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Correlation of Heart Rate Variability and Circadian Markers in Humans

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Abstract—**The frequency of adverse cardiovascular events is greater in the morning compared to its 24-hour average. A circadian variation in the regulation of the cardiovascular system could contribute to this increased cardiovascular risk in the morning. Indeed, circadian rhythms have been shown for a wide array of physiological processes. Using an ultradian sleep-wake cycle (USW) procedure, we sought to determine how heart rate (HR) and heart rate variability (HRV) correlate with the well-characterized circadian rhythms of cortisol and melatonin secretion. Specific HRV components, namely the low frequency (LF) power, high frequency (HF) power, and the LF:HF ratio can be used as markers of the autonomic modulation of the heart. Cross-correlation between HRV parameters and hormonal rhythms demonstrated that mean RR interval is significantly phase-advanced relative to salivary cortisol and urinary 6-sulfatoxy-melatonin (UaMt6s). Parasympathetic modulation of the heart (HF power) was phase-advanced relative to cortisol, but was in-phase with UaMt6s levels. Maximal correlation of the sympathovagal balance (the LF:HF ratio) had no significant lag compared to cortisol secretion and UaMt6s excretion. The protective effect of the parasympathetic nervous system at night, combined with the putative risk associated with the sympathetic nervous system peaking in the morning, could be associated with the increased cardiovascular risk observed in the morning hours.**

I. Introduction

MANY different epidemiologic factors contribute to the high incidence of cardiovascular diseases in our industrialized society. External factors such as diet, lack of exercise, and stressful work environment combined with internal physiological factors contribute to increased risk of cardiovascular diseases. Studies have reported a higher risk of suffering from different cardiovascular events in the morning as compared to the 24-hour average [1]. This diurnal distribution of cardiovascular events cannot be entirely explained by awakening in the morning and remains to be clarified.

In mammals, a wide range of physiological processes demonstrate circadian rhythms (i.e. rhythms of ~ 24 hours). These rhythms are driven by an endogenous central pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Indeed, the SCN acts as the major pacemaker to coordinate the diurnal variation of several physiological processes, including hormonal secretion, body temperature, and activity levels.

Circadian rhythms in humans are best measured in a highly controlled laboratory environment in order to limit/remove confounding factors such as light intensity, activity levels and social interactions. Using rigorous protocol designs, circadian rhythms of different physiological processes have been described. In humans, cortisol has a well-defined circadian rhythm with peak levels around awakening and minimal levels early at night. This hormone is released by the adrenal glands as part of the hypothalamic-pituitary-adrenal axis and is acutely responsive to stress. Melatonin is known to be secreted by the pineal gland at night, and is at low levels during the day. Melatonin secretion at night is highly inhibited by light.

Heart rate (HR) also follows a circadian rhythm, with higher levels during the day compared to the night [2]. Heart rate variability (HRV) can be used as a marker of the autonomic modulation of the heart. HRV is calculated using frequency or time domain analyses of the HR signal [3]. Several studies report circadian rhythms of HRV [4-8]. Thus, a circadian variation in autonomic cardiac modulation could contribute to the observed distribution of adverse cardiovascular events across the day.

The aim of this study is to use a rigorous laboratory protocol to investigate correlations of HR and HRV with well characterized circadian markers, namely cortisol and melatonin.

II. Methods

A. Subjects

Eight healthy young subjects (7 men, 2 women; mean age ± SD: 27.1 ± 4.4 years) were recruited to participate in a 5-day study. Exclusion criteria included sleep disorders, any medical conditions, illicit drug use, excessive tobacco or

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alcohol use, and night shift work or transmeridianal travel within the last 3 months. Women were excluded if they were using oral contraceptives or if they did not have a regular menstrual cycle. Women were studied during the follicular phase of their menstrual cycle and confirmed ovulation via plasma progesterone test on day 20 and 23 of their menstrual cycle prior to laboratory entry. For at least 3 weeks prior to admission, subjects maintained a regular sleep-wake cycle (8 hours of sleep per night) according to their habitual sleep-wake schedule. This was verified by daily phone calls to the laboratory, a sleep-wake log, and actigraphic monitoring during the week before admission (AW-64, Mini Mitter Respironics, Bend, Or, USA). All participants signed informed consent forms.

