

# Association of sleep-wake rhythm and sleep quality with endothelial function in young adults

Honoka Nakashima<sup>1</sup>  
Akiko Noda<sup>1\*</sup>  
Anna Tamura<sup>2</sup>  
Michiaki Nagai<sup>3</sup>  
Masato Okuda<sup>1</sup>  
Takahiro Okumura<sup>4</sup>  
Fumihiko Yasuma<sup>2</sup>  
Toyoaki Murohara<sup>4</sup>

<sup>1</sup>Chubu University Graduate School of Life and Health Sciences, Department of Biomedical Sciences - Kasugai - Aichi - Japan.

<sup>2</sup>Chubu University Collage of Life and Health Sciences, Department of Biomedical Sciences - Kasugai - Aichi - Japan.

<sup>3</sup>Hiroshima City Asa Hospital, Hiroshima, Department of Cardiology - Hiroshima - Japan.

<sup>4</sup>Nagoya University Graduate School of Medicine, Department of Cardiology - Nagoya - Aichi - Japan.

**\*Corresponding author:**

Akiko Noda  
E-mail: anoda@isc.chubu.ac.jp

Received: August 1, 2021;  
Accepted: December 13, 2021.

DOI: 10.5935/1984-0063.20220050

## ABSTRACT

**Objective:** The environment in modern society could disturb the sleep-wake rhythm. We aimed to study the association of sleep-wake rhythm with endothelial function and sleep quality. **Material and Methods:** Thirty-one healthy university students (mean age: 20.4±1.8 years) were enrolled. The endothelial function was evaluated with the percent endothelium-dependent flow-mediated dilation of the brachial artery [%FMD: (maximum diameter - baseline diameter)/baseline diameter x 100] using the high-resolution ultrasonography. We also measured the total sleep time (TST), sleep efficiency, and the standard deviation (SD) of sleep timing (midpoint between bedtime and wake-up time) using the actigraphy. The irregular sleep-wake rhythm was defined as having the shift of bedtime or wake-up time for two hours or longer. **Results:** The %FMD and sleep efficiency were significantly lower in the irregular group than regular group (%FMD: 6.1±2.4 vs. 10.9±2.3,  $p<0.001$ , sleep efficiency: 92.2±5.8 vs. 95.9±2.8%,  $p=0.027$ ), whereas there was no significant difference in %FMD between the two groups of TST <6 hours and TST ≥6 hours. The %FMD was significantly correlated with SD of sleep timing ( $r=-0.481$ ,  $p=0.006$ ). Multiple regression analyses, including age, sex, TST, sleep efficiency, and SD of sleep timing revealed that the SD of sleep timing was a significant factor associated with %FMD ( $\beta=-0.454$ ,  $p=0.017$ ). **Conclusion:** Our findings suggest that the irregular sleep-wake rhythm and poor sleep quality could have adverse effects on endothelial function in young adults.

**Keywords:** Circadian Rhythm; Young Adult; Actigraphy.

## INTRODUCTION

The environment in modern society could often disturb the sleep-wake rhythm<sup>1</sup>. Many of the university students have irregular schedules of classes<sup>2</sup>, and part-time works<sup>3</sup>. Moreover, they use the internet and social networking service on 24-hour basis<sup>4,5</sup>, with frequent shiftings of the bedtime and wake-up time. Accumulated sleep debt reduces their quality of sleep<sup>6</sup>, which has been identified as a major cause of fatigue, inattentiveness, anxiety<sup>7</sup>, and depression<sup>8</sup>. We recently showed that an irregular sleep-wake rhythm and a short sleep duration played a negative role in the brain activity of the university students using near infrared spectroscopy<sup>9</sup>. However, the association between the sleep-wake rhythm and endothelial function has not been clarified in young adults.

Endothelial dysfunction with impaired nitric oxide (NO) production is an early feature of atherosclerosis and cardiovascular diseases in human<sup>10</sup>. The NO is a product of endothelial cells that regulates the vascular tone and it plays a pivotal role for maintaining the homeostasis in hemodynamics. Since significant day-to-day variations in sleep duration and timing are assumed to be associated with the future risk of atherosclerosis<sup>11,12</sup>, lifestyle interventions for young adults could prevent occurrences of cardiovascular diseases in the long run.

