Supporting Information

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SI Methods

Supporting Information on Exercise Test Battery. On the first baseline day, subjects performed an initial test exercise session on the ergometer cycling at 70 rpm to determine the personalized workload to achieve a target HR level of ≈60% of the estimated maximum heart rate (HR) (using the formula: 220 − age). Because we wanted to test the physiological response to an identical workload at different circadian phases and because the HR during exercise could be different at different circadian phases, subjects performed each subsequent exercise test with this same and constant workload at 70 rpm.

Supporting Information on Blood/Plasma Assays. Plasma epinephrine and norepinephrine were assayed by RIA (IBL America; sensitivity: 1 and 40 pg/mL, respectively) and cortisol by chemiluminescent assay (Beckman Coulter, Access; sensitivity: 0.4 μg/dL). For whole blood platelet aggregability, 450 μL of whole blood (samples collected in sodium citrate tubes) was diluted with 450 μL of physiological saline (NaCl) at 37 °C. Platelet aggregability was assessed within 20 min of the blood draw as the area under the curve for the impedance signal (Ohm × min) for platelet aggregability across the 10 min after stimulation with 5 μg/mL collagen in duplicate by dual channel whole blood/optical lumi aggregometer (560VS; Chrono-Log) and analyzed with Chrono-Log software (Aggro/Link for Windows). For quality control, aggregometry data were excluded if duplicates differed by >25% from the mean (excluding 18% of data), resulting in a coefficient of variation of 7%. Platelet count was analyzed by FACSCalibur (Becton Dickinson) flow cytometer, as described (1).

1. Linden MD, et al. (2004) Application of flow cytometry to platelet disorders. *Semin Thromb Hemost* 30:501–511.

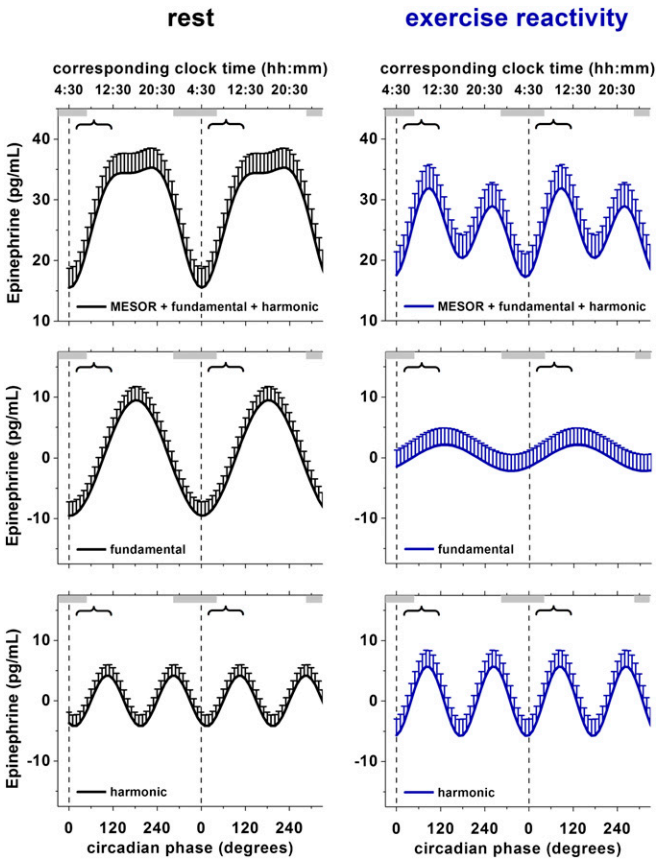

Fig. S1. Example of Cosinor model fit to circadian data. Shown are the cosinor fits to plasma epinephrine data at rest (*Left*) and cosinor fits to the change from rest induced by standardized exercise (exercise reactivity; *Right*). Each model reflects the summed MESOR, fundamental, and harmonic components (*Top*), the fundamental component alone (*Middle*), and the harmonic component alone (*Bottom*). Lines, model fit; error bars, SEM; gray bars, group average timing of habitual sleep episodes; vertical dotted lines, timing of the core body temperature minimum as circadian phase marker; curly brackets, most vulnerable period for adverse cardiovascular events observed in epidemiologic studies (≈6:00 AM–noon).