B. Protocol

Participants entered a time isolation suite on the evening of day 1. Recording equipment was installed and participants were familiarized with the laboratory procedures on the first day of the study. Participants were then prepared for their first sleep episode and lights were turned off based on their habitual bedtime, as reported in their sleep-wake log. After an 8-hour baseline sleep episode, subjects underwent an ultradian sleep-wake cycle (USW) procedure for 72 hours (Fig. 1). This procedure consisted of 60-minute wake episodes in dim light (<10 lux) alternating with 60-minute nap opportunities in total darkness. Throughout the USW procedure, participants remained in semi-recumbent position in bed, with low activity levels. Meals were replaced by balanced iso-caloric snacks administered during each wake episode. These conditions were imposed in order to limit the masking effects of activity, food intake, and light levels on

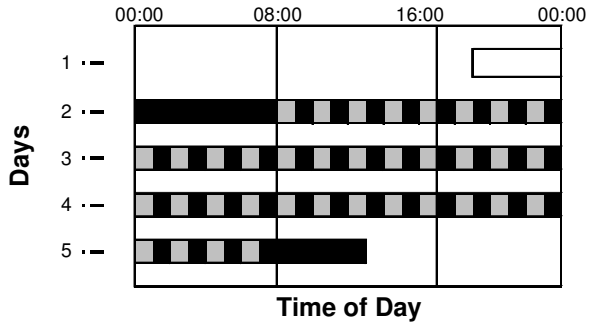

Fig. 1. USW procedure. Following an 8-hour baseline sleep episode (day 1-2), subjects began an USW cycle procedure for 72 hours (days 2-5). White bars represent waking episodes in 150 lux, grey bars represent waking episodes in dim light (<10 lux) and black bars represent sleep episodes in total darkness (<0.3 lux).

outcome measures. The USW procedure was concluded with an ad-libitum sleep episode on day 5.

C. Measurement and Data Processing

Core body temperature (CBT) was continuously recorded (4x/minute) throughout the experiment using a thermistor (Steri-Probe, Cincinnati Sub-Zero Products Inc., Cincinnati, OH, USA) inserted 10 cm into the rectum and connected to an in-house data acquisition system. Probe slips and malfunctions were removed by an ad-hoc program which excludes data < 36°C or > 38°C, or if the rate of change is > 0.2°C/minute. Data were visually inspected before discarding values. CBT data were subsequently averaged into 1-minute bins for further analysis.

Electrocardiogram (EKG) was continuously (200 Hz) monitored throughout the USW procedure using a special vest with built-in electrodes (LifeShirt, Vivometrics, Ventura, CA, USA). RR interval data was extracted from the EKG using an automatic-detection software (VivoLogic, Vivometrics, Ventura, CA, USA). Ectopic beats and artifacts were removed by visual inspection of the RR interval traces. RR interval time series were re-sampled at 2.4 Hz using cubic interpolation. Discrete wavelet transform (DWT) was applied to the RR interval signal in order to decompose it into the frequency domain. In DWT, a defined wavelet is slid along the signal at different scales, and a correlation coefficient is evaluated at each wavelet position. The Daubechies 12 wavelet was selected for use in the current DWT [9]. After reconstruction of the signal, HRV was described as the power density in two standard frequency bands (High frequency [HF]: 0.15-0.40 Hz; Low frequency [LF]: 0.04-0.15 Hz). The LF:HF ratio was also calculated. HF is considered to be a measure of the parasympathetic modulation of the heart whereas the LF:HF ratio is considered to reflect the sympathovagal balance [3]. HRV data were subsequently averaged into 1-minute bins and only data obtained during wake episodes were used for further analysis. HRV data is expressed as deviation from the average of the 24-hour period, in percentage. This average was based on data collected during all wake-nap episodes.

