Nutr Res Pract. 2020 Dec;14(6):606-620
https://doi.org/10.4162/nrp.2020.14.6.606
pISSN 1976-1457·eISSN 2005-6168

Nutrition Research and Practice

Original Research

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Effects of sleep-inducing juice on sleep quality and heart rate variability in adults with disturbed sleep

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OPEN ACCESS

Received: Dec 23, 2019
Revised: Feb 17, 2020
Accepted: Jun 29, 2020

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Funding
This research was supported by Hurom Co., Ltd. (No. 201703320000).

Conflict of Interest
The authors declare no potential conflicts of interests.

ABSTRACT

BACKGROUND/OBJECTIVES: Although some juices affect subjective sleep quality, there is a lack of information on the effect of a specific juice on objective sleep quality and heart rate variability (HRV) during sleep; thus the present study investigated whether a blended juice made from natural extracts influenced sleep quality and HRV during sleep in adults with disturbed sleep.
SUBJECTS/METHODS: A randomized, crossover study was conducted on twenty-five adults (15/10, female/male) complaining of difficulty initiating or maintaining nighttime sleep (Pittsburgh sleep quality index [PSQI] ≥ 5). During feeding sessions (FS), subjects received sleep-inducing juice made of natural ingredients (250 mL/trial) twice a day for 8 weeks or non-FS (N-FS) for 8 weeks while maintaining normal activities. Sleep quality and parameters were recorded via wearable actigraph for 7 consecutive days, and PSQI scores were assessed before and after the intervention. HRV was also monitored at rest and during sleep.
RESULTS: After receiving the sleep-inducing juice intervention (FS), PSQI scores were significantly decreased ($P < 0.001$) and correlated with a significant decline in fatigue severity scale and visual analogue scale levels ($P < 0.05$; both). HRV indices of vagal activity were significantly improved during FS ($P < 0.05$), and no significant differences in N-FS were observed. Sleep efficiency and total sleep time increased significantly ($P < 0.05$) and sleep latency, total counts, sleep fragmentation index, and movement index, decreased significantly ($P < 0.05$, all 4) during FS, with no significant differences-observed during N-FS.
CONCLUSIONS: This study results demonstrated that an 8-week course of sleep-inducing juice has led to improve sleep quality, suggesting an enhanced cardiac vagal tone during sleep. Thus, it could be a well-tolerated option for adults with disturbed sleep.

Keywords: Cross-over studies; sleep latency; autonomic nerves; heart rate

INTRODUCTION

Important biological functions, including physiological, cognition, and cardiovascular system processes [1] occur during sleep, yet more than 27% of adults worldwide suffer from sleep disorders, including difficulty in initiating and/or maintaining sleep [2]. The Korean National Health Insurance Corporation has reported an increasing trend in sleep disorders

Author Contributions

Conceptualization: Kim M; Formal analysis: Kim CS, Jung H, Kim MJ; Funding acquistion: Kim M; Investigation: Kim M, Kim CS; Methodology: Kim CS, Kim M; Supervision: Kim M; Writing - original draft: Kim CS, Kim MJ, Jung H, Kim M; Writing - review & editing: Kim M, Jung H, Kim CS, Kim MJ.

(disease code: G47) among young adults. Many studies have reported that poor sleep quality and reduced sleep time are related to a decline in overall quality of life and increased risk for further cardiovascular events [3]. As such, sleep itself is considered essential for overall quality of life and cardiovascular health. However, several studies rely upon subjective sleep surveys to investigate nocturnal profiles or intervention effectiveness, despite the fact that self-reported questionnaires introduce method bias that undermines their scientific validity [4]. Objective sleep assessments, in addition to self-reported surveys, are thereby necessary for an accurate sleep health evaluation.

The actigraph is a wearable device with a triaxial accelerometer and is widely used to evaluate sleep/wake cycles. Although polysomnography (pSG) is the predominant tool for diagnosing sleep disorders, it is challenging to use in research settings due to complicated and time-consuming procedures and the need for resource intensive data analysis [5]. A previous study monitored sleep patterns with an actigraph to uncover relationships between sleep and perceived quality of life and concluded that improvement of sleep parameters as detected by actigraph was accompanied by enhanced perceived quality of life [6].

Sleep deprivation is associated with increased cardiovascular disorder (CVD) risk. The Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands study conducted 10–15 yrs of research on 20,432 healthy adults (age 20–65 yrs) without any pathological disorders, and found that people with shorter sleep durations or poorer sleep quality were at greater risk for developing CVD [7]. Cardiac autonomic imbalance due to sleep disorders is associated with CVD incidence [8]. A reduction in vagal tone, including a decrease in parasympathetic activity during sleep, has also been associated with increased morbidity and mortality in CVD [9]. A recent study monitoring chronic fatigue syndrome (CFS) patients with poor sleep quality using heart rate variability (HRV) revealed that these patients demonstrated decreased vagal activity during nighttime sleep, suggesting the HRV index could be a significant predictor of cardiac autonomic imbalance caused by disrupted sleep [10]. As such, when assessing health problems related to sleep disorders, nocturnal HRV indices of cardiac autonomic regulation may provide critical information.

The recent intervention trial has examined alternative strategies such as nutritional [11,12] therapies to improve nighttime sleep. Nutritional therapy for induction of sleep could be used long-term without side effects and represents a potential alternative approach for sleep improvement [11]. However, many nutrition-related sleep studies were dependent on self-reporting of the effectiveness of interventions [13]. It remains unknown whether nutritional treatment can improve sleep quality or affect nocturnal HRV indices of cardiac autonomic regulation. New methods are thereby needed to evaluate the effects of sleep-inducing nutritional interventions, a need underscored by the rapid increase of sleep disorders and the relation of long-term sleep disorders to future CVD [2,3]. We have hypothesized that sleep-inducing juice which was made up of natural extracts will provide positive effects on sleep quality and enhance cardiac autonomic regulation for better-quality in sleep. To examine this hypothesis, the present study was performed to verify effectiveness of sleep-inducing juice composed of natural extracts on sleep quality and to examine whether an intervention to enhance sleep has influenced on HRV in adults who are suffering from sleep deprivation.

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SUBJECTS AND METHODS

Subjects

A total of 44 adults complaining of sleep difficulty were recruited for this study. Twelve subjects who did not meet the inclusion criteria were excluded. Inclusion criteria were over 18 yrs of age; difficulty in initiating or sustaining normal nighttime sleep, as evidenced by a score of at least 5 on the Pittsburgh sleep quality index (PSQI); freedom from overt cardiovascular, metabolic (body mass index [BMI] ≥ 30), and/or neurological disorders; a sedentary lifestyle for at least 12 months before the study; no history of sleep-inducing medication use; and no use of prescription medications and/or illicit drugs affecting cardiac autonomic nerve regulation.

