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Influence of sleep duration and sex on age-related differences in heart rate variability: Findings from program 4 of the HAIE study

Joaquin U. Gonzales [a, *], Steriani Elavsky [b, c], Lukáš Cipryan [b], Vera Jandačková [b, d], Michal Burda [e], Daniel Jandačka [b]

[a] Department of Kinesiology and Sport Management, Texas Tech University, Lubbock, TX, USA
[b] Department of Human Movement Studies, University of Ostrava, Ostrava, Czech Republic
[c] Department of Machine Learning and Data Processing, Masaryk University, Brno, Czech Republic
[d] Department of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic
[e] Institute for Research and Applications of Fuzzy Modeling, University of Ostrava, Czech Republic

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ABSTRACT

Heart rate variability (HRV) is an important marker of cardiac autonomic regulation and health. We examined the influence of sleep duration and sex on HRV in younger and middle-aged adults. Cross-sectional data (888 participants, 44% women) were analyzed from Program 4 of the Healthy Aging in Industrial Environment study (HAIE). Sleep duration was measured across 14 days using Fitbit Charge monitors. Short-term EKG recordings were used to evaluate HRV in the time (RMSSD) and frequency domains (low frequency (LF) and high frequency (HF) power). Regression analysis showed age was associated with lower HRV across all HRV variables (all $P < 0.001$). Sex was a significant predictor for LF ($\beta = 0.52$) and HF ($\beta = 0.54$; both $P < 0.001$) in normalized units. Similarly, sleep duration was only associated with HF in normalized units ($\beta = 0.06$, $P = 0.04$). To explore this finding further, participants within each sex were separated into groups based on age (<40 and ≥ 40y) and adequate sleep duration (<7 and ≥7 h). Middle-aged women with sleep durations <7 h, but not ≥7 h, had lower HRV than younger women after adjusting for medications, respiratory frequency, and cardiorespiratory fitness (peak VO_2). Middle-aged women with sleep durations <7 h also had lower RMSSD (33 ± 2 vs. 41±4 ms, $P = 0.04$), HF power (5.6 ± 0.1 vs. 6.0 ± 0.1 log ms^2, $P = 0.04$), and HF in normalized units (39 ± 1 vs. 48 ± 2, $P = 0.01$) than middle-aged women with sleep durations ≥7 h. In contrast, middle-aged men irrespective of sleep duration had lower HRV than younger men. These results suggest that adequate sleep duration may positively influence HRV in middle-aged women but not men.

1. Introduction

The National Sleep Foundation [1] and other health organizations including the American Heart Association [2] recommend at least 7 h of sleep per night in young and older adults. This duration of sleep is considered adequate to promote optimal health while sleeping less than 7 h is associated with an elevated risk of developing or dying from coronary heart disease or stroke [3,4]. Aging presents a challenge to this sleep recommendation as sleep duration is observed to decrease by about 10 min per decade with advancing age [5,6]. Thus, more research is needed to understand the health benefits of sleep duration in adults belonging to different age groups.

Heart rate variability (HRV) is a recognized indirect measure of the cardiac autonomic nervous system. Lower HRV has been reported to predict the development of cardiovascular disease [7] and new cardiac events in older adults [8]. Aging decreases HRV [9–12], often characterized as attenuated parasympathetic modulation of the heart. Sex differences in HRV have been reported such that young and middle-aged women tend to have higher parasympathetic modulation of the heart than age-matched men [10,11,13]. This sex difference in HRV, however, diminishes with advancing age [11,12] resulting in no differences in HRV between older women and men [9,14].

There have been few studies in the general population that have

* Corresponding author. Department of Kinesiology and Sport Management, Texas Tech University, Box 43011, Lubbock, TX, 79409-3011, USA.
E-mail address: joaquin.gonzales@ttu.edu (J.U. Gonzales).

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examined the influence of habitual sleep duration on HRV. The Multi-Ethnic Study of Atherosclerosis (MESA) objectively measured sleep duration using actigraphy and found adults that had inadequate sleep durations (>6 h but less than 7 h) had lower parasympathetic modulation of the heart as compared to adults that slept 7 h or more [15]. While sex was included as a covariate, the MESA study did not examine the influence of sleep duration on HRV within women and men separately. This is important since HRV differs between women and men [10,11,13], possibly based on age [9,14], which may introduce age-related variability in the relationship between sleep duration and HRV. Therefore, the purpose of this study was to examine the influence of sleep duration and sex on age-related differences in HRV.

2. Methods

2.1. Ethics

The study procedures were approved by the Ethics Committee at the University of Ostrava. Researchers reviewed the informed consent form with participant's in-person. Participants signed the consent form prior to data collection. This study and its procedures conformed to standards set by the Declaration of Helsinki.

2.2. Participants

Participants in this study were from Program 4 of the Healthy Aging in Industrial Environment study (4HAIE). The 4HAIE study was a 12-month prospective longitudinal study with four 2-week measurement bursts during which intensive survey data was collected using a mobile app. The primary aim of the 4HAIE study was focused on the links between air pollution, biomechanical, physiological, psychosocial, and sociodemographic variables (and their interaction) on the incidence of running-related injuries, physical (in) activity, health, and quality of life. Details about this prospective study can be read elsewhere [16,17]. The present study is a post-hoc analysis of 4HAIE study data to examine the relationship between sleep duration, age, and HRV. For this investigation, data from 1314 participants were reviewed for sleep and HRV data (Fig. 1). Sleep data was missing for 46 participants, another 366 participants lacked HRV data, and 14 participants were excluded due to quality-control issues related to HRV analysis. As a result, the present study analyzed data from 888 participants.