Accordingly, we investigated the effects of sleep-wake rhythm and sleep quality on endothelial function in the university students.

## MATERIAL AND METHODS

### Subjects

Thirty-one healthy university students (mean age:  $20.4 \pm 1.8$  years) were enrolled in this study. None of these subjects had any history of neurological disorder, substance abuse, head injury or major physical illness. They were free from smoking and did not use psychotropic medication. This study was approved by the ethics committee of Chubu University (Number 270098). After explaining the nature of the study and procedures involved, we obtained written informed consents from all participants.

### Actigraphy

Actigraphy for monitoring the activities and scoring the sleep-wake rhythm (Ambulatory Monitoring Inc., New York, NY, USA) was performed for 7 consecutive days. The actigraph was worn around the wrist of their non-dominant side to store the data in 1-min increments. Bedtime and wake-up time were derived from the sleep diary, with which the analysis interval of actigraphy was ascertained<sup>13</sup>. We used the algorithm supplied by the ActionW-2 clinical sleep analysis software package for Windows (Ambulatory Monitoring Inc., New York, NY, USA) to score the sleep/wakefulness according to the Cole-Kripke formula<sup>14</sup>.

We evaluated total sleep time (TST), sleep efficiency (calculated as  $TST/\text{time spent in bed} \times 100$ ), bedtime, wake-up time, sleep timing (midpoint between bedtime and wake-up time), and standard deviation (SD) of sleep timing<sup>15</sup>. The irregular sleep-wake rhythm was defined as having the shift of bedtime or wake-up time for two hours longer according to the International Classification of Sleep Disorders, 3<sup>rd</sup> edition<sup>16</sup>. We classified the participants into two groups of irregular group ( $n=16$ ) and regular group ( $n=15$ ) in sleep-wake rhythm.

### Brachial-ankle pulse wave velocity (baPWV)

The baPWV was measured using a plethysmograph (BP-203RPEIII, Omron Colin, Tokyo, Japan)<sup>17</sup>, and systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR), electrocardiogram (ECG), and heart sounds were simultaneously recorded with this instrument.

### Endothelium-dependent flow-mediated dilation (FMD)

The FMD of the brachial artery was assessed using the high-resolution ultrasonography equipped with a 12MHz linear array transducer (Prosound  $\alpha 7$ , Hitachi Aloka Medical, Tokyo, Japan)<sup>18,19</sup>. The diastolic time was identified continuously with three-lead ECG. A sphygmomanometric cuff on the right distal forearm was utilized for creating a flow stimulus to the brachial artery. After the diameter of brachial artery was measured at baseline at rest, the cuff was inflated with 50mmHg above the SBP to occlude the brachial artery for 5 minutes and subsequently deflated for 3 minutes to restore the flow. Endothelial function was assessed as a change in the diameter of brachial artery from baseline to peak expansion after cuff release. The %FMD was defined as:  $(\text{maximum diameter} - \text{baseline diameter})/\text{baseline diameter} \times 100$ .

### Statistical analysis

All data are expressed as mean  $\pm$  SD. We compared the data on SBP, DBP, HR, baPWV, %FMD, TST, sleep efficiency, bedtime, wake-up time, sleep timing, and SD of sleep timing between the groups (irregular sleep-wake rhythm vs. regular sleep-wake rhythm, and TST <6 hours vs. TST  $\geq$ 6 hours<sup>20</sup>) using the non-paired *t*-test. The Pearson's correlation analyses were performed to evaluate the relationships between the %FMD and sleep parameters. In addition, TST, sleep efficiency, and SD of sleep timing were included in multiple regression analyses to determine the independent parameters correlated with %FMD or baPWV. A probability value less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS Statistics version 25.0 (IBM Corporation, Armonk, New York, USA).

