Supplementary Methods

Participants: We studied 10 participants (6 female) during an established circadian 'forced desynchrony' (FD) protocol[1,2]. Participants were healthy except for mild untreated hypertension in one female. Studies were approved by the Institutional Review Board for human subject protection at Oregon Health & Science University and all participants provided written informed consent.

Clinical screening: Health status was based on self-report, physical and psychological examination by physician including a 12 lead electrocardiogram, three repeated blood pressure measurements in the laboratory plus 48-h blood pressure monitoring (Spacelabs Healthcare, WA, USA), at home sleep apnea screening (WatchPAT, Itamar Medical, Israel), and laboratory testing of hematological and metabolic measures (i.e., hemoglobin, hematocrit levels, basic blood chemistry, and blood glucose). Exclusion criteria included pregnancy, history of chronic disease and smoking, use of any prescription or non-prescription medications (Drugsmart 12 panel cup, Speares Medical, SC, USA) or nicotine products (cotinine: NicAlert®, Nymox Corporation, NJ, USA) and, to ensure stability of circadian rhythmicity, histories of travel across more than three time zones in the last 3 months or shift work for the past 6 months.

Home routine: For at least one week prior to entering the laboratory, participants maintained a constant self-selected 8-h sleep schedule to stabilize sleep-wake patterns and circadian rhythmicity (verified by actigraphy; ActiGraph wGT3X-BT, Actigraph Corporation, FL, USA; phone calls to a time stamped mailbox at bedtime and upon awakening; and a written sleep diary). For this at-home period, participants refrained from any medication, food supplements, caffeinated food and beverages, alcohol, all high intensity exercise, and light intensity physical activity for >45 min per day.

Circadian (Forced Desynchrony) Protocol: In order to study the endogenous circadian rhythmicity in rate of perceived exertion (RPE), participants completed an FD protocol in a time-isolated and temperature-controlled environment consisting of ten consecutive 5-h 20-min sleep/wake cycles (Research Letter: Figure 1A).[1,3] Upon admission to the Oregon Clinical and Translational Research Institute (OCTRI) laboratory, a drug screening test and a pregnancy test (in premenopausal females) were performed to ensure participants do not meet exclusion criteria. Subsequently, participants were instrumented with complete polysomnography and an IV–catheter was inserted in the non-dominant arm. The FD consisted of a baseline 8-h sleep opportunity at the participant's habitual sleep time and a baseline day followed by 10 identical recurring 5-h 20-min sleep/wake cycles (2-h 40-min sleep opportunity and 2-h 40-min scheduled wakefulness) to desynchronize the behaviors from the endogenous circadian system[4]. Light levels were <3 lux at the horizontal angle of gaze during scheduled wakefulness to prevent light-induced phase shifts of the circadian clock[5], and were <0.1 lux during scheduled sleep periods. At the end of each scheduled sleep episode, participants were gently awoken in a standardized fashion by use of a mild auditory stimulus. Thereafter, across each 2-h 40-min period of wakefulness, all activities were scheduled with the same sequence and included: a vascular endothelial function assessment, a short cognitive test battery, a 15-min period of mild intensity cycle-ergometer exercise (<50% max predicted heart rate using the Karvonen's formula[6]), and consumption of a small balanced meal representing 22% of the 24-h total isocaloric requirement.

Measurements:

Salivary melatonin: Saliva was regularly collected using a cotton swab (Salivette®, Starstedt Inc, NC, USA), spun and frozen until performance of radioimmunoassay for assessment of melatonin (Kennaway G280 anti-melatonin antibody; Bühlmann Laboratories, Schönenbuch, Switzerland). The in-house interassay CV was 11%.

Circadian phase and period in the FD Protocol: Dim-light melatonin onset (DLMO) was used as the circadian phase marker for alignment of all other data[7]. For each day we determined the DLMO as the linear interpolated time-point when salivary melatonin exceeded 3 pg/ml[7]. In one participant whose salivary melatonin never dipped below 3 pg/ml, 4 pg/ml[7] was used as the threshold. Circadian period was calculated for each day as the time between consecutive DLMOs. For each participant, their average circadian period and DLMO were computed.

References:

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