Participants were recruited from the Moravian-Silesian region (an area with high air pollution, n = 521) and the South Bohemian region (an area with low air pollution, n = 367) of the Czech Republic. Inclusion criteria consisted of age between 18 and 65 years, non-smoker, having internet access on a smartphone for software logging of sleep data, and no physician-diagnosed restrictions to physical activity. Exclusion criteria included being a smoker, pregnant, having an acute illness, a contraindication to magnetic resonance imaging, or a condition that limited physical activity. As planned, a large proportion of the participants (n = 543) were active runners defined by meeting the physical activity recommendation of the World Health Organization (WHO) to reach 150 or 75 min per week of moderate or vigorous physical activity, respectively, and must have reported running at least 10 km per week in the last six weeks. The other portion of participants (n = 345) were considered inactive as they reported not meeting the WHO recommendation for regular physical activity upon study entry.

2.3. Study procedures

Participants completed a series of online questionnaires after providing informed consent to participate. Relevant to this investigation, participants completed a medical history, the Pittsburgh Sleep Quality Index (PSQI), and Physical Activity Readiness (PARQ) questionnaires. Next, participants completed a 2-day in-person laboratory visit. Participants arrived at the laboratory in the afternoon after which the following measurements were performed: (1) resting seated blood pressure measured using a validated oscillometric device (Nissei DM 3000, Nihon Seimitsu Sokki Co. Ltd, Japan) [18]; and (2) a maximal treadmill exercise test for assessment of cardiorespiratory fitness. Participants slept in the laboratory with a set bedtime and wake time of 9:30 p.m. and 6:30 a.m., respectively. The next day HRV was measured followed by anthropometrics (height, weight). All HRV testing occurred in the morning within 45 min of awakening.

2.4. Cardiorespiratory fitness

Participants were instructed to avoid vigorous exercise for 24 h prior to testing, and to avoid food/caffeine for at least 3 h prior to testing. Maximal exercise testing was conducted using a motorized treadmill and an Ergostik metabolic system using BLUE Cherry software (Geratherm Medical AG, Germany). Expired air during exercise was analyzed for oxygen and carbon dioxide concentrations with each breath. Participants underwent an exercise test that consisted of a 3-min warmup at 5.0 km/h with 1% incline followed by the first stage of the test at 6.0 km/h with 1% incline. Treadmill incline did not change during exercise, but speed was increased by 1.0 km/h every minute until volitional fatigue. The highest 30-s average in relative oxygen uptake (peak VO_2 in ml/kg/min) was determined from the exercise test, and used as an index of cardiorespiratory fitness (higher values represent greater fitness). Missing peak VO_2 values were present for 78 participants (see Fig. 1) due to either not passing the PARQ, not getting permission by a medical doctor to perform the exercise test in the event of failing the PARQ, or seated resting blood pressure was greater than 150/90 mm Hg.

2.5. Heart rate variability

Short-term HRV was measured from a 10-min electrocardiographic (ECG) recording using a Bittium Faros device (Bittium Inc., Finland). This device collects a single-channel ECG from two electrodes placed on a chest belt. Participants rested in the supine position during the ECG recording and were asked to breathe normally. The last 5-min of the ECG recording was used for HRV analysis that was completed with Kubios Premium software after researchers filtered out artifacts and/or arrhythmias from the RR intervals. After software analysis, 14 participants were excluded (Fig. 1) due to artefact corrections higher than 5% (n = 9), ectopic beats ≥3 (n = 4), and high-frequency power out-of-range (n = 1). Variables of interest included mean heart rate and EKG-derived respiratory frequency from the Kubios software. In the time domain, root mean square of successive differences between normal heartbeats (RMSSD) was included as it is considered an estimate of parasympathetic mediated changes in heart rate [19]. In the frequency domain, we included total spectral power, low frequency power (LF, 0.04–0.15 Hz), and high frequency power (HF, 0.15–0.40 Hz) derived from the Fast Fourier Transform method. Low frequency power reflects both parasympathetic and sympathetic modulations in heart rate, while HF is considered an index of parasympathetic modulation of heart rate [19]. Natural logarithms of LF and HF power in milliseconds squared (ms^2) are reported as these values were normally distributed. Normalized units for LF (LFnu) and HF (HFnu) are also reported. These ratio-based HRV measures are mathematically redundant (e.g., HFnu = HF/(LF + HF)), as such, it is not advised to interpret LFnu and HFnu