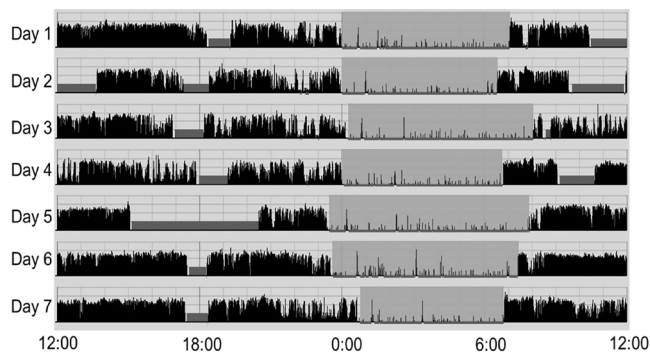
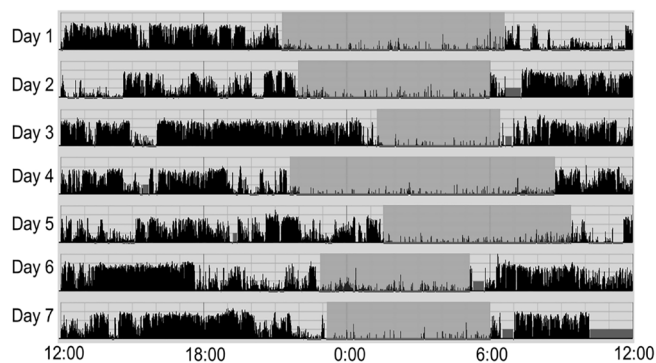
## RESULTS



Table 1 shows the characteristics, vascular function and sleep parameters of the participants. Figure 1 shows two representative actigrams of regular sleep-wake rhythm (Case A) and irregular sleep-wake rhythm (Case B).

**Table 1.** Demographics and vascular/sleep parameters.

Demographics	
Gender (male/female)	9/22
Age (years)	20.4 ± 1.8
Height (cm)	161.0 ± 7.4
Weight (kg)	54.2 ± 10.2
BMI (kg/m <sup>2</sup> )	20.9 ± 2.7
Vascular parameters	
SBP (mmHg)	112.2 ± 11.5
DBP (mmHg)	61.9 ± 5.2
HR (bpm)	67.3 ± 9.2
baPWV (cm/s)	973.5 ± 198.3
%FMD	8.4 ± 3.4
Sleep parameters	
TST (min)	366.3 ± 58.5
Sleep efficiency (%)	94.2 ± 4.9
Bedtime	1:13 ± 0:58
Wake-up time	7:52 ± 0:52
Sleep timing	4:39 ± 0:55
SD of sleep timing (min)	69.7 ± 42.5

Notes: Data are expressed as mean ± standard deviation. BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; HR = Heart rate; baPWV = Brachial-ankle pulse wave velocity; FMD = Flow-mediated dilation; TST = Total sleep time; SD = Standard deviation.

**Case A****Case B****Figure 1.** Actigram of two representative cases of regular sleep-wake rhythm (case A) and irregular sleep-wake rhythm (case B).

Notes: The horizontal axis represents the clock over 24 hours (12 p.m. to 12 p.m.). The vertical axis represents the amount of activity recorded by the actigraph, with the black bars indicating the movement in 1 min. The section of  indicates the period when the participants were in bed and the section of  indicates the period when the participants apparently removed the actigraphy instrument.

The SD of sleep timing was significantly greater in the group of TST <6 hours than group of TST ≥6 hours (86.2±49.2 vs. 56.0±28.5min,  $p=0.041$ ). No significant difference was observed in %FMD between the groups (Table 2).

**Table 2.** Comparison of demographics and vascular/sleep parameters by total sleep time.

	TST <6 hours (n=14)	TST ≥6 hours (n=17)	p-value
Demographics			
Age (years)	20.1 ± 1.7	20.6 ± 1.8	0.494
BMI (kg/m <sup>2</sup> )	20.6 ± 1.9	21.2 ± 3.3	0.525
Vascular parameters			
SBP (mmHg)	108.8 ± 7.6	115.0 ± 13.8	0.143
DBP (mmHg)	60.9 ± 5.4	62.7 ± 6.6	0.440
HR (bpm)	65.9 ± 10.1	68.5 ± 8.5	0.452
baPWV (cm/s)	986.3 ± 125.7	963.1 ± 245.9	0.751
%FMD	8.1 ± 3.5	8.7 ± 3.3	0.645
Sleep parameters			
Sleep efficiency (%)	94.1 ± 4.9	94.3 ± 4.7	0.932
Bedtime	1:24 ± 0:43	1:04 ± 1:05	0.662
Wake-up time	7:47 ± 1:02	7:56 ± 0:49	0.321
Sleep timing	4:52 ± 0:57	4:28 ± 0:52	0.221
SD of sleep timing (min)	86.2 ± 49.2	56.0 ± 28.5	0.041

Notes: Data are expressed as mean ± standard deviation. TST = Total sleep time; BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; HR = Heart rate; baPWV = Brachial-ankle pulse wave velocity; FMD = Flow-mediated dilation; SD = Standard deviation.