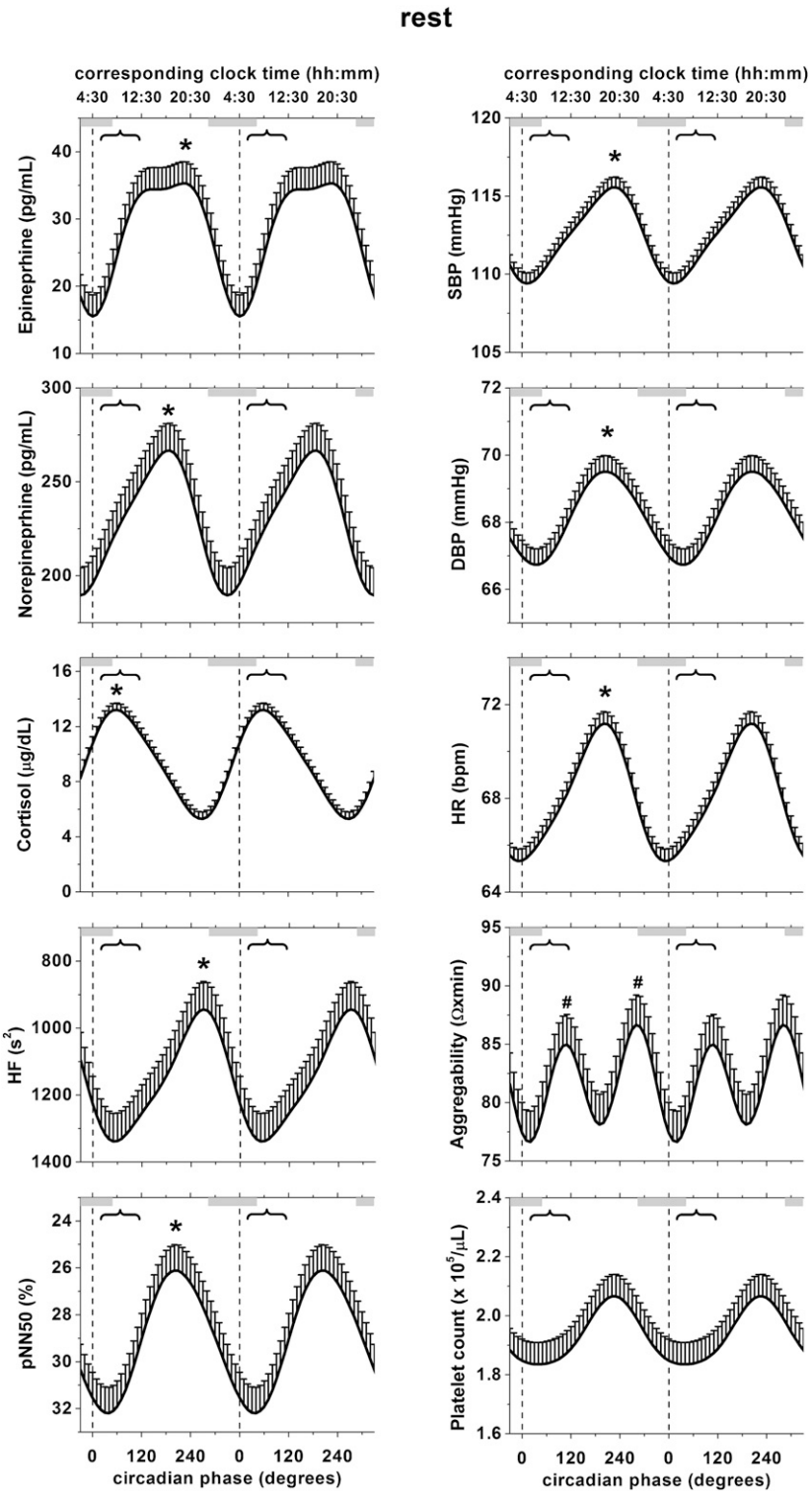

Fig. S2. Influence of the endogenous circadian system on cardiovascular function at rest. Cosine models of variables at rest revealed a significant fundamental circadian rhythm for epinephrine, norepinephrine, cortisol, HF, pNN50, SBP, DBP, and HR, and a harmonic rhythm (i.e., with a period of ≈12 h) for platelet aggregability. Platelet count did not show significant rhythmicity. Note, so that higher putative risk is upwards for all variables, the *y* axes for the vagal markers HF and pNN50 (*Bottom Left*) are inverted, i.e., upwards corresponds to vagal withdrawal. This is different from the orientation of these variables in Fig. 2 of the main manuscript. Symbols as in Fig. S1. *Timing of the peak for significant fundamental circadian cycle; #timing of the peaks for significant second harmonic of circadian cycle.