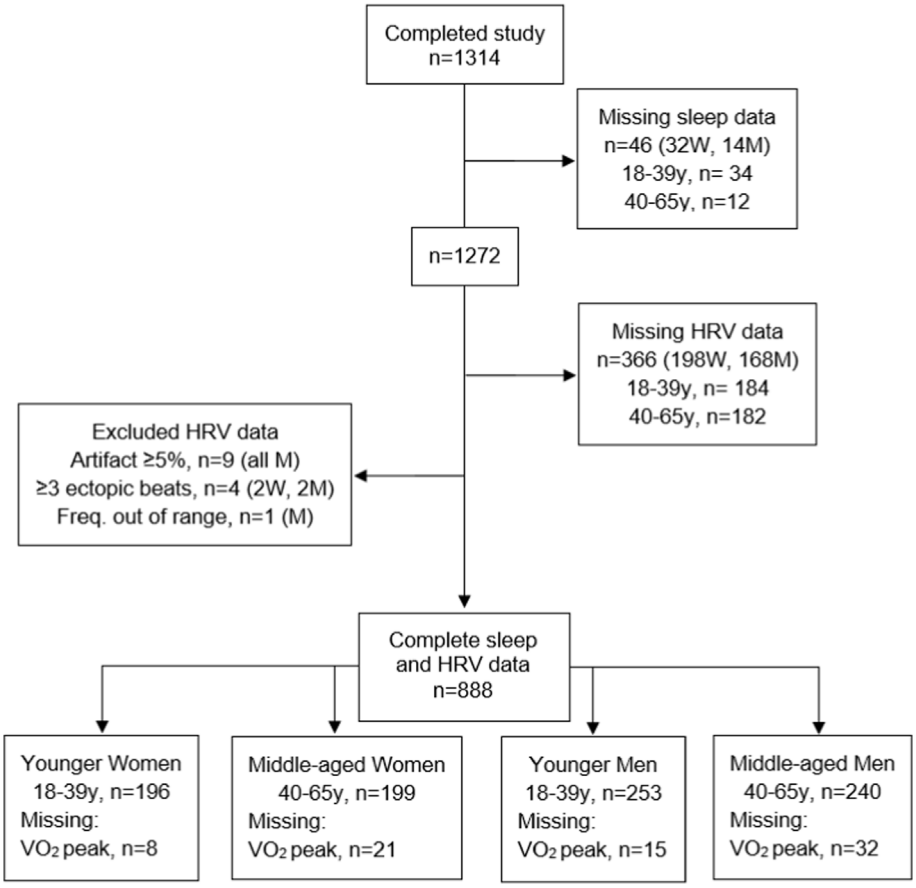

Fig. 1. Flowchart of the study sample selection.

separately from one another [20]. Ratio-based HRV measures are considered by some to reflect sympathovagal balance [19].

2.6. Sleep tracking

Sleep was objectively measured using wrist actigraphy. Two models of Fitbit Charge devices were used to measure sleep duration in this study. Fitbit Charge 3 was utilized initially until they were discontinued, after which time Fitbit Charge 4 was worn by participants. In this study sample 883 participants wore Fitbit Charge 3 (99.4%) and 5 participants wore Fitbit Charge 4 (0.6%). The change in models did not alter device size, motion sensors, or sleep algorithms. Rather, the major difference between models was the introduction of an on-board GPS. Participants were instructed to wear their Fitbit Charge device on the non-dominant wrist all day and night, and to only remove the device for charging. Sleep data from the device was uploaded through the Fitbit app of each participant's smartphone using Bluetooth into a Fitbit data cloud. Researchers were able to access and download this data. For this investigation, we analyzed data from the first 2-week measurement burst which started always on Saturday following the in-person laboratory visit to remain close in time with the laboratory testing of HRV and cardiorespiratory fitness. The average number of nights analyzed for sleep was 13.1 ± 2.1 (median = 14). Variables of interest from the automated Fitbit sleep analysis included time in bed, sleep duration, sleep efficiency (sleep duration/time in bed), and wake after sleep onset. Sleep data was missing for 46 participants (see Fig. 1) because some participants refused to wear the device at night or encountered technical problems with synchronization of the device with the mobile app that led to data loss.

Global sleep score from the PSQI that was administered online as part of the baseline questionnaire battery (see Study Procedures above) was included in the analysis as it has been shown to be a valid self-reported measure of sleep quality [21]. The total global score ranges from 0 to 21, with a total score of 5 or greater indicating poor sleep quality. In this study sample, 378 participants (43%) had a global score considered to reflect poor sleep quality.

2.7. Statistical analysis

Prior to regression analysis, all numerical variables were normalized by selecting an optimal normalizing transformation from a list of most commonly used transformation functions and estimated using the out-of-sample estimate of the Pearson P statistic computed by the repeated cross-validation (see the bestNormalize R package) [22]. Namely, the following transformations were applied: ordered quantile normalization (age, peak VO_2, LFnu, time in bed), Yeo-Johnson (BMI, sleep duration), arcsinh (systolic BP, mean HR, efficiency), Box-Cox (HFnu), logarithmic (LF and HF), and the square root transformation (PSQI score). Multivariable regression analysis was used to assess the relationship between sleep duration and HRV after controlling for variance from other predictor or potentially confounding variables (air pollution region, sex, age, body mass index, medications, peak VO_2, blood pressure, and mean heart rate). A linear regression model was fitted for confounder variables then single-term additions of sleep variables were performed with F-test being applied as a model improvement

significance test. In a separate analysis, participants were divided into age (younger and middle-aged, <40 and ≥ 40 years) and sleep duration groups (<7 and ≥ 7 h) within each sex. Two-way analysis of variance (ANOVA) was used to compare variables between age and sleep duration groups. Analysis of covariance (ANCOVA) was also used to adjust for medications, respiratory frequency, and peak VO_2. In the ANCOVA analysis, missing peak VO_2 values were substituted by group (age, sex, active or inactive) specific averages. Tukey post-hoc testing was used for pairwise comparisons if a significant interaction (age x sleep duration) was present. Statistical significance was set a priori at $P < 0.05$.

3. Results

3.1. Participant medical history

Relevant to this HRV study, only cardiovascular conditions are reported here. No participant reported having a history of myocardial infarction or diabetes, although three participants reported taking metformin (1 younger women, 1 younger man, 1 middle-aged man). Two participants reported having ischemic heart disease (1 younger woman, 1 middle-aged man). Lastly, 20 women (1 younger, 19 middle-aged) and 31 men (6 younger, 25 middle-aged) reported having hypertension with 35 of these adults taking antihypertensive medications (16 middle-aged women, 1 younger man, 18 middle-aged men). Other medications recorded and included in the analysis were use of adrenergic drugs, corticosteroids, antidepressants, and medications for anxiety and/or mood disorders due to their influence on HRV (Tables 1–3).

3.2. Regression analysis

Table 1 presents the results from the multivariable regression analysis. Chronological age, resting mean heart rate, and use of vasoactive medications were significant predictors of RMSSD (adj. $r^2 = 0.17$, F-statistic = 15.08, $P < 0.001$), HF power (adj. $r^2 = 0.13$, F-statistic = 11.80, $P < 0.001$), and LF power (adj. $r^2 = 0.10$, F-statistic = 8.80, $P < 0.001$). In addition to these predictors, sex and peak VO_2 were also included as significant predictors for LF nu (adj. $r^2 = 0.21$, F-statistic = 19.74, $P < 0.001$) and HFnu (adj. $r^2 = 0.22$, F-statistic = 20.58, $P < 0.001$). After adjusting for these predictors and other possible confounding variables, sleep duration was found to significantly associate with HFnu ($\beta = 0.06$, $P = 0.04$), but not for RMSSD ($P = 0.77$), HF power ($P = 0.79$), LF power ($P = 0.48$), or LFnu ($P = 0.058$). Time in bed, wake after sleep onset, sleep efficiency, and PSQI global sleep score were not associated with HRV after adjusting for significant predictors and other possible confounding variables (Table 1).

