1 independent of the behavioral cycle, as we have previously reported (66). Furthermore, the 24-h PAI-1 rhythm was severely blunted after repeated circadian misalignment exposure, reminiscent of the blunting of the rhythm of the circadian markers melatonin and cortisol in this protocol (35).

Strengths, Limitations, and Future Directions. Strengths of our study include the highly controlled laboratory protocol, which was able to determine the independent impact of circadian misalignment—similar to that typically experienced by shift workers—on cardiovascular disease risk factors assessed repeatedly across 24 h. Limitations of the study should also be considered. First, our participants were healthy and had little or no shift work experience. The effect of circadian misalignment on cardiovascular function and inflammatory markers may be different in people with hypertension and in shift workers. Second, our sample size was relatively small, although in keeping with similar highly controlled, within-participant studies. Thus, we may have been statistically underpowered to detect the impact of circadian misalignment on some of the outcome variables. Moreover, we were likely statistically underpowered to detect certain correlations between circadian misalignment-mediated changes in our primary outcome variables (e.g., blood pressure and inflammatory makers) and circadian misalignment-mediated changes in our explanatory/secondary outcome variables (i.e., sleep duration and autonomic markers). Third, we investigated the effect of circadian misalignment on a selection of cardiovascular- and inflammatory-related variables. Further studies are needed to test the impact of circadian misalignment on other cardiovascular (e.g., baroreflex sensitivity and endothelial function) and inflammatory markers (e.g., IL-8 and monocyte chemoattractant protein-1). Fourth, we assessed the impact only of short-term circadian misalignment on cardiovascular disease risk factors. The independent effect of chronic circadian misalignment on cardiovascular disease risk factors still needs to be studied. Future studies are warranted to identify countermeasures for the adverse effect of circadian misalignment on blood pressure and inflammatory markers, such as timing of exercise and diet. For example, we have shown that evening exercise decreases blood pressure during a subsequent simulated night shift (67).

Summary

We demonstrate that circadian misalignment per se increases blood pressure and inflammatory markers. Circadian misalignment caused by rapidly shifting the behavioral and light/dark cycles relative to the endogenous circadian system is typical in shift workers. Thus, our findings may help explain why shift work is a risk factor for elevated blood pressure, hypertension, and cardiovascular disease.

Methods

Other aspects of this study—which was designed to test separate hypotheses—have previously been published (35, 68).

Participants. Fourteen healthy, nonsmoking, drug- and medication-free (except for oral contraceptives) adults completed this study [mean ± SD (range) age, 28 ± 9 y (20–49 y); body mass index, 25.4 ± 2.6 kg/m^2 (21–29.5 kg/m^2); eight men]. The Partners Human Research Committee approved this research, which was conducted in the Center for Clinical Investigation at Brigham and Women's Hospital (Boston, MA). All participants provided written informed consent. See *SI Methods* for details.

Experimental Protocol. Each participant underwent two 8-d laboratory protocols, according to a cross-over design, to test the impact of circadian misalignment on cardiovascular disease risk factors (Fig. 1). One protocol included circadian misalignment, and the other maintained circadian alignment. The visits were separated by 2–8 wk [mean, 4 wk (SD, 2)]. "Minimization" was used to minimize imbalance—according to age, gender and body mass index—in the order of laboratory visits (circadian alignment protocol first, $n = 7$; circadian misalignment protocol first, $n = 7$) (69).

Preinpatient Study Conditions. Participants selected and maintained a normal sleep/wake schedule, with an 8-h sleep opportunity, for ≥11 d [mean, 17 d (SD, 3)] before each laboratory visit. Compliance was assessed with wrist actigraphy (Actiwatch Spectrum, Philips-Respironics; or Actiwatch-L, Mini Mitter), sleep diary, and daily bedtime and wake time calls to a time-stamped voicemail system [mean bedtime, 23:30 hh:mm (SD, 0:48); mean wake time, 7:21 hh:mm (SD, 0:41); data from seven sleep periods preceding the final ambulatory sleep period before both inpatient admissions]. On the night preceding each inpatient admission, participants were instructed to sleep between 11:00 PM and 7:00 AM to aid the adaptation of participants' endogenous circadian system to the initial laboratory sleep/wake schedule (sleep opportunity, 11:00 PM to 7:00 AM).

Inpatient Study Conditions. On the first day of each 8-d laboratory protocol, participants were admitted to the Center for Clinical Investigation at ∼10:30 AM, to undertake either the circadian alignment protocol or circadian misalignment protocol, in a cross-over design (Fig. 1). Participants remained in a private laboratory room throughout each laboratory protocol to allow strict control of environmental conditions. Participants were not permitted to exercise while in the laboratory. Participants completed computerized tests each "day" in the laboratory. In the circadian alignment protocol, participants' sleep opportunity occurred between 11:00 PM and 7:00 AM for days 1–8. In the circadian misalignment protocol, participants' sleep opportunity occurred between 11:00 PM and 7:00 AM for days 1–3. On day 4 of the circadian misalignment protocol, participants' behavioral cycle was shifted by 12 h, and this inverted cycle was maintained until the end of that protocol (day 8). The 12-h shift on day 4 was achieved by including an 8-h wake episode and a 4-h sleep opportunity, thereby maintaining the same sleep opportunity to wake ratio (1:2) in the circadian alignment and misalignment protocols. Light levels during the protocols are shown in Fig. 1.