The %FMD and sleep efficiency were significantly lower in the irregular group than regular group in sleep-wake rhythm (%FMD: 6.1±2.4 vs. 10.9±2.3,  $p<0.001$ , sleep efficiency: 92.2±5.8 vs. 95.9±2.8%,  $p=0.027$ ). Moreover, the sleep timing and SD of sleep timing were significantly greater in the irregular group than regular group (sleep timing: 4:58±0:52 vs. 4:18±0:52,  $p=0.042$ , SD of sleep timing: 94.3±29.6 vs. 43.3±36.0min,  $p<0.001$ ) (Table 3).

**Table 3.** Comparison of demographics and vascular/sleep parameters by sleep-wake rhythm.

	Irregular group (n=16)	Regular group (n=15)	p-value
Demographics			
Age (years)	20.9 ± 1.7	20.0 ± 1.7	0.113
BMI (kg/m <sup>2</sup> )	20.3 ± 1.9	21.6 ± 3.3	0.196
Vascular parameters			
SBP (mmHg)	108.9 ± 8.3	115.7 ± 13.9	0.110
DBP (mmHg)	61.3 ± 6.6	62.5 ± 5.5	0.604
HR (bpm)	67.6 ± 9.0	67.0 ± 9.6	0.853
baPWV (cm/s)	918.3 ± 249.2	1032.5 ± 101.6	0.110
%FMD	6.1 ± 2.4	10.9 ± 2.3	< 0.001
Sleep parameters			
TST (min)	353.8 ± 70.2	379.7 ± 51.4	0.253
Sleep efficiency (%)	92.2 ± 5.8	95.9 ± 2.8	0.027
Bedtime	1:28 ± 0:54	0:56 ± 0:58	0.132
Wake-up time	8:08 ± 0:58	7:35 ± 0:47	0.105
Sleep timing	4:58 ± 0:52	4:18 ± 0:52	0.042
SD of sleep timing (min)	94.3 ± 29.6	43.3 ± 36.0	< 0.001

Notes: Data are expressed as mean ± standard deviation. BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; HR = Heart rate; baPWV = Brachial-ankle pulse wave velocity; FMD = Flow-mediated dilation; TST = Total sleep time; SD = Standard deviation.

Significant correlation was observed between %FMD and the SD of sleep timing ( $r=-0.481$ ,  $p=0.006$ ) in the simple correlation analysis. In the multiple regression analysis, the SD of sleep timing was a significant factor associated with %FMD ( $\beta=-0.454$ ,  $p=0.017$ ) (Table 4).

**Table 4.** Relationships among vascular and sleep parameters.

	Simple correlation analysis		Multiple regression analysis	
	<i>r</i>	<i>p</i> -value	$\beta$	<i>p</i> -value
<b>%FMD</b>				
TST	0.186	0.315	0.001	0.996
Sleep efficiency	0.225	0.224	0.138	0.434
SD of sleep timing	-0.481	0.006	-0.454	0.017
<b>baPWV</b>				
TST	-0.227	0.219	-0.287	0.165
Sleep efficiency	0.061	0.743	0.119	0.542
SD of sleep timing	-0.015	0.937	-0.086	0.665

Notes: FMD = Flow-mediated dilation; TST = Total sleep time; SD = Standard deviation; baPWV = Brachial-ankle pulse wave velocity.

## DISCUSSION

We found that an irregular sleep-wake rhythm was adversely affecting endothelial function. Moreover, sleep efficiency was significantly lower in young adults with irregular sleep-wake rhythm than those with regular sleep-wake rhythm. Our findings suggest that irregular sleep-wake rhythm may be associated with reduced endothelial function and poor sleep quality.

Many of the Japanese university students devote their free time to a part-time job, use social networking and other site on a 24-hour basis<sup>4,6</sup>, or engage in-out-of class activities. Thus, irregular sleep schedule may be a result of these lifestyle choices. Irregular sleep-wake rhythm might disturb behavioral rhythms in regards to timing/amount of eating, which can pose even higher cardiovascular risks to irregular sleepers of nocturnal food intake and breakfast skipping<sup>21-23</sup> or reduced physical activity<sup>24,25</sup>.