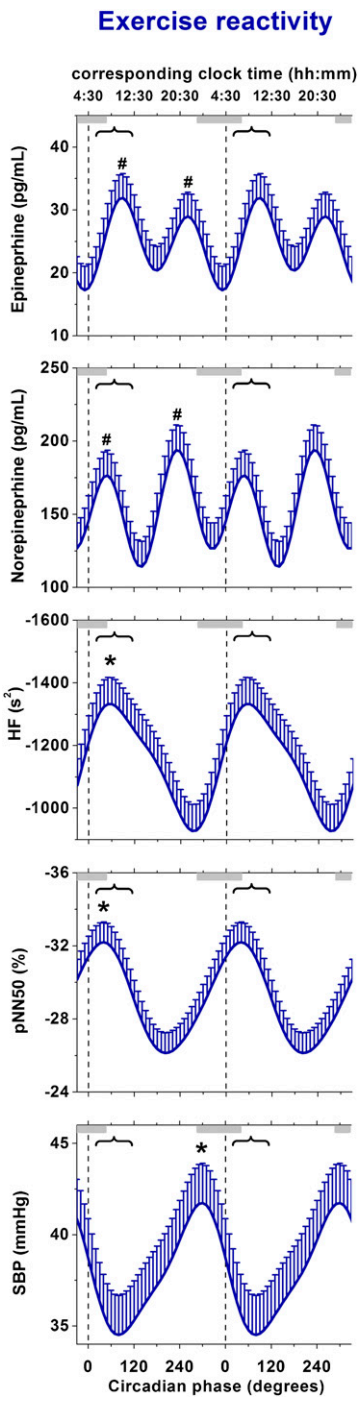

Fig. S3. Influence of the endogenous circadian system on cardiovascular reactivity to exercise. Cosine models of reactivity to exercise revealed significant circadian rhythmicity for HF, pNN50, and SBP, and significant second harmonics of the circadian rhythmicity for epinephrine and norepinephrine. The other variables did not have statistically significant rhythmicity ($P > 0.05$). Although a circadian variation in the magnitude of reactivity to exercise (vertical difference between red and black line) can be observed in Fig. 2, the statistically significant 12-h rhythmicity is more apparent in this figure because this is based on the cosine model that uses circadian phase data with a resolution of 1 circadian degree, whereas Fig. 2 shows the data grouped into 60° windows, which causes slight visual differences that do not affect the statistical analysis (all statistical analysis based on cosinor models). Note, so that higher putative risk is upwards for all variables, the *y* axes for the vagal markers HF and pNN50 are inverted, i.e., upwards corresponds to vagal withdrawal. This is different from the orientation of these variables in Fig. 2 of the main manuscript. Symbols as in Fig. 2. *Timing of the peak position for significant fundamental circadian cycle; #timing of the peak positions for significant second harmonic of circadian cycle.

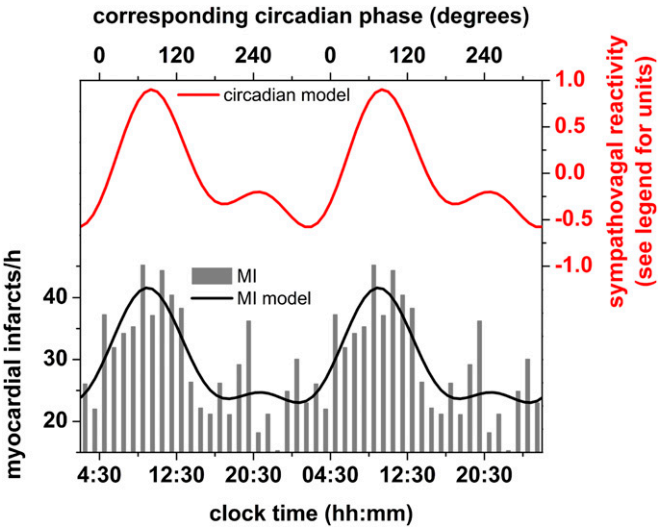

Fig. S4. Conceptual model of multiple circadian factors potentially contributing to day/night pattern of cardiovascular risk. In our data, no single variable exhibited a circadian profile that, when normally aligned with the wake/sleep cycle, matched the day/night pattern in cardiovascular events (primary morning peak, secondary evening peak, and overnight trough). Thus, we developed a simplified conceptual model in which combinations of risk factors could summate to produce the day/night pattern in cardiovascular events. Gray vertical bars, distribution of myocardial infarctions (MI) across the day/night cycle from epidemiological data (redrawn from ref. 1); black line, cosinor model of the distribution of myocardial infarctions (fundamental plus one harmonic; MI model; left axis); red line, the summation of cosinor fits of a sympathetic marker (epinephrine responses to exercise across the circadian cycle; Epi; see Fig. S3, *Top Upper*) plus an inverted parasympathetic marker (high frequency power; HF; see Fig. S3, *Middle*) derived from spectral analysis of R-R interval data. HF is inverted on the *y* axis so that higher putative risk (i.e., vagal withdrawal) is upwards as in Figs. S2 and S3. In this example, the sympathetic and inverted vagal measures were summated after normalization of both measures by subtracting the mean and setting the peak-to-trough difference at 1 (right axis). In this conceptual model, the endogenous sympathovagal changes in response to exercise across the circadian cycle yield a day/night pattern of ostensible risk (red line) that is similar to the day/night pattern in myocardial infarctions (MI) in epidemiological data (black line; MI model; assuming that average wake time is approximately 7:00 AM). This model is a simple conceptual model, and we acknowledge that there are several limitations. For instance, we have simply normalized and summated the sympathetic and vagal markers, whereas most cardiovascular variables interact. In addition, we do not know the timing and amplitude of the circadian modulation of sympathovagal balance in vulnerable subjects. It is conceivable that similar profiles could be achieved with other variables. Finally, the day/night distribution of behavior may play an important contributing role in the profile of adverse cardiovascular events.

1. Muller JE, et al. (1985) Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med* 313:1315–1322.