Diet. Participants were given an ad libitum lunch around noon on the first day of each laboratory protocol. Thereafter, participants received an isocaloric diet, calculated according to the Harris–Benedict equation with an activity factor of 1.4. The diet consisted of 45–50% carbohydrate, 30–35% fat, and 15–20% protein, 150 mEq Na^+ (±20%), 100 mEq K^+ (±20%), and at least 2.5 L of water per 24 h. Participants were instructed to consume all food provided (verified by checking their food trays). Diet was identical within each participant between laboratory visits, except for the required and prorated additional food and water given during the 12-h behavioral cycle (day 4) in the circadian misalignment protocol (50% of the intake compared with the 24-h cycles). In the 24-h periods preceding test period 1 and 3 in the circadian alignment and circadian misalignment protocol, the participants' diet was identical, including the same exact meals (including the same calories, macronutrients, etc.) given at the same time relative to scheduled awakening.

Twenty-Four-Hour Blood Pressure and Heart Rate Measurements. Blood pressure and heart rate measurements started shortly after scheduled wake time until scheduled wake time 24 h later: i.e., between 7:00 AM and 7:00 AM in the circadian alignment protocol (wake and sleep period 5, and wake and sleep period 7) and between 7:00 PM and 7:00 PM in the circadian misalignment protocol (wake and sleep period 6, and wake and sleep period 8). Measurements were obtained with a Spacelabs 90217 ambulatory blood pressure monitor (Spacelabs Medical, Inc.), which has been validated according to the Association for the Advancement of Medical Instrumentation's standards (70).

Urine and Blood Sampling. Twenty-four-hour urinary epinephrine and norepinephrine levels were determined by collecting urine voids shortly after scheduled wake time until shortly after scheduled wake time 24 h later: i.e., between ∼7:00 AM and 7:00 AM in the circadian alignment protocol (wake period 5 and 6 and wake period 7 and 8) and between ∼7:00 PM and 7:00 PM in the circadian misalignment protocol (wake period 6 and 7 and wake period 8 and 9). Urine voids were scheduled every 4 h during the wake episodes and once after the 8-h sleep opportunities. Any extra voids were stored at 4 °C and pooled with the subsequent scheduled voids. See *SI Methods* for details.

To assess levels of inflammatory markers, PAI-1 and tPA activity, blood samples were collected every 4 hours between 11:53 PM and 7:53 PM in the circadian alignment protocol (sleep period 4 and wake period 5, and sleep period 6 and wake period 7) and every 4 hours between 11:53 AM and 7:53 AM in the circadian misalignment protocol (sleep period 5 and wake period 6, and sleep period 7 and wake period 8). See *SI Methods* for details.

Heart Rate Variability. For assessment of heart rate variability, a three-lead electrocardiogram was recorded on a Vitaport (TEMEC Instruments) between 10:40 PM and 10:35 PM in the circadian alignment protocol (sleep period 4 and wake period 5, and sleep period 6 and wake period 7) and 10:40 AM and 10:35 AM in the circadian misalignment protocol (sleep period 5 and wake

period 6, and sleep period 7 and wake period 8). Participants underwent 7 min of voluntary paced breathing at 10:20 AM and 10:20 PM in the circadian alignment protocol (wake period 5 and 7) and at 10:20 PM and 10:20 AM in the circadian misalignment protocol (wake period 6 and 8). Heart rate variability analyses were performed according to the standards of the Task Force (71). See *SI Methods* for details.

Polysomnography and Actigraphy. Sleep was recorded by PSG during sleep periods 1, 4, and 6 in the circadian alignment protocol and during sleep periods 1, 5, and 7 in the circadian misalignment protocol, as detailed previously (35). We used actigraphy data to estimate sleep duration for sleep periods in which PSG assessments did not occur (Actiwatch Spectrum; Philips-Respironics). See *SI Methods* for details.

Statistics. Statistical tests were performed with linear mixed models, with participant included as a random factor. We tested the effects of condition (circadian misalignment vs. circadian alignment), time into the behavioral cycle, and their individual and combined interaction with test period (1 vs. 3) (Fig. 1) on blood pressure, heart rate, cardiac vagal markers, epinephrine and norepinephrine excretion rates, and IL-6, CRP, resistin, PAI-1, and tPA concentrations. We also tested the effect of condition and its interaction with test period on the sleep opportunity-associated dipping in blood pressure and heart rate (calculated as the decrease from the wake period to the sleep opportunity as a percentage of the wake period) and 24-h area under the curve—calculated using the trapezoidal method—for epinephrine and norepinephrine excretion rates. Within-participant correlations were performed as previously described (72). Linear mixed models (participant included as a random factor) were also used for mediation analyses (73). Where appropriate, Bonferroni-adjusted multiple comparisons were performed. Where necessary, analysis was performed on log-transformed data. Statistical significance was accepted as $P < 0.05$. Data are presented as mean ± SEM, unless otherwise indicated.

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