The irregular sleep-wake rhythm of a long-term intermittent night shift worker was a factor of impaired endothelial function<sup>26,27</sup>, with most shift workers exhibiting a significant increase in arterial stiffness. Moreover, in a multicenter, cross-sectional, population-based study, the late timing of sleep and irregular sleep-wake rhythm were associated with hypertension, suggesting that interventions to adjust the timing of sleep and obtain a regular sleep-wake rhythm may be important targets for cardiovascular health<sup>28</sup>. These findings may explain the relationship between the irregular sleep-wake rhythm and endothelial dysfunction in young adults.

In a study of BMAL1-knockout and clock mutant mice, aberrant circadian rhythms impaired the endothelial function presumably via a decrease in NO production<sup>29</sup>. Other studies on myocardial ischemia-induced mice demonstrated that recovery from ischemia was delayed by the aberrant circadian rhythms<sup>30</sup>, and the tolerance for myocardial infarction was improved after modifying of circadian rhythms<sup>31</sup>.

Disturbed diurnal rhythm was found to alter gene expression, which affected the cardiovascular system adversely<sup>32</sup>. Hence, the disruption of sleep-wake rhythms may precipitate endothelial dysfunction, which would lead to future occurrence of cardiovascular diseases.

An irregular sleep-wake rhythm was associated with reduced endothelial function and lower sleep efficiency. Hypothalamic-pituitary-adrenal axis dysregulation was associated with the sympathovagal imbalance in sleep deprivation<sup>33,34</sup>. A disruption of circadian rhythm and poor sleep quality have also been found to influence the rhythms of the autonomic nervous system<sup>35,36</sup>, which directly govern normal cardiac function. Thus, the sympathovagal imbalance may be involved in endothelial dysfunction in young adults. Lowered sleep efficiency stimulated the secretions of Interleukin-6 and C-reactive protein, which were known to impair the endothelial function<sup>37,38</sup>. Moreover, long-term inflammation and excessive reactive oxygen species promote oxidative stress, which could lead to the endothelial dysfunction<sup>39</sup>. A previous study assessed FMD in adults free from routine work and showed that poor sleep quality was associated with the endothelial dysfunction<sup>40</sup>. Therefore, a regular sleep-wake rhythm may play an important role in maintaining the sleep quality, sympathovagal balance, and cardiovascular health in even young adults.

In this study, we showed that the TST was not a significant factor affecting the FMD. In a cross-sectional study of 684 subjects (32% male, 68% female) aged 37 to 60 years, there was a significant relationship only seen between the FMD and component 1 (sleep quality) of Pittsburgh sleep quality index, but not with sleep duration<sup>41</sup>. Short sleep duration was not associated with a dysfunction of circulating endothelial progenitor cells in thirty-seven healthy adults (age range: 43-65 years)<sup>42</sup>. In addition, the short sleep duration was associated with in reduced FMD in healthy adults, but not with the reduced FMD after adjusting for sex, age, body mass index, smoking, and other complications<sup>43</sup>. Moreover, the TST in the polysomnographic study was not associated with FMD<sup>40</sup>. Our present results were consistent with the relationship between sleep duration and endothelial function in the previous studies.

The present study has methodological limitations. The study population was relatively small and the sample size was not sufficient to examine the gender differences. Especially, hormones and the menstrual cycle might have biased the results<sup>44</sup>. In the future, prospective or interventional trials with larger sample sizes are required to elucidate the causal relationship between sleep parameters and endothelial function.

## CONCLUSION

An irregular sleep-wake rhythm was associated with the reduced endothelial function and lower sleep efficiency in young adults. Thus, a lifestyle promoting regular sleep-wake rhythm is important for benefits such as better sleep quality and cardiovascular health.