Saliva (1x/hour) and urine (1x/ 2 hours) samples were collected throughout the USW procedure. Saliva samples were assayed for their content in cortisol using a sensitive enzyme immunoassay kit (Salimetrics, State College, PA, USA). All cortisol assays were done in duplicate. The limit of detection of this assay was 0.012 ug/dl for a range of 0.012-3 ug/dl. The intra-assay coefficient of variation was 2.14%. Urine samples were assayed for they content in 6-sulfatoxy-melatonin (UaMt6s), the main melatonin metabolite. UaMt6s concentration was measured in duplicate using commercially-available radioimmunoassay kit (Stockgrand Ltd, Surrey, UK). The sensitivity of the assay was 0.05 ng/ml, with a coefficient of variation ranging from 11.3 to 12.4%.

D. Analysis

A dual-harmonic regression model without serial correlated noise (courtesy of C.A. Czeisler, Brigham and Women's Hospital, Boston, MA, USA) was used to assess the CBT minimum of each subject. HRV measurements and hormonal data were assigned a circadian phase between 0° to 359.9° relative to the individual CBT minimum (set at 0°).

HRV and hormonal data were subsequently grouped in

	Salivary cortisol		*UaMt6s*	
	Phase Difference (h)	Correllation Coefficient	Phase Difference (h)	Correllation Coefficient
RR interval	6.25 ± 0.70 *	0.375	2.57 ± 0.84 *	0.483
LF power	5.75 ± 1.53 *	0.391	2.57 ± 1.36	0.474
HF power	8.25 ± 1.91 *	0.388	1.71 ± 2.11	0.478
LF:HF ratio	2.25 ± 2.09	0.439	-0.86 ± 1.62	0.428

Table 1. Maximal correlation and correlation coefficient between the HRV parameters, and salivary cortisol and UaMt6s. Values are expressed in decimal hours. A positive phase difference indicates an advance of the HRV parameter series compared to the hormonal series. * indicates phase difference significantly different than zero. Values are expressed as mean ± SEM.

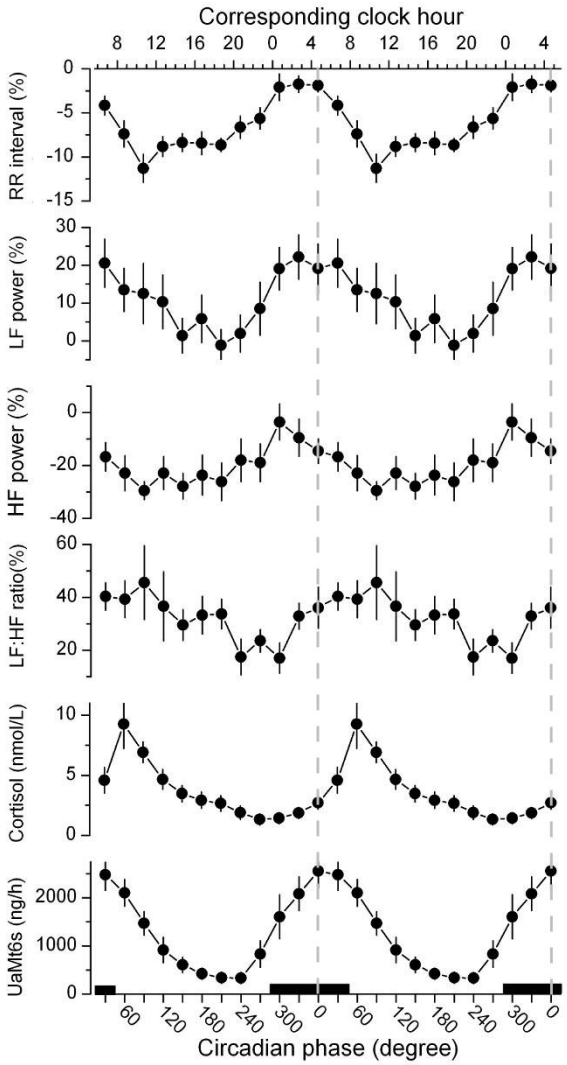

Fig. 2. HRV and hormonal data during the USW procedure. HRV component are expressed as percentage of the total 24-hour mean. Black bars represent time of projected habitual nocturnal sleep episode. Values are expressed as mean ± SEM.

