Human circadian system causes a morning peak in prothrombotic plasminogen activator inhibitor-1 (PAI-1) independent of the sleep/wake cycle

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Key Points

- The human circadian system causes a morning peak in circulating levels of PAI-1, independent of any behavioral or environmental influences.
- The circadian system determines to a large extent the PAI-1 rhythm observed during a regular sleep/wake cycle.

Introduction

The ability to clot blood can be life-saving after an injury, but thrombi within vessels can contribute to myocardial infarction, ischemic stroke, and sudden cardiac death.¹² Ongoing fibrinolytic activity helps break down thrombi and maintain vessel patency and is largely managed by circulating tissue plasminogen activator, which converts plasminogen to plasmin.¹³ Fibrinolytic activity is significantly reduced in the morning due to an increase in plasminogen activator inhibitor-1 (PAI-1), the primary inhibitor of tissue plasminogen activator.¹³ Thus, increased PAI-1 and decreased fibrinolysis in the morning may increase the risk for development of occlusive thrombi and could help explain the morning peak in adverse cardiovascular events.¹² To begin to understand potential underlying mechanisms in humans, we tested the degree to which the morning increase in PAI-1 is caused by a direct endogenous circadian rhythm in PAI-1 vs influences from the daily behavioral/environmental changes across the night and day (eg, rest/activity, fasting/feeding, dark/light, and ambient-temperature cycles).

Methods

We studied 12 healthy volunteers taking no medications (mean [range], 25.8 [20-42] years; body mass index, 23.6 [19.9-29.6] kg/m²; 6 women). Studies were approved by the Partners Human Research Committee, and participants gave written informed consent in accordance with the Declaration of Helsinki.

Participants were assessed throughout a 2-week laboratory protocol designed to desynchronize daily behavioral rhythms from internal circadian rhythms, while maintaining environmental factors constant. Participants had 2 baseline 24-hour days in normal room lighting conditions (~90 lux wake episodes/0 lux sleep episodes), followed by a forced desynchrony protocol (FD) consisting of 12 standardized 20-hour “days” with controlled activity, posture, meals, sleep, room temperature, and light (<4 lux), as previously published.⁹¹² On the second baseline day and each 20-hour day, participants underwent a standardized test battery,¹² which included a 15-minute 60° passive head-up tilt test and a 15-minute steady-state cycle ergometer test at 60% of maximal predicted heart rate. Core body temperature derived from a rectal thermistor was continuously recorded and used to determine circadian period and phase, with minimum core body temperature assigned as 0° (equivalent to ~4:30 AM in these participants).⁸ Blood samples were collected by intravenous catheter in EDTA tubes on ice, immediately spun down in a precooled centrifuge (4°C) for 10 min at 2200 rpm, and frozen at ~80°C until assayed for PAI-1 antigen by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). We note that PAI-1 antigen and PAI-1 activity are highly correlated.¹¹ The normal daily pattern of plasma PAI-1 antigen was assessed from samples taken hourly across 24 hours on the second baseline day and night. Thereafter, the effect on PAI-1 of the endogenous circadian system, standardized activities, and their interaction was assessed from 4 samples taken across a range of activities on each of the 12 test batteries (so that all circadian phases were represented). The 4 standardized activities each day throughout the FD were supine rest (after 15
Results and discussion

From group analysis of the FD data, there was a large amplitude endogenous circadian rhythm in PAI-1 with a peak at a circadian phase of 30°, corresponding to ~6:30 AM (~1 hour prior to habitual wake time) and a trough at a phase corresponding to ~3:30 PM (peak-to-trough amplitude = 1.24 ng/mL; 82% of mean; P < .0001; Figure 1A,C) with an increase from trough to peak of 124%. Although there was an effect in anticipation of the stress battery and not before, we likely missed any such anticipation-triggered PAI-1 changes in the circadian analyses.

Previous studies have shown a significant daily rhythm in PAI-1 in humans, but the role of the endogenous circadian system vs behaviors was not established. Our main finding is that the endogenous circadian system appears to be the major contributor to the daily changes in PAI-1 seen during a normal sleep/wake cycle in humans. Although behavioral or environmental factors such as activity level could also influence PAI-1, our 15-minute mild stress tests likely did not capture the full range of PAI-1 changes that can be induced by behavior.

This complexity is highlighted by a study that found a blunted PAI-1 rhythm in 2 blind individuals who were not synchronized to the 24-hour light/dark cycle. In animals, lesioning the central circadian pacemaker (suprachiasmatic nucleus) abolished the daily rhythm in PAI-1 but also abolished behavioral rhythmicity so it could not be determined if the PAI-1 rhythm is driven directly by the circadian system or indirectly through behavior. Nonetheless, recent genetic studies in animals have demonstrated that the PAI-1 promoter is under direct control of core clock genes and, in humans, a PAI-1 promoter polymorphism (4G5G) was found to increase morning PAI-1. The complexity is further highlighted by a study that found a blunted PAI-1 rhythm in 2 blind individuals who were not synchronized to the 24-hour light/dark cycle. In animals, lesioning the central circadian pacemaker (suprachiasmatic nucleus) abolished the daily rhythm in PAI-1 but also abolished behavioral rhythmicity so it could not be determined if the PAI-1 rhythm is driven directly by the circadian system or indirectly through behavior. Nonetheless, recent genetic studies in animals have demonstrated that the PAI-1 promoter is under direct control of core clock genes and, in humans, a PAI-1 promoter polymorphism (4G5G) was found to increase morning PAI-1.

Furthermore, a genome-wide association study identified a common variant in the core clock gene BMAL-1 (ARNTL) to be robustly associated with elevated morning PAI-1 levels. Building on this work, we have now discovered that there is a true endogenous circadian rhythm in circulating PAI-1 in humans, independent of the behavioral and environmental factors. Moreover, the circadian peak in PAI-1 at ~6:30 AM is at the start of the vulnerable window for adverse cardiovascular events (6 AM to noon), as we hypothesized based on the possibility that the circadian system causes a prothrombotic state in the morning. One could speculate that evolutionary pressures shaped the circadian influences to favor blood clotting (ie, elevated PAI-1) at times of increased activity after sleep when risk of blood loss due to laceration injury was increased, eg, due to predator/prey/competitor encounters. On the other hand, this large endogenous circadian
We previously found that other cardiovascular risk factors are under direct circadian control, including platelet activation, count, and aggregability, plasma epinephrine and norepinephrine, plasma cortisol, systolic and diastolic blood pressure, heart rate, and vagal cardiac modulation.\textsuperscript{9,12} Of these factors, platelet activation and cortisol had endogenous circadian peaks, and plasma epinephrine the steepest circadian increase, during the vulnerable window for adverse cardiovascular events, around 9 AM. Furthermore, in response to exercise, the largest circadian increase in epinephrine and largest circadian decrease in cardiac vagal modulation also occurred around 9 AM. Together with these previous data, our current data suggest that the circadian system could contribute to the increased risk for cardiovascular events in the morning via a number of related cardiovascular mechanisms. Such mechanisms may have homeostatic advantage in most circumstances in anticipation of the expected behavioral changes in the morning, such as vagal withdrawal, sympathetic activation, and increased blood clotting. However, these same physiological adaptations could exacerbate existing risk factors in certain individuals, such as those with vulnerable atherosclerotic plaques in a coronary artery.\textsuperscript{7}

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**Authorship**

Contribution: F.A.J.L.S. and S.A.S. designed and performed research and wrote the paper; and F.A.J.L.S. analyzed data.

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**References**


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