## REFERENCES

1. Touitou Y, Reinberg A, Touitou D. Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: health impacts and mechanisms of circadian disruption. *Life Sci*. 2017 Mar;173:94-106.
2. Oginska H, Pokorski J. Fatigue and mood correlates of sleep length in three age-social groups: School children, students, and employees. *Chronobiol Int*. 2006;23(6):1317-28.
3. Ferreira LRC, Martino MMF. Sleep patterns and fatigue of nursing students who work. *Rev Esc Enferm USP*. 2012;46(5):1178-83.
4. Levenson JC, Shensa A, Sidani JE, Colditz JB, Primack BA. The association between social media use and sleep disturbance among young adults. *Prev Med*. 2016;85:36-41.
5. Lin LY, Sidani JE, Shensa A, Radovic A, Miller E, Colditz JB, et al. Association between social media use and depression among U.S. young adults. *Depress Anxiety*. 2016 Apr;33(4):323-31.
6. Zhai X, Ye M, Wang C, Gu Q, Huang T, Wang K, et al. Associations among physical activity and smartphone use with perceived stress and sleep quality of Chinese college students. *Ment Health Phys Act*. 2020;18:100323.
7. Pires GN, Bezerra AG, Tufik S, Andersen ML. Effects of acute sleep deprivation on state anxiety levels: a systematic review and meta-analysis. *Sleep Med*. 2016;24:109-18.
8. Eisenberg D, Chung H. Adequacy of depression treatment among college students in the United States. *Gen Hosp Psychiatry*. 2012 May/ Jun;34(3):213-20.
9. Miyata S, Noda A, Kawai S, Honda K, Iwamoto K, Ozaki N, et al. Delayed sleep/wake rhythm and excessive daytime sleepiness correlate with decreased daytime brain activity during cognitive task in university students. *Biol Rhythm Res*. 2019 Feb;50(2):171-9.
10. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. *Circ J*. 2009 Mar;73(3):411-8.
11. Huang T, Mariani S, Redline S. Sleep irregularity and risk of cardiovascular events: the multi-ethnic study of atherosclerosis. *J Am Coll Cardiol*. 2020 Mar;75(9):991-9.
12. Yin J, Jin X, Shan Z, Li S, Huang H, Li P, et al. Relationship of sleep duration with all-cause mortality and cardiovascular events: a systematic review and dose-response meta-analysis of prospective cohort studies. *J Am Heart Assoc*. 2017 Sep;6(9):e005947.
13. Morgenthaler T, Alessi C, Friedman L, Owens J, Kapur V, Boehlecke B, et al. Practice parameters for the use of actigraphy in the assessment of sleep and sleep disorders: an update for 2007. *Sleep*. 2007 Apr;30(4):519-29.
14. Cole RJ, Kripke DF, Gruen W, Mullaney DJ, Gillin JC. Automatic sleep/wake identification from wrist activity. *Sleep*. 1992 Oct;15(5):461-9.
15. Youngstedt SD, Kripke DF, Elliott JA, Klauber MR. Circadian abnormalities in older adults. *J Pineal Res*. 2001 Oct;31(3):264-72.
16. American Academy of Sleep Medicine (AASM). International Classification of Sleep Disorders. 3<sup>rd</sup> ed. Darien: AASM; 2014.
17. Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. *Hypertens Res*. 2002 May;25(3):359-64.
18. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002 Jan;39(2):257-65.
19. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol*. 2011 Jan;300(1):H2-12.
20. Van Dongen HP, Maislin G, Mullington JM, Dinges DF. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*. 2003 Mar;26(2):117-26.
21. Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian timing of food intake contributes to weight gain. *Obesity (Silver Spring)*. 2009 Nov;17(11):2100-2.
22. Garaulet M, Gómez-Abellán P, Alburquerque-Béjar JJ, Lee YC, Ordovás JM, Scheer FA. Timing of food intake predicts weight loss effectiveness. *Int J Obes (Lond)*. 2013 Apr;37(4):604-11.
23. Rong S, Snetselaar LG, Xu G, Sun Y, Liu B, Wallace RB, et al. Association of skipping breakfast with cardiovascular and all-cause mortality. *J Am Coll Cardiol*. 2019 Apr;73(16):2025-32.
24. Davies KAB, Pickles S, Sprung VS, Kemp GJ, Alam U, Moore DR, et al. Reduced physical activity in young and older adults: metabolic and musculoskeletal implications. *Ther Adv Endocrinol Metab*. 2019 Nov;10:2042018819888824.