3.3. Age and sleep group comparisons

Comparisons between age and sleep duration groups are presented in Table 2 for women and Table 3 for men. Middle-aged adults, irrespective of sex, had higher body mass index and resting blood pressure but lower peak VO_2 (main effect for age, all $P < 0.001$). In addition, middle-aged women and men took more vasoactive medications than younger adults (both $P < 0.05$). Sleep duration tended to be lower but only marginally statistically significant in middle-aged as compared to younger women ($P = 0.052$) and men ($P = 0.07$). Sleep efficiency was lower in middle-aged men as compared to younger men ($P = 0.03$). In terms of HRV, in both women and men, LFnu was higher while all other HRV variables (RMSSD, total power, LF power, HF power, HFnu) were lower in middle-aged as compared to younger adults (all $P \leq 0.01$).

In addition to longer sleep duration, women and men in the ≥7 h sleep duration group also spent more time in bed and more time awake after sleep onset (see main effects for sleep group, all $P < 0.001$; Tables 2 and 3). There were no differences between sleep duration groups in age, body mass index, peak VO_2, resting blood pressure, use of vasoactive medications, or HRV. Women that slept ≥7 h tended ($P = 0.058$) to have a higher prevalence of corticosteroid users as compared to women that slept <7 h (Table 2). While men that slept ≥7 h had a higher prevalence of adults taking antidepressant medications than men that slept <7 h ($P = 0.01$; Table 3).

3.4. Interactions between age and sleep duration groups

Table 2 presents the unadjusted comparison of HRV between age and sleep duration groups in women. Only normalized units of LF and HF power showed an influence of sleep duration on HRV such that LFnu was higher and HFnu lower in middle-aged women

Table 1
Relationship with HRV in pooled analysis.

	RMSSD	*P*-value	LF power	*P*-value	HF power	*P*-value	HFnu	*P*-value
Values are parameter estimate (Std. Error)								
Location (unpolluted/polluted)	−0.02 (0.06)	0.70	−0.05 (0.07)	0.46	−0.01 (0.06)	0.91	0.02 (0.06)	0.69
Sex (1 = women, 2 = men)	0.07 (0.09)	0.40	−0.15 (0.09)	0.12	0.05 (0.09)	0.57	0.55 (0.09)	<0.001
Age	−0.27 (0.04)	<0.001	−0.21 (0.04)	<0.001	−0.25 (0.04)	<0.001	−0.29 (0.04)	<0.001
Body mass index	−0.04 (0.04)	0.36	0.01 (0.04)	0.84	−0.04 (0.04)	0.33	0.03 (0.04)	0.46
Peak VO_2	−0.06 (0.05)	0.22	−0.01 (0.05)	0.93	−0.06 (0.05)	0.24	−0.12 (0.05)	0.02
Resting systolic BP	0.03 (0.05)	0.43	−0.01 (0.05)	0.73	−0.01 (0.05)	0.86	−0.04 (0.04)	0.39
Resting diastolic BP	−0.08 (0.05)	0.08	−0.05 (0.05)	0.30	−0.06 (0.05)	0.17	−0.02 (0.05)	0.69
Resting mean heart rate	−0.31 (0.03)	<0.001	−0.16 (0.03)	<0.001	−0.25 (0.03)	<0.001	−0.39 (0.03)	<0.001
Vasoactive medications†	−0.40 (0.15)	0.01	−0.43 (0.16)	0.007	−0.31 (0.15)	0.04	0.28 (0.15)	0.06
Corticosteroid use	−0.16 (0.20)	0.42	−0.10 (0.21)	0.63	−0.16 (0.21)	0.43	−0.03 (0.20)	0.84
Antidepressant use‡	−0.19 (0.21)	0.36	−0.13 (0.22)	0.55	−0.22 (0.22)	0.31	−0.07 (0.21)	0.73
Values are Sum of Squares (F value) after adjusting for all variables above.								
PSQI sleep score	0.03 (0.04)	0.83	0.07 (0.08)	0.76	0.001 (0.001)	0.97	0.05 (0.07)	0.78
Time in bed	0.09 (0.11)	0.73	0.18 (0.20)	0.65	0.01 (0.01)	0.91	1.99 (2.58)	0.10
Sleep duration	0.06 (0.07)	0.77	0.43 (0.47)	0.48	0.05 (0.06)	0.79	3.02 (3.91)	0.04
Wake after sleep onset	0.001 (0.001)	0.96	0.39 (0.44)	0.50	0.01 (0.01)	0.92	0.15 (0.20)	0.65
Sleep efficiency	0.001 (0.002)	0.96	1.33 (1.49)	0.22	0.01 (0.01)	0.91	2.91 (3.77)	0.052

VO_2, maximal oxygen uptake; BP, blood pressure; PSQI, Pittsburgh Sleep Quality Index; †, antihypertensives, beta agonists, alpha blockers. ‡, medications for depression, anxiety, and/or mood.

Table 2
Comparison between age and sleep duration groups in women.