30° bins, then folder every 360° in order to obtain one complete 360° (i.e. 24 hours) cycle for each subject.

In order to define phase difference between hormonal and HRV rhythms, cross-correlation analysis was used. Specifically, maximal cross-correlation coefficients were calculated between 30° binned cortisol and HRV data for each subject. Maximal cross-correlation coefficients were also individually calculated between UaMt6s and HRV data for each subject. Phase differences corresponding to the maximum correlation were then averaged across subjects. Maximum correlation phase-lags are reported in decimal hours (15°=1 hour). A positive phase-lag indicates an advance of the HRV rhythm relative to the hormonal circadian rhythm, whereas negative lag indicates the opposite.

Paired t-tests were used to compare the maximum cross-correlation coefficients to zero in order to assess if there was a significant phase-lag between HRV and hormonal data. Significance level was set at $p \leq 0.05$. All results are expressed as mean ± SEM.

III. Results

Maximum correlation of RR interval series, LF power, and HF power showed significant phase leads relative to cortisol secretion ($p \leq 0.004$). These HRV parameters were phase-advanced by 5.75–8.25 hours relative to salivary cortisol (Table 1, Fig. 2). In comparison the LF:HF ratio rhythm had no significant phase difference compared to that of salivary cortisol ($p=0.158$).

The rhythm of mean RR interval was phase-advanced by 2.57 hours relative to the UaMt6s excretion rhythm ($p=0.022$, Table 1, Fig. 2). The rhythm of LF power ($p=0.108$), HF power ($p=0.448$) and the LF:HF ratio ($p=0.617$) had no significant phase-lag relative to the UaMt6s excretion rhythm.

UaMt6s excretion was significantly phase-advanced relative to salivary cortisol secretion by 3.71 ± 0.52 hours ($p<0.001$).

IV. Conclusion

Mean RR interval was advanced relative to the salivary cortisol and UaMt6s rhythms. Mean RR interval was higher at night compared to daytime. A circadian rhythm of the mean RR interval series has been shown in many studies, with high levels at night and low levels during the day [2, 5-8, 10-13].

Our results indicate that the variation in parasympathetic modulation of the heart (HF power) is phase-locked to that of UaMt6s excretion (lag ~ 0°), but phase-advanced relative to salivary cortisol secretion. These results are consistent with other studies using protocols such as sleep deprivation [12, 14], constant routine [4, 5, 8, 15], or forced desynchrony [6, 13]. These studies showed a circadian rhythm in parasympathetic modulation of the heart, with higher levels at night. Combined with our results, these observations suggest that peak parasympathetic modulation of the heart is coincidental with peak UaMt6s excretion and with the nocturnal sleep period.

The phase-lag between salivary cortisol and the sympathovagal balance (the LF:HF ratio) was not different than zero, suggesting that both parameters vary in phase with each other. Similar results were reported by Anders and colleagues [7] using a 40-hour constant routine protocol where the LF:HF ratio peaked around the time of habitual awakening. Our results and those of Anders et al. [7] suggest that cortisol secretion and the sympathetic activation of the heart coincide and peak in the morning near the habitual wake time. Consistent with our results, a recent study showed the greatest rise in plasma epinephrine, a marker of sympathetic activity, in the morning [13]. Our experiment did not reveal a significant phase-lag between the rhythm of UaMt6s excretion and that of the LF:HF ratio. However, the UaMt6s rhythm was significantly phase-advanced relative to that of salivary cortisol. These results suggest peak LF:HF ratio occurring between peak UaMt6s and cortisol levels. It is thus difficult to draw definitive conclusion from our study regarding the specific association between peak sympathetic tone and either melatonin or cortisol secretion.

Throughout the present study, subjects had an opportunity to sleep every other hour. Even though data were collected during wake periods, sleep could exert a non-linear masking effect on HRV. Despite this limitation, our results support those observed by other groups using various experimental approaches.

Our results are consistent with Sheer et al. [13] interpretation that the reduction in the protective effect of the parasympathetic modulation of the heart combined with the potentially harmful effect of sympathetic activation at the end of the night can contribute to the circadian pattern in cardiovascular adverse events.

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