25. Sugiura M, Noda A, Miyata S, Kojima J, Hara Y, Minoshima M, et al. The effect of habitual physical training on left ventricular function during exercise assessed by three-dimensional echocardiography. *Echocardiography*. 2015 Nov;32(11):1670-5.
26. Lunsford-Avery JR, Engelhard MM, Navar AM, Kollins SH. Validation of the sleep regularity index in older adults and associations with cardiometabolic risk. *Sci Rep*. 2018 Sep;8(1):14158.
27. Wehrens SM, Hampton SM, Skene DJ. Heart rate variability and endothelial function after sleep deprivation and recovery sleep among male shift and non-shift workers. *Scand J Work Environ Health*. 2012 Mar;38(2):171-81.
28. Abbott SM, Weng J, Reid KJ, Daviglius ML, Gallo LC, Loreda JS, et al. Sleep timing, stability, and BP in the sueno ancillary study of the hispanic community health study/study of Latinos. *Chest*. 2019 Jan;155(1):60-8.
29. Anea CB, Zhang M, Stepp DW, Simkins GB, Reed G, Fulton DJ, et al. Vascular disease in mice with a dysfunctional circadian clock. *Circulation*. 2009 Mar;119(11):1510-7.
30. Hurd MW, Ralph MR. The significance of circadian organization for longevity in the golden hamster. *J Biol Rhythms*. 1998 Oct;13(5):430-6.
31. Durgan DJ, Pulinilkunnil T, Villegas-Montoya C, Garvey ME, Frangogiannis NG, Michael LH, et al. Short communication: ischemia/reperfusion tolerance is time-of-day-dependent: mediation by the cardiomyocyte circadian clock. *Circ Res*. 2010 Feb;106(3):546-50.
32. Martino TA, Tata N, Belsham DD, Chalmers J, Straume M, Lee P, et al. Disturbed diurnal rhythm alters gene expression and exacerbates cardiovascular disease with rescue by resynchronization. *Hypertension*. 2007;49(5):1104-13.
33. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. *Lancet*. 1999 Oct;354(9188):1435-9.
34. Nagai K, Nagai N, Shimizu K, Chun S, Nakagawa H, Nijijima A. SCN output drives the autonomic nervous system: with special reference to the autonomic function related to the regulation of glucose metabolism. *Prog Brain Res*. 1996;111:253-72.
35. Burgess HJ, Trinder J, Kim Y, Luke D. Sleep and circadian influences on cardiac autonomic nervous system activity. *Am J Physiol*. 1997 Oct;273(4):H1761-8.
36. Huang T, Poole EM, Vetter C, Rexrode KM, Kubzansky LD, Schernhammer E, et al. Habitual sleep quality and diurnal rhythms of salivary cortisol and dehydroepiandrosterone in postmenopausal women. *Psychoneuroendocrinology*. 2017 Oct;84:172-80.
37. Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. *J Clin Sleep Med*. 2007 Aug;3(5):519-28.
38. Nowakowski S, Matthews KA, Von Känel R, Hall MH, Thurston RC. Sleep characteristics and inflammatory biomarkers among midlife women. *Sleep*. 2018 May;41(5):zsy049.
39. Agita A, Alsagaff MT. Inflammation, immunity, and hypertension. *Acta Med Indones*. 2017 Apr;49(2):158-65.
40. Cooper DC, Ziegler MG, Milic MS, Ancoli-Israel S, Mills PJ, Loreda JS, et al. Endothelial function and sleep: associations of flow-mediated dilation with perceived sleep quality and rapid eye movement (REM) sleep. *J Sleep Res*. 2014 Feb;23(1):84-93.
41. Behl M, Bliwise D, Veledar E, Cunningham L, Vazquez J, Brigham K, et al. Vascular endothelial function and self-reported sleep. *Am J Med Sci*. 2014 Jun;347(6):425-8.
42. Weil BR, Maceneaney OJ, Stauffer BL, Desouza CA. Habitual short sleep duration and circulating endothelial progenitor cells. *J Cardiovasc Dis Res*. 2011 Apr/ Jun;2(2):110-4.
43. Hall MH, Mulukutla S, Kline CE, Samuelsson LB, Taylor BJ, Thayer JF, et al. Objective sleep duration is prospectively associated with endothelial health. *Sleep*. 2017 Jan;40(1):zsw003.
44. Williams MR, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K, et al. Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab*. 2001 Nov;86(11):5389-95.