Participants of this study were recruited 2 ways. Firstly, we have explained the benefits of applying to join this study using a web bulletin board of Kyungpook National University, from April to May, 2017. And then all volunteers who want to take part in the current study were recruited by telephone counseling. Secondly, we had selected employees who are suffering from sleep deprivation in South Gyeongsang Province-based Hurom Corporation. After selection of experimental subjects who meet the requirements of inclusion criteria for this study through self-reported questionnaire and interviews, order and procedure for each session were designed. The first session was conducted from May to July, 2017. And then second session was carried out from July to September, 2017, following the 2-week washout period. After a comprehensive examination, 32 men and women were enrolled in the study (**Fig. 1**). The clinical characteristics of the participants are presented in **Table 1**. After being

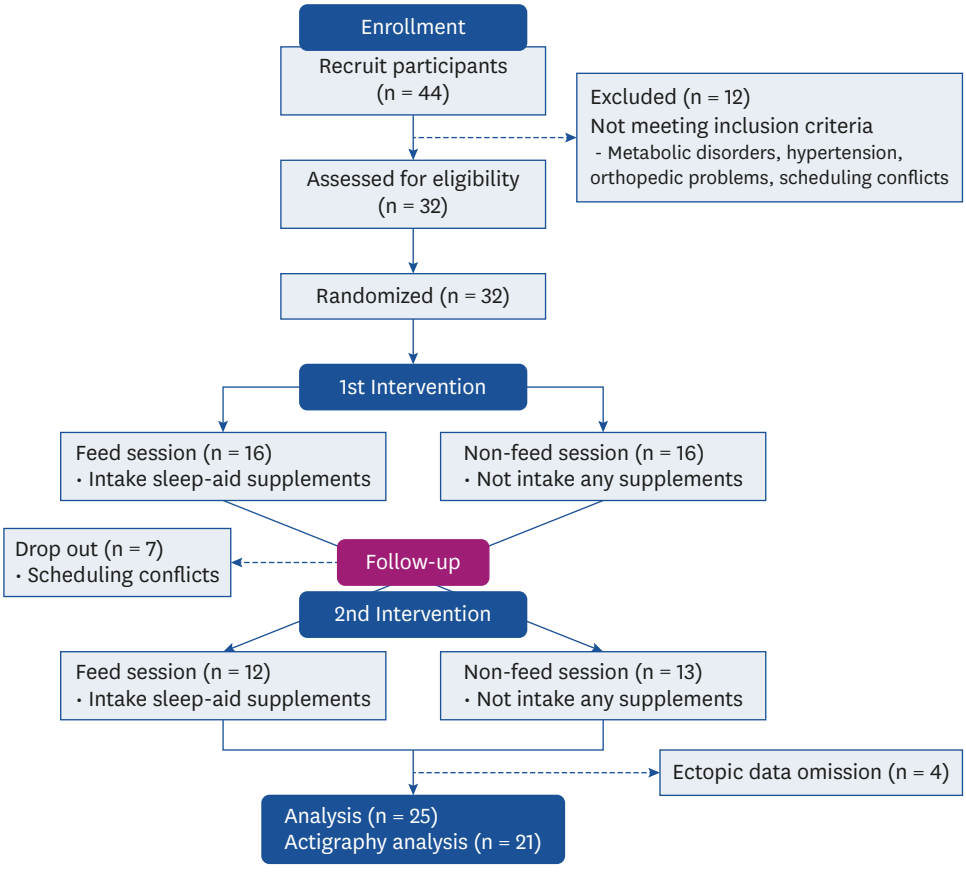

Fig. 1. Flow chart outlining participation.

https://doi.org/10.4162/nrp.2020.14.6.606

Table 1. Changes in characteristics of subjects and questionnaire scores

Variables	N-FS (n = 25)			FS (n = 25)			Time × session
	Pre	Post	*P*-value	Pre	Post	*P*-value	
Age (yrs)	25.04 ± 1.08	-	-	-	-	-	-
Body composition							
Height (cm)	165.88 ± 1.47	166.32 ± 1.42	0.951	165.97 ± 1.47	165.88 ± 1.49	0.415	0.244
Weight (kg)	60.39 ± 2.27	59.71 ± 2.37	0.679	60.08 ± 2.22	60.42 ± 2.21	0.211	0.237
BMI (kg/m^2)	22.04 ± 0.66	21.45 ± 0.64	0.855	21.72 ± 0.63	21.87 ± 0.62	0.247	0.133
WHR	0.83 ± 0.01	0.83 ± 0.01	0.906	0.83 ± 0.01	0.83 ± 0.01	0.686	0.968
Body fat (%)	20.71 ± 1.42	20.43 ± 1.29	0.616	20.24 ± 1.44	20.72 ± 1.45	0.484	0.305
Sleep quality index and fatigue index							
PSQI (score)	8.32 ± 0.56	7.84 ± 0.53	0.821	9.96 ± 0.57	6.52 ± 0.39	0.000*	0.008*
FSS (score)	35.24 ± 1.80	35.76 ± 1.71	0.605	37.96 ± 1.83	29.68 ± 1.49	0.001*	0.008*
VAS (score)	4.40 ± 0.40	4.32 ± 0.35	0.904	4.74 ± 0.35	2.96 ± 0.30	0.000*	0.006*

Values are mean ± SEM. *P*-values calculated by Wilcoxon's signed-rank test.
Time × session values calculated by repeated measure. N-FS, non-feeding session; FS, feeding session; BMI, body mass index; WHR, waist-to-hip ratio; PSQI, Pittsburgh sleep quality index; FSS, fatigue severity scale; VAS, visual analog scale; SEM, standard error of mean.
*$P < 0.05$.

informed of the experimental procedures, all participants provided written informed consent indicating voluntary participation before beginning the study. This study was approved by the Kyungpook National University Life Science Ethics Committee (IRB: 2017-0033).

Trial protocol

An unblinded randomized crossover trial consisting of 8 weeks with (feeding sessions [FS]) or without (non-FS [N-FS]) sleep-inducing juice supplementation, a 2-week wash-out period, and then another 8 weeks in the other (N-FS or FS) group, was conducted. FS received sleep-inducing juice (250 mL/trial) developed from natural ingredients twice per day (morning and afternoon, respectively) for 8 weeks while maintaining their usual lifestyle and diet. Because of relatively a short expiration period of sleep-inducing juice used in the current study, we had to visit at least every 3 days to the home of all subject to supply it. For this reason, whenever we have visited their home, we were allowed to review daily life activities and dietary intake for each subject. In particular, when participants have visited the laboratory due to nocturnal HRV assessment and actigraphy monitoring for 7 consecutive days, we tried more detailed interviews to find whether daily life patterns were altered. None has been reported any marked alteration for daily life patterns, including dietary intake throughout all experimental sessions (data not shown).

For at least 48 hours prior to physical examination at pre- and post- intervention, we instructed all subjects to abstain from strenuous physical activity and intake of alcoholic or caffeinated beverages. After overnight fasting, all subjects visited the laboratory between 9 A.M. and 12 P.M., to exclude circadian variation effects, followed by measurements of anthropometric and hemodynamic indices and HRV, and completion of subjective sleep quality and fatigue surveys (**Fig. 2**).