	Younger Women (<40y)		Middle-aged Women (≥40y)		Age Group	Sleep Group	Interaction
	≥7 h	<7 h	≥7 h	<7 h	*P*-value	*P*-value	*P*-value
Sample size (n =)	90	106	53	146			
Age (y)	27 ± 6	27 ± 6	48 ± 6	47 ± 6	<0.001	0.47	0.86
Body mass index (kg/m^2)	22.4 ± 4.4	22.8 ± 3.7	24.7 ± 4.3	24.7 ± 4.6	<0.001	0.27	0.34
Peak VO_2 (mL/kg/min)	39 ± 6	40 ± 6	32 ± 7	34 ± 8	<0.001	0.13	0.95
Systolic BP (mm Hg)	120 ± 13	118 ± 10	129 ± 16	131 ± 15	<0.001	0.98	0.17
Diastolic BP (mm Hg)	75 ± 9	73 ± 8	80 ± 10	81 ± 9	<0.001	0.77	0.07
Vasoactive† drug users, n (%)	6 (6)	1 (1)	5 (9)	15 (10)	0.02	0.36	0.22
Corticosteroid users, n (%)	5 (5)	1 (1)	4 (7)	6 (4)	0.22	0.058	0.78
Antidepressant‡ users, n (%)	2 (2)	2 (2)	1 (2)	6 (4)	0.59	0.59	0.47
Sleep Variables							
PSQI Sleep Score	5.0 ± 3.3	4.8 ± 2.2	4.7 ± 2.5	4.9 ± 2.3	0.74	0.84	0.54
Nights analyzed (#)	12.8 ± 2.8	12.8 ± 2.5	13.5 ± 0.8	13.3 ± 1.6	<0.01	0.61	0.66
Time in bed (min)	508 ± 24	442 ± 31	507 ± 31	430 ± 39	0.053	<0.001	0.17
Sleep duration (min)	447 ± 19	387 ± 27	446 ± 25	377 ± 34	0.052	<0.001	0.21
WASO (min)	61 ± 12	54 ± 9	60 ± 10	52 ± 9	0.36	<0.001	0.48
Sleep efficiency (%)	88 ± 2	87 ± 1	88 ± 1	87 ± 1	0.89	0.09	0.89
HRV Variables							
Mean heart rate (bpm)	64 ± 9	63 ± 8	60 ± 7	62 ± 8	0.01	0.31	0.10
Respiratory freq. (Hz)	0.23 ± 0.04	0.24 ± 0.04	0.23 ± 0.04	0.23 ± 0.05	0.11	0.22	0.60
RMSSD (ms)	54 ± 33	61 ± 39	37 ± 20[a,b]	31 ± 18[a,b]	<0.001	0.42	0.01
Total power (log ms^2)	7.5 ± 0.9	7.7 ± 1.0	6.8 ± 0.8	6.7 ± 0.9	<0.001	0.62	0.14
LF power (log ms^2)	6.7 ± 0.9	6.8 ± 1.0	6.0 ± 0.8	6.0 ± 1.0	<0.001	0.42	0.62
LFnu	51 ± 17	49 ± 19	52 ± 18	61 ± 20[a,b,c]	0.001	0.08	0.008
HF power (log ms^2)	6.6 ± 1.1	6.8 ± 1.2	5.9 ± 0.9[a,b]	5.4 ± 1.1[a,b]	<0.001	0.43	0.01
HFnu	48 ± 17	50 ± 19	47 ± 18	38 ± 20[a,b,c]	0.001	0.08	0.009

Data presented as mean ± SD. VO_2, maximal oxygen uptake; BP, blood pressure; PSQI, Pittsburgh Sleep Quality Index; WASO, wake after sleep onset. †, antihypertensives, beta agonists, alpha blockers. ‡, medications for depression, anxiety, and/or mood. [a], different from younger women with ≥7 h of sleep; [b], different from younger women with <7 h of sleep; [c], different from middle-aged women with ≥7 h of sleep.

Table 3
Comparison between age and sleep duration groups in men.

	Younger Men (<40y)		Middle-aged Men (≥40y)		Age Group	Sleep Group	Interaction
	≥7 h	<7 h	≥7 h	<7 h	*P*-value	*P*-value	*P*-value
Sample size (n =)	84	169	41	199			
Age (y)	27 ± 6	28 ± 6	47 ± 6	48 ± 6	<0.001	0.49	0.75
Body mass index (kg/m^2)	23.7 ± 3.2	24.3 ± 2.9	25.2 ± 2.9	25.8 ± 3.3	<0.001	0.08	0.80
Peak VO_2 (mL/kg/min)	49 ± 8	50 ± 8	42 ± 9	43 ± 9	<0.001	0.27	0.95
Systolic BP (mm Hg)	129 ± 12	131 ± 11	138 ± 15	136 ± 14	<0.001	0.62	0.16
Diastolic BP (mm Hg)	77 ± 8	76 ± 9	86 ± 8	84 ± 8	<0.001	0.28	0.60
Vasoactive† drug users, n (%)	2 (2)	3 (2)	2 (5)	23 (11)	0.01	0.23	0.15
Corticosteroid users, n (%)	3 (3)	2 (1)	0 (0)	5 (2)	0.46	0.96	0.11
Antidepressant‡ users, n (%)	3 (3)	1 (<1)	3 (7)	4 (2)	0.10	0.01	0.46
Sleep Variables							
PSQI Sleep Score	4.4 ± 2.3	4.6 ± 2.0	4.5 ± 2.2	4.5 ± 2.6	0.99	0.65	0.72
Nights analyzed (#)	12.4 ± 2.9	12.7 ± 2.3	13.6 ± 1.0	13.3 ± 1.5	<0.001	0.66	0.22
Time in bed (min)	507 ± 27	428 ± 44	504 ± 32	420 ± 55	0.23	<0.001	0.75
Sleep duration (min)	444 ± 23	374 ± 37	439 ± 23	365 ± 47	0.07	<0.001	0.84
WASO (min)	63 ± 8	54 ± 10	65 ± 12	54 ± 11	0.34	<0.001	0.64
Sleep efficiency (%)	87 ± 1	87 ± 1	87 ± 1	86 ± 1	0.03	0.44	0.91
HRV Variables							
Mean heart rate (bpm)	58 ± 8	58 ± 9	59 ± 9	57 ± 7	0.50	0.38	0.39
Respiratory freq. (Hz)	0.22 ± 0.04	0.23 ± 0.05	0.22 ± 0.06	0.22 ± 0.05	0.65	0.20	0.88
RMSSD (ms)	67 ± 42	61 ± 41	33 ± 26	35 ± 22	<0.001	0.83	0.06
Total power (log ms^2)	8.0 ± 0.9	7.9 ± 0.9	6.7 ± 1.1	6.9 ± 0.9	<0.001	0.42	0.16
LF power (log ms^2)	7.3 ± 0.9	7.3 ± 1.0	6.2 ± 1.2	6.4 ± 1.0	<0.001	0.52	0.54
LFnu (%)	57 ± 18	62 ± 17	71 ± 18[a,b]	66 ± 18[a,b]	<0.001	0.99	0.02
HF power (log ms^2)	6.9 ± 1.1	6.7 ± 1.2	5.1 ± 1.2[a,b]	5.5 ± 1.2[a,b]	<0.001	0.52	0.01
HFnu (%)	42 ± 18	37 ± 17	28 ± 18[a,b]	33 ± 18[a,b]	<0.001	0.99	0.02