Objective sleep parameters were obtained by asking participants to wear the ActiGraph GT3X (Actigraph, LLC., Pensacola, FL, USA) during sleep along with recording their sleep daily in a diary for 7 days before and after the intervention. Nocturnal RR intervals were collected by Polar RS800CX (polar® Electro Őy, Kemple, Finland) over a single night along with objective sleep evaluation to assess the effects of sleep-inducing juice on cardiac autonomic regulation. Subjects received a comprehensive demonstration and explanation of proper use of these devices to wear at home, and proper use was verified prior to embarking on the study. Experimental procedures are described below (**Fig. 2**).

https://doi.org/10.4162/nrp.2020.14.6.606

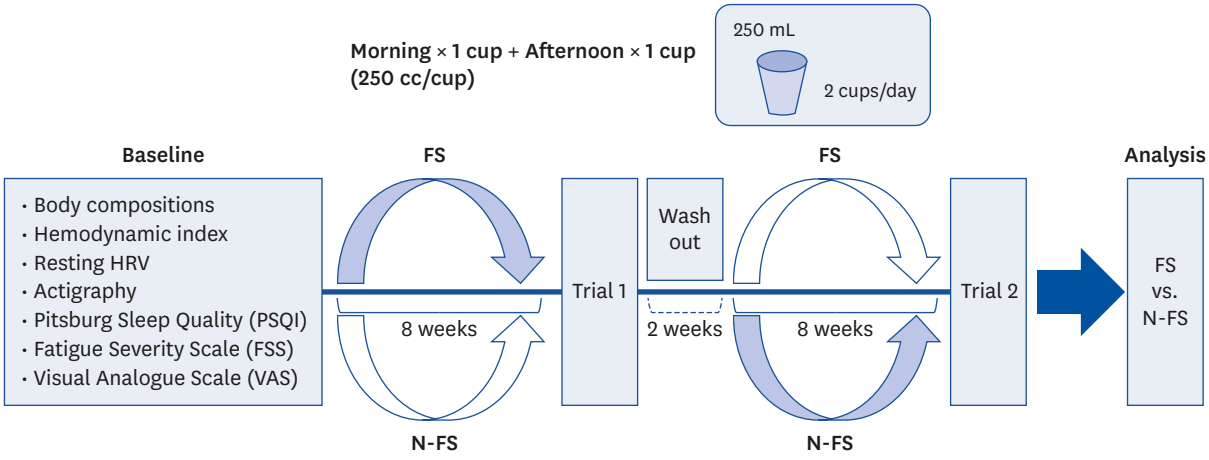

Fig. 2. Schematic outlining interventions.
FS, feeding sessions; N-FS, non-feeding sessions.

Interventions

Sleep-inducing ingredients were obtained as follows: lettuce (*Lactuca sativa* L.) was supplied by Jeonnam Agricultural Research and Extension Services in Naju, Korea. Oranges, pineapples, and frozen cherries were purchased from a local market. The lettuce, orange, pineapple and frozen cherry were extracted with a vertical-type low-speed masticating juicer (HWS-SBF18; Hurom Co., Ltd., Gimhae, Korea) at a ratio of 28, 44, 14 and 14% respectively. The extracted juice was mixed with 0.1% dried bonnet bellflower and 250 mL of juice placed in a bottle for high pressure sterilization at 550 MPa for 60 s (QFP 350L; Avure Technologies, Kent, WA, USA). Extraction and processing details, such as blending technique, are proprietary and confidential per the manufacturer's instructions.

Anthropometry and hemodynamics

Height and weight were measured to the nearest 0.1 cm and 0.01 kg using an automatic digital stadiometer (DS-102; JENIX Co., Seoul, Korea) and a digital electronic weighing scale (GS 36; Beurer GmBH & Co., Ulm, Germany), respectively. BMI was calculated by dividing weight by height squared (kg/m^2). Waist-to-hip ratio was calculated from waist circumference divided by that of the hips. In accordance with the American College of Sports Medicine guidelines [14], body density was calculated by Jackson and Pollock equation following skinfold measurements of 3 sites with caliper (Dynatronics Corp., Salt Lake City, UT, USA) and body fat percentages were estimated by Siri equation. Participants remained in a seated position for 20 min or more, after which systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean heart rate were obtained on their non-dominant arm using a BP monitor (BM 16; Beurer GmBH & Co.). Mean arterial pressure was estimated as follows: [(2 × DBP) + SBP]/3.

Subjective sleep quality and fatigue assessments

The PSQI is a 24-item questionnaire designed for participants to self-evaluate their sleep/wake patterns across 7 different components: subjective sleep quality, sleep latency (SL), sleep duration, habitual sleep efficiency (SE), sleep disturbances, use of sleeping medication, and daytime dysfunction [15]. Each dimension is scored on a Likert-type scale from 0 to 3, with 0 representing normal, and 3 representing extremely disrupted. Global PSQI scores range from 0 to 21. A score of at least 5 indicates a poor sleeper who suffers from

https://doi.org/10.4162/nrp.2020.14.6.606

serious difficulties in 2 domains or moderate difficulties in 3 domains of sleep. In addition, the Pittsburgh sleep diary consists of bedtime and wake-time sections [16]. Subjects were instructed to complete the both every day when they wore the actigraph. Bedtime questionnaires involved timing of breakfast, lunch and dinner; caffeine, alcohol and tobacco product consumption; use of prescribed and over-the-counter medications; and timing and duration of daytime exercise and naps. Wake-time questionnaires contained timing of going to bed, turning out lights, minutes to sleep onset; final wake-time, method of final waking; incidence, duration, and reasons for wake after sleep onset (WASO); and subjective quality of sleep episode, mood on awakening (tense vs. calm), and alertness on awakening, marked on 10 cm visual analogue scale (VAS). The 9-item fatigue severity scale (FSS) and VAS scores were combined to assess the subjective intensity of fatigue on functional and behavioral aspects of daily life [17]. The Korean version of the FSS presented a high internal consistency (Cronbach's $\alpha = 0.93$), and the item-total correlation ranged from 0.56 to 0.90 [18]. Each item was expressed on a scale from 1 (strongly disagree) to 7 (strongly agree). Possible scoring range was 9 to 63, with higher scores (≥ 36) reflecting greater levels of fatigue. The 10 cm VAS was also quantified and contributed to calculations of overall fatigue status.

HRV analysis

We evaluated resting and nocturnal cardiac autonomic regulation through HRV, measured at 1,000 Hz by Polar RS800CX (polar® Electro Őy), which records normal-to-normal RR intervals from a wireless chest strap (2-lead) heart rate monitor. Raw data were extracted as a text file (.hrm) by Pro-trainer Polar 5 software (version 5.40.171; polar® Electro Őy) and then imported into HRV analysis software (Kubios HRV version 2.1, 2012; Biosignal Analysis and Medical Imaging Group, University of Kuopio, Kuopio, Finland, MATLAB). Prior to HRV analysis, ectopic beats were excluded by artifact correction. Following a standardized short-term protocol (5 min), a series of RR intervals at 256 points/Hz were selected and re-extracted via detrended method using smoothness priors ($\lambda/500$) technique [19]. Presently, a standardized protocol for nocturnal HRV analysis during sleep has not been established. However, previous studies have proposed recording starting one hour after going to bed [20,21]. Considering the shortest duration of sleep among study participants, nocturnal cardiac autonomic regulation was evaluated over a 240 min period at least one hour after bedtime. Time-course changes of HRV indices during sleep were assessed by dividing total sleep periods into 30 min intervals (60, 90, 120, 150, 180, 210, and 240 min after sleep). We computed time, frequency, and nonlinear indices using the first 5 min of each period. Time domain index, square root of the mean squared differences between successive RR intervals (rMSSD), and NN50 divided by the total number of RR intervals, which reflect vagal activity, were calculated. Low frequency (0.04–0.15 Hz) and high frequency (HF; 0.15–0.4 Hz) values were extracted via power spectrum density analysis by fast Fourier transform method [22]. Nonlinear techniques have increased in importance in recent studies because non-invasive bio-signals commonly present with non-stationary characteristics. A Poincaré plot applicable to short-term HRV measures schematized the association between continuous RR intervals. This plot is derived from a nonlinear index such as SD of the instantaneous beat-to-beat variability of data (SD1), or deviation of instantaneous RR interval variability by short diameter of ellipse, and transverse axis.

Actigraphy

Objective parameters of the nighttime sleep pattern were assessed by an ActiGraph GT3X+ (ActiGraph, LLC.) equipped with a triaxial accelerometer. All patients were instructed to secure the wristwatch actigraph (4.6 × 3.3 × 1.5 cm, 19 g) on their non-dominant wrist before bedtime

https://doi.org/10.4162/nrp.2020.14.6.606

over 7 consecutive days (6 nights) to assess nocturnal sleep quality. The following objective sleep parameters were taken: SL, total counts (TC) of movement, SE, sleep fragmentation index (SFI), total sleep time (TST), WASO, number of awakenings, average awakening length, movement index (MI), and fragmentation index. Raw actigraph data stored as digitized computer data were analyzed by ActiLife version 6.9.2 software (ActiGraph, LLC.), wherein the actigraph accelerometer was initialized to collect data and automatically scored sleep in 60 s epochs at 80 Hz [23]. The Sadeh algorithm previously validated on a sample of young adults, was used to calculate objective sleep measures [24]. Validity and reliability were improved by analyzing 4 days, with the 2 least reliable days excluded.

Statistical analysis

Data were analyzed using descriptive statistics performed by SPSS 25.0 (SPSS software; IBM Corp., IL, USA). All variables are represented as mean ± standard error of the mean. The Shapiro-Wilk test was used to assess the normality of the distribution. Changes in independent variables pre- and post-intervention were examined by Wilcoxon signed-rank test for each session. Finally, repeated measurement of variance analysis was conducted to identify potential interaction effects between time and sessions. Significance was set at $\alpha = 0.05$.

RESULTS

Seven participants with scheduling conflicts dropped out of the second trial after the 2-week wash out following the first trial. In the second trial, there were twelve participants in FS, and thirteen participants in N-FS. In addition, 4 participants were excluded from the objective sleep assessment due to missing actigraph recording data. As a result, the present study analyzed 25 participants, but only 21 participants included an analysis of actigraphy data.

Anthropometrics and hemodynamic indices

Measured variables demonstrated no significant differences between baselines for the 2 sessions ($P > 0.05$; data not shown). No statistical differences in resting blood pressure (data not shown) and body composition ($P > 0.05$, **Table 1**) were associated with either group.

Subjective sleep quality and fatigue index

There were no differences between sessions for baseline subjective measures of sleep quality and perceived fatigue. However, self-reported PSQI and FSS scores, including VAS were significantly decreased after FS ($P < 0.01$, **Table 1**), while no significant differences in these values were observed for N-FS ($P > 0.05$, **Table 1**). Significant session × time period interaction effects were observed on subjective measures of sleep quality and perceived fatigue (PSQI and FSS scores with VAS, $P < 0.01$; respectively).

Resting and nocturnal cardiac autonomic regulation

There were no significant differences between sessions for resting HRV indices such as time, frequency, domain, and nonlinear parameters in baseline levels ($P > 0.05$; data not shown). However, resting HF component reflecting vagal tone was significantly increased in FS, while that of N-FS were not statistically different ($P < 0.05$, **Table 2**). A significant session × time period interaction effect was observed on HF at rest ($P < 0.05$, **Table 2**).

Nocturnal HRV indices had no baseline statistical differences between sessions ($P > 0.05$; data not shown). However, enhanced vagal activity accompanied by sympathetic withdrawal

https://doi.org/10.4162/nrp.2020.14.6.606

Table 2. Changes in resting heart rate variability parameters

Variables	N-FS (n = 25)			FS (n = 25)			Time × session
	Pre	Post	*P*-value	Pre	Post	*P*-value	
Time domain							
rMSSD	39.39 ± 3.49	36.63 ± 3.44	0.183	39.52 ± 3.58	41.65 ± 3.30	0.253	0.061
pNN50	18.83 ± 3.14	16.24 ± 3.21	0.221	20.35 ± 3.43	21.07 ± 3.11	0.412	0.263
Frequency domain							
HF	543.38 ± 117.44	421.66 ± 67.66	0.339	519.66 ± 78.00	718.09 ± 134.84	0.030*	0.028
LF/HF ratio	4.29 ± 0.74	4.83 ± 0.75	0.253	4.93 ± 0.79	3.87 ± 0.90	0.201	0.106
LF nu	0.70 ± 0.04	0.72 ± 0.04	0.231	0.70 ± 0.05	0.63 ± 0.05	0.109	0.094
HF nu	0.30 ± 0.04	0.28 ± 0.04	0.231	0.30 ± 0.05	0.37 ± 0.05	0.109	0.094
Nonlinear							
SD1	27.89 ± 2.47	25.94 ± 2.44	0.183	27.99 ± 2.54	29.49 ± 2.33	0.253	0.061
SampEn	1.53 ± 0.06	1.49 ± 0.06	0.150	1.46 ± 0.05	1.53 ± 0.06	0.276	0.214
DFA1	1.15 ± 0.06	1.15 ± 0.06	0.493	1.13 ± 0.06	1.08 ± 0.07	0.397	0.274

Values are means ± SEM. *P*-values are calculated by Wilcoxon's signed-rank test.
N-FS, non-feeding session; FS, feeding session; rMSSD, square root of the mean squared differences between successive RR intervals; pNN50, NN50 (number of successive RR interval pairs that differ more than 50 ms) divided by the total number of RR interval; HF, high frequency(0.15–0.4 Hz); LF/HF ratio, ratio between low frequency (0.04–0.15 Hz) and high frequency; LF nu, low frequency normalized unit; HF nu, high frequency normalized unit; SD1, SD of the instantaneous beat-to-beat variability of data; SampEn, sample entropy; DFA1, detrended fluctuation analysis index alpha1 short term fractal scaling exponent; SEM, standard error of mean.
*$P < 0.05$.

during sleep were found after sleep-inducing juice intake. We calculated nocturnal HRV indices to assess cardiac autonomic regulation during sleep over a total of 7 periods (60, 90, 120, 150, 180, 210, and 240 min).

Results of this study indicated that the HRV indices of vagal activity rMSSD, HF, and SD1 were increased ($P < 0.05$; all 3) following sleep-inducing juice intake at 210 min during sleep (**Table 3**); those of the N-FS did not show significant differences in total periods of nocturnal cardiac autonomic regulation (data not shown). Significant session × time period interaction effects on HRV indices reflecting vagal activity at 210 min during sleep were also observed (rMSSD, HF, and SD1, $P < 0.05$; all 3).

Objective sleep quality

There were no significant differences between sessions for baseline objective measures of sleep quality (data not shown). However, TST was statistically increased over baseline in FS ($P < 0.05$), while that of N-FS tended to decrease compared to baseline (**Table 4**). In contrast, MI was significantly decreased in FS from baseline ($P < 0.05$), while that of N-FS tended to increase compared to baseline (**Table 4**). Significant session × time period interaction effects on TST and MI were also observed (TST and MI, $P < 0.01$; respectively).