Data presented as mean ± SD. VO_2, maximal oxygen uptake; BP, blood pressure; PSQI, Pittsburgh Sleep Quality Index; WASO, wake after sleep onset. †, antihypertensives, beta agonists, alpha blockers. ‡, medications for depression, anxiety, and/or mood. [a], different from younger men with ≥7 h of sleep; [b], different from younger men with <7 h of sleep.

that slept <7 h as compared to middle-aged women that slept ≥7 h ($P = 0.02$) and younger women ($P < 0.001$). After adjusting for medications, respiratory frequency, and peak VO_2, it was observed that RMSSD, HF power, HFnu, and LFnu all showed an influence of sleep duration on HRV as shown in Fig. 2. Specifically, middle-aged women with sleep durations <7 h had lower RMSSD (difference = −7.47, t-value = −1.62, $P = 0.04$), lower HF power (difference = −0.48, t-value = −2.62, $P = 0.04$), lower HFnu (difference = −7.47, t-value = −3.03, $P = 0.01$), and higher LFnu (difference = 9.26, t-value = 3.04, $P = 0.01$) than middle-aged

women with sleep durations ≥7 h. In addition, middle-aged women with sleep durations <7 h (but not ≥7 h) showed statistically significant age-related differences in RMSSD, HF power, HFnu, and LFnu as compared to younger women (Fig. 2).

Table 3 presents the unadjusted comparison of HRV in men, while Fig. 3 shows the comparison of HRV between age and sleep duration groups in men after adjusting for medications, respiratory frequency, and peak VO_2. Both unadjusted and adjusted comparisons did not show sleep durations ≥7 h to influence HRV in comparison to sleeping <7 h in younger or middle-aged men.

4. Discussion

The aim of this study was to examine the influence of sleep duration and sex on the age-related difference in HRV. Our main finding was that adequate sleep duration did not influence HRV in younger or middle-aged men, but in contrast, sleep duration influenced HRV in women such that only middle-aged women with average sleep durations <7 h showed significantly lower HRV than younger women after adjusting for potentially confounding variables. These results provide support for the postulation that adequate sleep duration may have a positive influence on autonomic regulation of the heart in middle-aged women, and advances the field by showing sex is an important factor to consider in this area of investigation.

In agreement with past research [10–12,23], time and frequency-domain measures of HRV were observed to be different between middle-aged as compared to younger adults in the present study. Age held a significant relationship with HRV such that advancing age was associated with lower parasympathetic modulation of the heart as indicated by RMSSD and HF power as well as sympathovagal imbalance as reflected by lower HFnu. Lower RMSSD and HF power are reported by others to associate with a higher risk of sudden cardiac death in middle-aged adults [24], thus these measures of HRV are important to study in relation to sleep. Sleep duration has been studied previously in relation to HRV under conditions of experimental sleep loss [25], but few studies have examined the association between habitual sleep duration on HRV in the general population. In a twin study of older adults that objectively measured sleep duration using wrist actigraphy across