SL, TC, and SFI were significantly increased over baseline in FS ($P < 0.01$), while values for N-FS showed no significant differences compared to baseline (**Fig. 3**). Significant session × time period interaction effects on SL and TC were also observed (SL and TC, $P < 0.01$; respectively). SFI did not have a statistically significant interaction effect ($P > 0.05$, **Fig. 3**). SE was statistically increased over baseline in FS ($P < 0.01$), while that of N-FS tended to decrease compared to baseline (**Fig. 4**). A significant session × time period interaction effect was also observed for SE ($P < 0.01$, **Fig. 4**).

https://doi.org/10.4162/nrp.2020.14.6.606

Table 3. Changes in nocturnal heart rate variability parameters

Variables	Session	180	*P*-value	Time × session	210	*P*-value	Time × session	240	*P*-value	Time × session
Time domain										
rMSSD	N-FS	−3.94 ± 4.99	0.104	0.060	−2.61 ± 5.64	0.065	0.043*	2.46 ± 5.91	0.798	0.842
	FS	8.69 ± 4.52			13.01 ± 3.59			4.19 ± 4.60		
pNN50	N-FS	−2.01 ± 3.78	0.231	0.187	−0.99 ± 4.46	0.135	0.130	4.32 ± 4.78	0.563	0.807
	FS	5.38 ± 3.88			7.84 ± 3.49			2.68 ± 4.13		
Frequency domain										
HF	N-FS	−485.55 ± 288.15	0.007*	0.006*	−262.27 ± 307.57	0.019*	0.018*	−147.25 ± 340.37	0.840	0.394
	FS	572.39 ± 262.72			669.12 ± 192.31			244.95 ± 248.54		
LF/HF ratio	N-FS	0.32 ± 0.24	0.035*	0.030*	0.28 ± 0.18	0.037*	0.108	0.06 ± 0.29	0.904	0.918
	FS	−0.35 ± 0.19			−0.15 ± 0.14			0.09 ± 0.26		
LF nu	N-FS	0.07 ± 0.05	0.069	0.056	0.06 ± 0.04	0.026*	0.048*	0.00 ± 0.06	0.510	0.771
	FS	−0.06 ± 0.05			−0.06 ± 0.03			−0.02 ± 0.05		
HF nu	N-FS	−0.07 ± 0.05	0.069	0.056	−0.06 ± 0.04	0.026*	0.048*	0.00 ± 0.06	0.510	0.771
	FS	0.06 ± 0.05			0.06 ± 0.03			0.02 ± 0.05		
Nonlinear domain										
SD1	N-FS	−2.79 ± 3.54	0.104	0.060	−1.85 ± 3.99	0.065	0.043*	1.74 ± 4.19	0.798	0.842
	FS	6.16 ± 3.20			9.22 ± 2.54			2.97 ± 3.26		
SampEn	N-FS	−0.04 ± 0.04	0.166	0.143	0.05 ± 0.04	0.619	0.776	0.03 ± 0.06	0.798	0.935
	FS	0.10 ± 0.07			0.03 ± 0.05			0.04 ± 0.06		
DFA1	N-FS	0.11 ± 0.07	0.042*	0.029*	0.05 ± 0.05	0.069	0.105	0.00 ± 0.09	0.638	0.684
	FS	−0.10 ± 0.06			−0.08 ± 0.05			−0.03 ± 0.07		

Values are means ± SEM. *P*-values are calculated by Wilcoxon's signed-rank test.
rMSSD, square root of the mean squared differences between successive RR intervals; pNN50, NN50 (number of successive RR interval pairs that differ more than 50 ms) divided by the total number of RR interval; N-FS, non-feeding session; FS, feeding session; HF, high frequency (0.15–0.4 Hz); LF/HF ratio, ratio between low frequency (0.04–0.15 Hz) and high frequency; LF nu, low frequency normalized unit; HF nu, high frequency normalized unit; SD1, SD of the instantaneous beat-to-beat variability of data; SampEn, smaple entropy; DFA1, detrended fluctuation analysis index alpha1 short term fractal scaling exponent; SEM, standard error of mean.
*$P < 0.05$.

Table 4. Changes in objective sleep quality index

Variables	N-FS (n = 21)			FS (n = 21)			Time × session
	Pre	Post	*P*-value	Pre	Post	*P*-value	
TST (min)	183.36 ± 4.63	180.84 ± 3.67	0.590	178.23 ± 4.10	193.73 ± 3.32	0.001**	0.007**
WASO (min)	39.89 ± 4.09	42.13 ± 3.36	0.765	42.63 ± 3.89	36.51 ± 3.57	0.079	0.144
NOA (no/night)	10.85 ± 0.75	11.33 ± 0.59	0.422	10.98 ± 0.59	10.31 ± 0.61	0.322	0.290
AAL (min)	4.01 ± 0.46	3.71 ± 0.25	0.550	4.24 ± 0.43	3.66 ± 0.39	0.170	0.581
MI	19.27 ± 1.58	20.10 ± 1.23	0.498	20.27 ± 0.96	15.29 ± 0.71	0.001**	0.008**
FI	13.00 ± 1.74	13.63 ± 1.14	0.543	11.81 ± 1.00	11.80 ± 1.11	0.664	0.772

Values are means ± SEM. *P*-values calculated by Wilcoxon's signed-rank test.
N-FS, non-feeding session; FS, feeding session TST, total sleep time; WASO, wake after sleep onset; NOA, number of awakenings; AAL, average awakening length; MI, movement index; FI, fragmentation index; SEM, standard error of mean.
**$P < 0.01$.

DISCUSSION

Human beings spend approximately one-third of their lives sleeping. Despite the importance of sleep health, it is often overlooked. Numbers of patients who suffer from sleep disorders involving reduced sleep time and/or quality are on the increase. The 2004 International Journal of Sleep reported that people who sleep less than 5 hours a night have a significantly higher death rate than those who sleep less than 7 hours a night, indicating that sleep health is important to overall health [25]. The current study examined the effects of sleep-inducing juice made of natural extracts on sleep quality and cardiac autonomic regulation in adults suffering from sleep difficulties.

https://doi.org/10.4162/nrp.2020.14.6.606

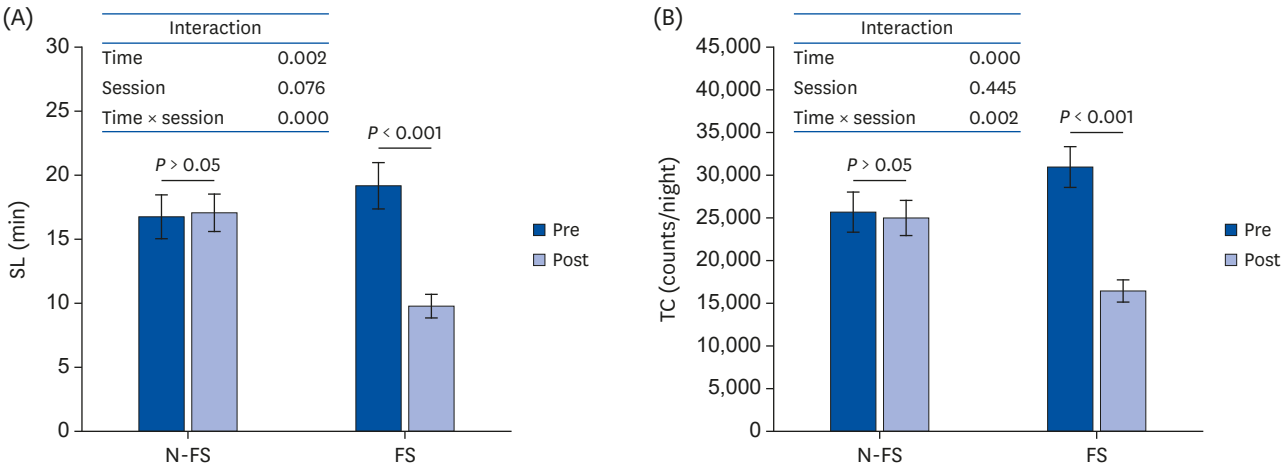

Fig. 3. Comparison between pre- and post-objective sleep quality index in SL and TC. Values are means ± SEM. *P*-values calculated by Wilcoxon's signed-rank test. SL, sleep latency; TC, total counts; N-FS, non-feeding session; FS, feeding session; SEM, standard error of mean.

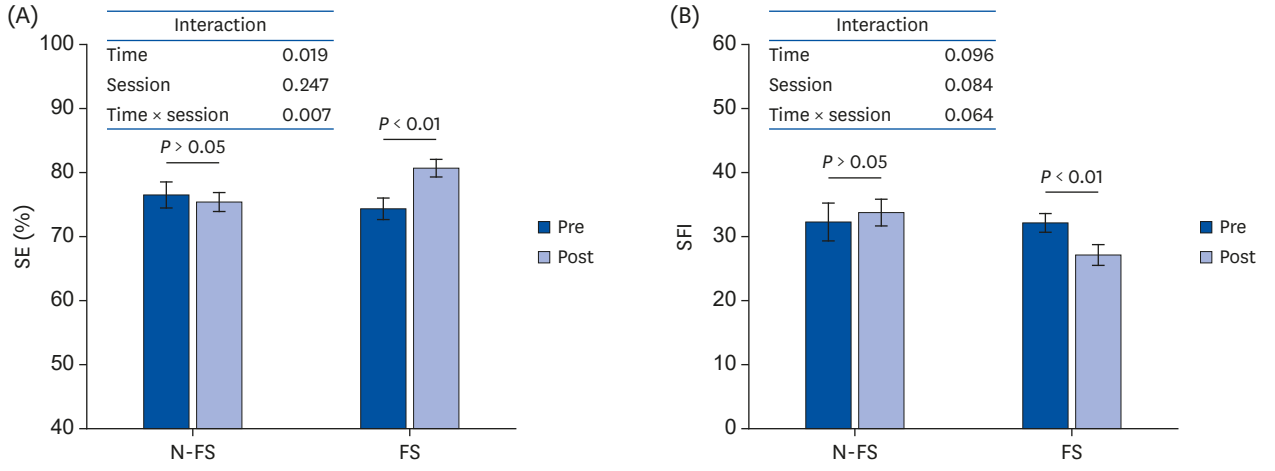

Fig. 4. Comparison between pre- and post-objective sleep quality index in SE and SFI. Values are means ± SEM. *P*-values calculated by Wilcoxon's signed-rank test. SE, sleep efficiency; SFI, sleep fragmentation index; N-FS, non-feeding session; FS, feeding session; SEM, standard error of mean.

The sleep-inducing juice employed in the current study was associated with enhanced resting parasympathetic activation and increased vagal tone at specific time points during sleep. Findings suggest that an 8-week course of sleep-inducing juice could improve sleep quality as assessed by self-reported and objective sleep measures. Sleep quality is important for maintaining innate biological rhythms and physiological homeostasis, and many individuals with sleep disorders or poor sleep quality present with other health problems, as well [26]. Persons with poor sleep quality are also more likely to experience cardiovascular events due to autonomic imbalance. The cardiac sympathetic hyperactivity and parasympathetic withdrawal often observed in those with primary insomnia are warning signs indicating an increased risk of morbidity and mortality from CVD [27].

Results from the present study also showed that the 8-week course of sleep-inducing juice was associated with enhanced resting vagal tone, including increased parasympathetic activity. Since participants were asked to maintain their normal lifestyle throughout the trial, it may be that improved nocturnal sleep was related to the changes in resting cardiac autonomic

https://doi.org/10.4162/nrp.2020.14.6.606

regulation observed in this study. While there are many potential mechanisms by which changes in nocturnal sleep quality could influence resting HRV indices, no physiological markers were measured in this study, and future research will be needed to uncover such mechanisms.

CFS patients with poor sleep quality [10] and individuals suffering from primary insomnia [27] may present with reduced nocturnal HRV due to autonomic imbalances defined as sympathetic hyperactivity and parasympathetic withdrawal. Studies on nocturnal HRV conducted by Tobaldini *et al.* [28] demonstrated that poor sleep quality induced by pathological sleep conditions, such as sleep breathing disorders, could cause significant cardiac autonomic imbalances during sleep. However, since a standardized protocol for measurement of nocturnal HRV has yet to be established, the effects of sleep quality on cardiac autonomic regulation during night sleep tend to be inconsistent among studies.

Burton *et al.* [10] conducted a nocturnal HRV analysis based on TST starting one hour after bedtime and found that rMSSD and HF in HRV, measurements of vagal activity, were decreased in patients with CFS. Conversely, Manconi *et al.* [29] investigated the influence of periodic leg movements during sleep on cardiac autonomic regulation and found no significant differences between patients with restless leg syndrome and healthy subjects for all nocturnal HRV indices. However, unlike Burton *et al.* [10], Manconi *et al.* [29] used only 5-min epochs of electrocardiograph signal during non-rapid eye movement sleep stage 2 when comparing differences in nocturnal HRV indices between groups. Another study by Manconi *et al.* [29] also found no significant results associated with sleep disorders in cardiac autonomic regulation during sleep using 5 min periods of RR intervals in accordance with the standardized protocol for short term HRV analysis. While the different clinical and pathological features of participants may also affect results, HRV analysis protocol can have a significant impact on cardiac autonomic regulation profiles during sleep. The novel findings observed in this study regarding changes in HRV indices suggesting enhanced nocturnal cardiac vagal tone after sleep-inducing juice may be due to the HRV analysis protocol used. The current study used short-term HRV recordings at 7 time points at 30 min intervals starting one hour after bedtime to explore temporal changes in cardiac autonomic regulation during sleep following sleep-inducing juice intake. Parasympathetic activity is key to the induction and maintenance of sleep [10], and HRV indices indicating increased parasympathetic activity during sleep suggest that sleep-inducing juice may contribute to improved sleep quality.

Many studies employ subjective measures, such as PSQI scores, to assess sleep quality or intervention effects under different clinical conditions [30]. Since poor sleep quality may also lead to increased daytime sleepiness and fatigue, the FSS is used to assess subjective fatigue due to disrupted nighttime sleep [31]. The results of the present study indicated that the 8-week intervention with sleep-inducing juice was associated with increased subjective sleep quality and decreased perceived fatigue levels. The PSQI has demonstrated high test–retest reliability and good validity in patients with primary insomnia [32] and has also been used to identify the effects of intervention therapy on sleep quality [33]. However, self-reported questionnaires introduce a certain amount of subjectivity due to differences in personal experiences and emotions. We thereby employed an objective data assessment technique to accompany self-reported estimations of sleep quality.