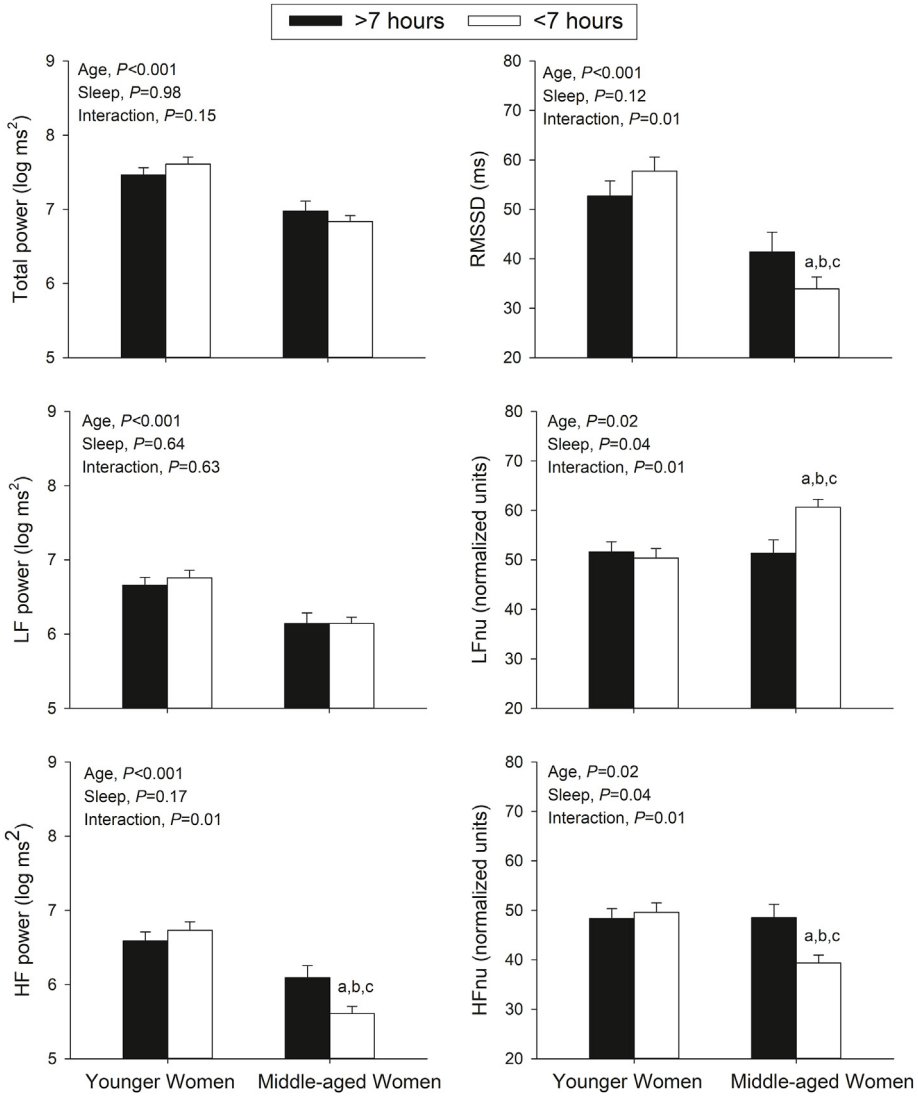

Fig. 2. Comparison of HRV between younger and middle-aged women based on habitual sleep duration. Values are adjusted mean ± SEM after controlling for variance explained by medications (antihypertensives, beta agonists, alpha blockers, corticosteroids, antidepressants), respiratory frequency, and peak VO_2 using analysis of covariance. [a], different from younger women with ≥7 h of sleep; [b], different from younger women with <7 h of sleep; [c], different from middle-aged women with ≥7 h of sleep.

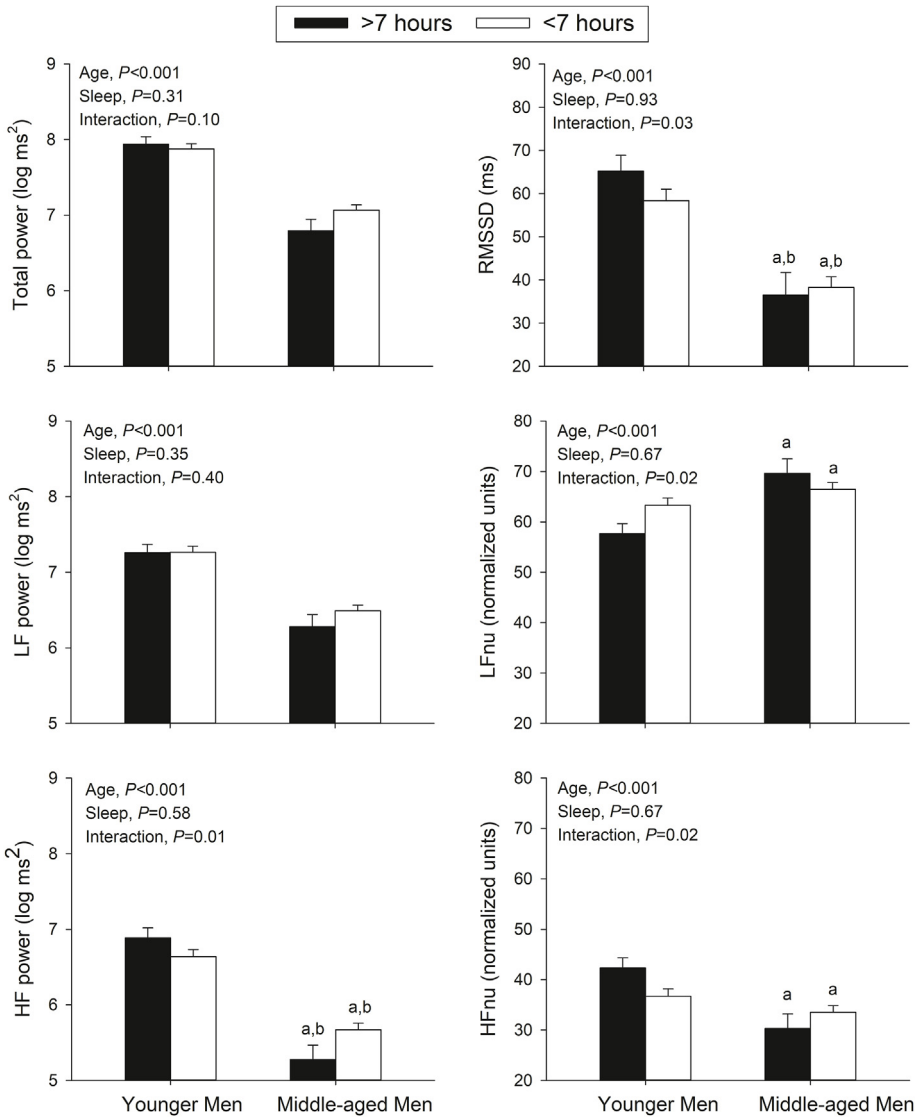

Fig. 3. Comparison of HRV between younger and middle-aged men based on habitual sleep duration. Values are adjusted mean ± SEM after controlling for variance explained by medications (antihypertensives, beta agonists, alpha blockers, corticosteroids, antidepressants), respiratory frequency, and peak VO_2 using analysis of covariance. [a], different from younger men with ≥7 h of sleep; [b], different from younger women with <7 h of sleep.

7-days, Huang et al. [26] found longer sleep durations predicted higher LF power the following day. In a separate study, Castro-Dehl et al. [15] used data from the Multi-Ethnic Study of Atherosclerosis (MESA) to demonstrate that middle-aged to older adults with average sleep durations less than 7 h (but >6 h) had lower HF power as compared to adults that slept 7 h or more after adjusting for factors known to affect heart rate. Our results in middle-aged women after adjusting for potentially confounding variables are in agreement with this finding. We observed middle-aged women with sleep durations ≥7 h had higher RMSSD, HF power, and HFnu than middle-aged women with sleep durations <7 h in our adjusted comparison. The MESA study did not separate women and men in their analysis, so it's unclear whether a similar result would have been found in their adult sample. Thus, the present study sheds new information that sex may play an important role in the association between habitual sleep duration and HRV in middle-aged adults. Moreover, we also show that middle-aged women with sleep durations ≥7 h had similar HRV than younger women suggesting adequate sleep duration may promote parasympathetic modulation of the heart and sympathovagal balance in women with aging.

Why middle-aged men did not present with a similar association between sleep duration and HRV as middle-aged women is unclear from the present study. Middle-aged men with sleep durations ≥7 h had similar total sleep time than younger men with sleep durations ≥7 h (about 7 h and 20 min), yet HRV was lower in middle-aged men. Wake after sleep onset, sleep efficiency, and self-reported Pittsburgh Sleep Quality Index global score were also similar between middle-aged and younger men with sleep durations ≥7 h. This suggests that adequate sleep duration (and quality of sleep) had no impact on age-related differences in HRV in men. The relatively low sample size in middle-aged men with sleep durations ≥7 h is a consideration, but the values for HRV exhibited by this group did not reveal any trend that would indicate better HRV than middle-aged men with sleep durations <7 h. One explanation for our results may be that sleep duration alters sympathetic activity differently in women than men [27]. Carter et al. [28] directly measured sympathetic activity through multifiber

recordings of muscle sympathetic nerve activity (MSNA) in older women and men before and after a single night of 24-h sleep deprivation. Only older women in their experiment exhibited elevated MSNA while older men showed no change in MSNA demonstrating an absent sympathoexcitatory response to sleep loss. Sleep deprivation is different than inadequate sleep duration (<7 h) studied here, but our indirect measure of the cardiac autonomic nervous system using HRV showed a similar result such that middle-aged women with inadequate sleep durations presented with sympathovagal imbalance (higher LFnu & lower HFnu) as compared to middle-aged women with adequate sleep durations, while middle-aged men did not present a similar pattern. More studies are needed to understand how biological sex influences the association between sleep duration and HRV.

Several interesting points should be highlighted from this study. First, younger women and men did not show a significant influence of sleep duration on HRV. This result is consistent with past research in young adults that report no correlation between sleep duration and resting daytime HRV [29,30]. This observation may be due to a greater ability of younger adults to buffer stress on cardiac autonomic regulation that results from inadequate sleep durations, while middle-aged adults may have a lower ability to counter sleep-related stress. Second, this study analyzed data collected from Program 4 of the Healthy Aging in Industrial Environment study (4HAIE) [16,17]. Participants were adults who lived for at least five years in a high air-polluted (Moravian-Silesian) or low air-polluted (South Bohemia) region of the Czech Republic. Our regression analysis did not find residence in an air-polluted region to associate with HRV. Third, cardiorespiratory fitness (i.e., peak VO_2) was directly measured using indirect calorimetry during maximal treadmill exercise in the present study. Peak VO_2 was associated with HFnu in our regression analysis, but it should be noted that peak VO_2 was not correlated with sleep duration (pooled sample, $r = -0.04$, $P = 0.21$). Our approach to statistically control for variance in HRV explained by peak VO_2 is a major strength of this study since identified differences in HRV between age and sleep duration groups can be interpreted without the well-known confounding influence of cardiorespiratory fitness on HRV [31].

Limitations of this study include a healthy cohort comprised of majority active adults. While this type of sample reduces the influence of confounding variables when examining the association between sleep duration and HRV, it does reduce the relevance of the present results to populations with overt cardiovascular disease. Moreover, we did not directly ask about sleep disorders in our medical history questionnaire nor is this a question in the PSQI questionnaire. However, our medical history questionnaire did ask participants to list the presence of other conditions or illnesses where no person responded with a sleep problem or sleep disorder. Another limitation of this study is the use of FitBit monitors to measure sleep duration rather than polysomnography. While FitBit Charge monitors show good accuracy in measuring sleep duration as compared to in-lab polysomnography [32], it is possible that some participants were incorrectly stratified into sleep duration groups. Lastly, one night of in-laboratory sleep (time in bed, 9:30 p.m.–6:30 a.m.) was required of all participants prior to the HRV measurement. This step was meant to reduce the influence of short sleep the night prior to testing on lowering HRV [33] as well as standardize everyone to a similar sleep opportunity. However, we did not have actual sleep duration in the lab to assess the impact of sleep duration the night prior to testing on HRV.

In summary, this cross-sectional study of healthy adults found sleep duration influenced HRV in a sex-specific manner such that middle-aged women with adequate sleep durations did not show significantly lower HRV as compared to younger women, while men showed the expected significant age-related difference in HRV irrespective of sleep duration. These results suggest that meeting the recommended amount of sleep (7 h per night) may positively impact cardiac autonomic regulation in middle-aged women by maintaining higher vagal modulation of parasympathetic activity and sympathovagal balance from younger age.

Author contributions

All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. JG conceptualized the research question. JG, SE, and MB analyzed the data. LC, VJ, and DJ collected and/or populated the data. JG wrote the manuscript and all authors read and approved the final version.

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Data availability statement

Data analyzed for this are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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