The pSG is the predominant method used to assess sleep-related disorders, but it is time-consuming and expensive, with difficulties related to procedures and interpretation. As

https://doi.org/10.4162/nrp.2020.14.6.606

such, many studies have employed an actigraphy monitor with accelerometer algorithms to objectively measure sleep quality [34]. Actigraphy is closely related to subjective sleep quality and perceived fatigue and has been reported as useful in identifying whether an intervention improved nighttime sleep [24]. Recently, the American Academy of Sleep Medicine Clinical Practice guidelines have suggested the use of actigraphy to assess certain sleep disorders such as insomnia, circadian rhythm sleep-wake disorder, sleep-disordered breathing, hypersomnolence, and insufficient sleep syndrome [35]. We thereby employed actigraphy to objectively detect potential effects of sleep-inducing juice on sleep quality.

The actigraphy results of the present study indicated that sleep-inducing juice might contribute to increased SE and decreased sleep onset latency. Another study used actigraphy to assess whether an intervention with natural extracts was associated with improved nighttime sleep. Howatson *et al.* [12] reported that TST and efficiency as measured by actigraphy were increased, including elevation of urinary melatonin after tart cherry juice intake. Tart cherry juice is known to have anti-oxidative stress and anti-inflammatory properties and high concentrations of melatonin [36]. Melatonin produced by the pineal gland is critical to endogenous circadian clock regulation of the sleep-wake cycle [34]. Thus, the increase of exogenous melatonin by tart cherry juice intake might have contributed to improvements in actigraphy sleep measures [12].

The sleep-inducing juice used in the present study was developed using natural fruit and vegetable extracts, but the major constituents and blend methods were proprietary and confidential per the manufacturer's instructions. This precludes the estimation of physiological mechanisms that could explain positive changes in sleep parameters obtained from actigraphy following sleep-inducing juice intake. The core material constituents of the sleep-inducing juice were theorized to increase the secretion of sleep-promoting hormones such as serotonin and melatonin, and enhance the biological availability of tryptophan [37].

Findings from this study suggest that TST is related to fruit and vegetable consumption in adults [37]. Other studies have shown that individuals with shorter sleep durations also reported lower fruit and vegetable consumption and lower levels of associated biomarkers such as lycopene, vitamin C, total carotenoids, α-carotene, and β-carotene [38]. The sleep-inducing juice evaluated in the present study was manufactured using fruits and vegetables as main ingredients, and increased synthesis of sleep-promoting hormones and changes in fruit and vegetable-associated biomarkers is to be expected from sleep-inducing juice consumption, and may be one of the mechanisms leading to the enhanced objective sleep quality assessed by actigraphy in this study.

There are several limitations of the current study. Since only young, healthy adults in a narrow age range participated in this study, results do not extend to differences associated with age or pathological conditions. Sleep characteristics such as TST and sleep-wake cycle vary based on age, and there are many different types of sleep disorders, rendering results from the present study difficult to generalize. We focused on examining the effectiveness of sleep-inducing juice itself by selecting individuals with mild-to-moderate sleep complaints (global PSQI score > 5) while excluding pathological conditions that might impact sleep quality. However, we did not control for differences in sleep hygiene, such as exercise, stress management, noise, sleep timing, avoidance of caffeine, nicotine, alcohol, and daytime napping [39].

https://doi.org/10.4162/nrp.2020.14.6.606

Although water intake may affect the sleep quantity, results from recently published study reported that controlled mild dehydration does not affect sleep quality and quantity in young adults [40]. Thus, we do not think that the differences of water intake supplied to both groups have shown different patterns of sleep. Because, FS group only consumed 500 cc per day. In both group, there was no significant difference in body weight and body composition which affect sleep quality and quantity, as showed in **Table 1**, during intervention as well. Moreover, with monitoring the confounding factors, such as caffeine intake, alcohol consumption, and physical activity including exercise, the experimental condition in this study was conducted with a strictly controlled experiment by crossover design. Behavioral and environmental recommendations for normal nighttime sleep indicate sleep hygiene improvements, while critical for promoting sleep, can also be used to treat mild or moderate insomnia. We examined the effects of sleep-inducing juice only, instructing participants to continue their usual lifestyle habits over the entire experimental period.

In conclusion, the present study investigated the effects of sleep-inducing juice developed from natural extracts from fruits and vegetables on improvements in sleep quality and changes in nocturnal cardiac autonomic regulation associated with enhanced nighttime sleep in adults suffering from mild-to-moderate sleep problems. Results indicated that both subjective and objective measures of sleep quality were significantly improved by an 8-week course of sleep-inducing juice. The juice was also associated with increases in resting and nocturnal HRV indices of vagal activity, which may be associated with enhanced sleep quality.

ACKNOWLEDGMENTS

We would like to thank for all the participants in this study.

REFERENCES

1. Cirelli C, Tononi G. Is sleep essential? PLoS Biol 2008;6:e216.
PUBMED | CROSSREF
2. Ghorbani A, Rakhshandeh H, Sadeghnia HR. Potentiating effects of *Lactuca sativa* on pentobarbital-induced sleep. Iran J Pharm Res 2013;12:401-6.
PUBMED
3. Dodds KL, Miller CB, Kyle SD, Marshall NS, Gordon CJ. Heart rate variability in insomnia patients: a critical review of the literature. Sleep Med Rev 2017;33:88-100.
PUBMED | CROSSREF
4. Podsakoff PM, MacKenzie SB, Lee JY, Podsakoff NP. Common method biases in behavioral research: a critical review of the literature and recommended remedies. J Appl Psychol 2003;88:879-903.
PUBMED | CROSSREF
5. Pallin M, O'Hare E, Zaffaroni A, Boyle P, Fagan C, Kent B, Heneghan C, de Chazal P, McNicholas WT. Comparison of a novel non-contact biomotion sensor with wrist actigraphy in estimating sleep quality in patients with obstructive sleep apnoea. J Sleep Res 2014;23:475-84.
PUBMED | CROSSREF
6. Lemola S, Ledermann T, Friedman EM. Variability of sleep duration is related to subjective sleep quality and subjective well-being: an actigraphy study. PLoS One 2013;8:e71292.
PUBMED | CROSSREF
7. Hoevenaar-Blom MP, Spijkerman AM, Kromhout D, van den Berg JF, Verschuren WM. Sleep duration and sleep quality in relation to 12-year cardiovascular disease incidence: the MORGEN study. Sleep (Basel) 2011;34:1487-92.
PUBMED | CROSSREF

8. Greco C, Spallone V. Obstructive sleep apnoea syndrome and diabetes. Fortuitous association or interaction? Curr Diabetes Rev 2015;12:129-55.
PUBMED | CROSSREF
9. Thayer JF, Lane RD. The role of vagal function in the risk for cardiovascular disease and mortality. Biol Psychol 2007;74:224-42.
PUBMED | CROSSREF
10. Burton AR, Rahman K, Kadota Y, Lloyd A, Vollmer-Conna U. Reduced heart rate variability predicts poor sleep quality in a case-control study of chronic fatigue syndrome. Exp Brain Res 2010;204:71-8.
PUBMED | CROSSREF
11. Pigeon WR, Carr M, Gorman C, Perlis ML. Effects of a tart cherry juice beverage on the sleep of older adults with insomnia: a pilot study. J Med Food 2010;13:579-83.
PUBMED | CROSSREF
12. Howatson G, Bell PG, Tallent J, Middleton B, McHugh MP, Ellis J. Effect of tart cherry juice (*Prunus cerasus*) on melatonin levels and enhanced sleep quality. Eur J Nutr 2012;51:909-16.
PUBMED | CROSSREF
13. Peuhkuri K, Sihvola N, Korpela R. Diet promotes sleep duration and quality. Nutr Res 2012;32:309-19.
PUBMED | CROSSREF
14. American College of Sports Medicine. ACSM's Guidelines for Exercise Testing and Prescription. Philadelphia (PA): Lippincott Williams & Wilkins; 2013.
15. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213.
PUBMED | CROSSREF
16. Monk TH, Reynolds CF 3rd, Kupfer DJ, Buysse DJ, Coble PA, Hayes AJ, MacHen MA, Petrie SR, Ritenour AM. The Pittsburgh sleep diary. J Sleep Res 1994;3:111-20.
CROSSREF
17. Valko PO, Bassetti CL, Bloch KE, Held U, Baumann CR. Validation of the fatigue severity scale in a Swiss cohort. Sleep 2008;31:1601-7.
PUBMED | CROSSREF
18. Lee JH, Jeong HS, Lim SM, Cho HB, Ma JY, Ko E, Im JJ, Lee SH, Bae S, Lee YJ, Lyoo IK, Jeong DU. Reliability and validity of the fatigue severity scale among university student in South Korea. Korean J Biol Psychiatry 2013;20:6-11.
19. Kim CS, Kim MK, Jung HY, Kim MJ. Effects of exercise training intensity on cardiac autonomic regulation in habitual smokers. Ann Noninvasive Electrocardiol 2017;22:e12434.
PUBMED | CROSSREF
20. Brosschot JF, Van Dijk E, Thayer JF. Daily worry is related to low heart rate variability during waking and the subsequent nocturnal sleep period. Int J Psychophysiol 2007;63:39-47.
PUBMED | CROSSREF
21. Hynynen E, Uusitalo A, Konttinen N, Rusko H. Heart rate variability during night sleep and after awakening in overtrained athletes. Med Sci Sports Exerc 2006;38:313-7.
PUBMED | CROSSREF
22. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93:1043-65.
PUBMED | CROSSREF
23. Kim M, Sasai H, Kojima N, Kim H. Objectively measured night-to-night sleep variations are associated with body composition in very elderly women. J Sleep Res 2015;24:639-47.
PUBMED | CROSSREF
24. Sadeh A. The role and validity of actigraphy in sleep medicine: an update. Sleep Med Rev 2011;15:259-67.
PUBMED | CROSSREF
25. Patel SR, Ayas NT, Malhotra MR, White DP, Schernhammer ES, Speizer FE, Stampfer MJ, Hu FB. A prospective study of sleep duration and mortality risk in women. Sleep 2004;27:440-4.
PUBMED | CROSSREF
26. Medic G, Wille M, Hemels ME. Short- and long-term health consequences of sleep disruption. Nat Sci Sleep 2017;9:151-61.
PUBMED | CROSSREF
27. Spiegelhalder K, Scholtes C, Riemann D. The association between insomnia and cardiovascular diseases. Nat Sci Sleep 2010;2:71-8.
PUBMED | CROSSREF

28. Tobaldini E, Nobili L, Strada S, Casali KR, Braghiroli A, Montano N. Heart rate variability in normal and pathological sleep. Front Physiol 2013;4:294.
PUBMED | CROSSREF

29. Manconi M, Ferri R, Zucconi M, Clemens S, Rundo F, Oldani A, Ferini-Strambi L. Effects of acute dopamine-agonist treatment in restless legs syndrome on heart rate variability during sleep. Sleep Med 2011;12:47-55.
PUBMED | CROSSREF

30. Yang PY, Ho KH, Chen HC, Chien MY. Exercise training improves sleep quality in middle-aged and older adults with sleep problems: a systematic review. J Physiother 2012;58:157-63.
PUBMED | CROSSREF

31. Kaminska M, Kimoff RJ, Benedetti A, Robinson A, Bar-Or A, Lapierre Y, Schwartzman K, Trojan DA. Obstructive sleep apnea is associated with fatigue in multiple sclerosis. Mult Scler 2012;18:1159-69.
PUBMED | CROSSREF

32. Backhaus J, Junghanns K, Broocks A, Riemann D, Hohagen F. Test-retest reliability and validity of the Pittsburgh sleep quality index in primary insomnia. J Psychosom Res 2002;53:737-40.
PUBMED | CROSSREF

33. Berger AM, Kuhn BR, Farr LA, Von Essen SG, Chamberlain J, Lynch JC, Agrawal S. One-year outcomes of a behavioral therapy intervention trial on sleep quality and cancer-related fatigue. J Clin Oncol 2009;27:6033-40.
PUBMED | CROSSREF

34. Cornu C, Remontet L, Noel-Baron F, Nicolas A, Feugier-Favier N, Roy P, Claustrat B, Saadatian-Elahi M, Kassaï B. A dietary supplement to improve the quality of sleep: a randomized placebo controlled trial. BMC Complement Altern Med 2010;10:29.
PUBMED | CROSSREF

35. Smith MT, McCrae CS, Cheung J, Martin JL, Harrod CG, Heald JL, Carden KA. Use of actigraphy for the evaluation of sleep disorders and circadian rhythm sleep-wake disorders: an American Academy of Sleep Medicine systematic review, meta-analysis, and GRADE assessment. J Clin Sleep Med 2018;14:1209-30.
PUBMED | CROSSREF

36. Burkhardt S, Tan DX, Manchester LC, Hardeland R, Reiter RJ. Detection and quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (*Prunus cerasus*). J Agric Food Chem 2001;49:4898-902.
PUBMED | CROSSREF

37. Noorwali EA, Cade JE, Burley VJ, Hardie LJ. The relationship between sleep duration and fruit/vegetable intakes in UK adults: a cross-sectional study from the National Diet and Nutrition Survey. BMJ Open 2018;8:e020810.
PUBMED | CROSSREF

38. Beydoun MA, Gamaldo AA, Canas JA, Beydoun HA, Shah MT, McNeely JM, Zonderman AB. Serum nutritional biomarkers and their associations with sleep among US adults in recent national surveys. PLoS One 2014;9:e103490.
PUBMED | CROSSREF

39. Irish LA, Kline CE, Gunn HE, Buysse DJ, Hall MH. The role of sleep hygiene in promoting public health: a review of empirical evidence. Sleep Med Rev 2015;22:23-36.
PUBMED | CROSSREF

40. Aristotelous P, Aphamis G, Sakkas GK, Andreou E, Pantzaris M, Kyprianou T, Hadjigeorgiou GM, Manconi M, Giannaki CD. Effects of controlled dehydration on sleep quality and quantity: a polysomnographic study in healthy young adults. J Sleep Res 2019;28:e12662.
PUBMED | CROSSREF