**ABSTRACT**

**Objective:** Recent community-based studies have identified sleep deprivation (SD) as an important modifiable risk factor for hypertension. However, the underlying mechanisms linking SD to hypertension remain elusive. Thus, this study investigates blood pressure (BP) responses to cardiac autonomic stress tests in the presence of SD. Furthermore, we analyzed vascular inflammatory biomarkers as a possible underlying factor linking SD to increased BP.

**Methods:** Ten healthy male volunteers (age, 21.6±1.2 years) underwent repeated autonomic stress tests for three consecutive days (baseline, SD, and recovery). The autonomic stress tests included the Valsalva maneuver, mental arithmetic, isometric handgrip, and cold pressor tests. Each day, resting BPs were measured, venous blood samples were collected for intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin measurements, and stress tests were performed between 0900 and 1100. Ambulatory BP was recorded during the entire SD period (24 h).

**Results:** One-night SD abolished BP reactivity to the Valsalva maneuver, isometric hand grip, and cold pressor tests, which returned after recovery sleep. Ambulatory BP monitoring showed that the mean systolic and diastolic BPs were 121.1±8.5 mm Hg and 72.8±6.3 mm Hg, respectively, between 0700 and 2300 and 120.3±9.6 mm Hg and 74.1±6.1 mm Hg, respectively, between 2300 and 0700 during the SD day (p>0.05 for both). Vascular inflammatory markers seemed unrelated to BP changes.

**Conclusion:** Acute SD altered BP responses to cardiac autonomic stress tests in healthy men without affecting resting BP levels. SD led to a non-dipping pattern in BP oscillation. Collectively, these findings highlight the importance of sleep in regulating BP.

**Keywords:** cell adhesion molecules, stress tests, E-selectin, sleep loss, Valsalva maneuver.

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**Introduction**

Hypertension is the first-ranked cause of death and leading preventable risk factor for cardiovascular diseases. The prevalence of hypertension is rising and has exceeded 30% of the global adult population (1). In Turkey, hypertension is one of the most common medical diagnoses affecting 15 million adults, and the number of patients with uncontrolled hypertension is approximately 11 million (2). Although prevention and treatment strategies put emphasis on diet and physical activity, growing evidence suggests that adequate sleep represents a behavioral target in managing hypertension (3). Adequate sleep includes several dimensions including quality, duration, regularity, and timing as well as lack of sleep disorders (4).

Sleep duration is the most widely studied dimension in relation to cardiovascular health. A 10-year follow-up analysis has shown that persons with a sleep duration of 5 h or less are 32% more likely to develop hypertension than individuals who sleep 7 to 8 h (5). A meta-analysis of prospective cohort studies has found that short sleep duration, typically defined as less than 6 h per night, is associated with an increased risk of hypertension incidence (6). In a study, 22 subjects with prehypertension or stage 1 hypertension and habitual sleep durations of less than 7 h received a 6-week intervention to increase sleep duration (7).
Daily 1-h increase in sleep led to a significant decrease in 24-h systolic and diastolic beat-to-beat BPs by an average of 14 and 8 mm Hg, respectively (7). In addition, every 1-h reduction in sleep duration was associated with a 37% increase in the odds of hypertension occurring (8). In brief, a large body of evidence comprising longitudinal and cross-sectional studies reveals an increased risk of hypertension in individuals with insufficient sleep (3, 9, 10).

Experimental studies examining the effect of sleep deprivation (SD) on BP are limited. In one of the earliest studies, one-night SD resulted in increased resting BP along with decreased muscle sympathetic nerve activity in healthy subjects (11). Authors have suggested that an increased sympathetic drive is unlikely to be the predominant factor mediating BP increase following SD (11). Finally, one-night acute SD in a real-life model increased cardiac autonomic modulation without causing significant changes in BP and BP variability (12). Taken together, BP response to SD and the role of the autonomic nervous system in this relationship remain elusive. Recently, serum intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) levels were related to 24-h BP variability (13). Here we investigated the effect of SD on BP in the presence of autonomic challenge. Furthermore, we analyzed vascular inflammatory biomarkers as possible underlying factors linking SD to increased BP.

Methods

Subjects
Ten healthy young adult male subjects (mean age ± standard deviation: 21.6±1.2 years, age range: 21–25 years) completed the study. All subjects were nonsmokers and physically and psychologically healthy as assessed by history taking and physical examination. The exclusion criteria were the presence of cardiac disease, sleep disturbance, chronic drug use, familial dysautonomia, and other autonomic nervous system disorders. The baseline evaluations included subjective sleep quality assessed using the Pittsburgh Sleep Quality Index (PSQI) and cardiological examination using electrocardiography and echocardiography. All subjects reported regular sleep habits. In addition, initial venous blood samples were collected for blood biochemistry. The study was reviewed and approved by the Institutional Review Board of our institution in accordance with the Declaration of Helsinki and other regulations. After approval, the subjects were informed about the study and provided written informed consent before participation.

The PSQI
This self-report, 19-item retrospective questionnaire measures a person’s subjective sleep quality within the last month. The reliability and validity of the Turkish version of the PSQI was verified (14). The PSQI measures subjective sleep quality, sleep latency, sleep duration, habitual sleep activity, sleep disorders, use of sleep drugs, and daytime dysfunction by using component scores ranging from 0 (no disturbance) to 3 (severe disturbance). The global PSQI is scored between 0 and 21. A global score higher than 5 indicates a poor sleep quality.

Study design
The subjects were taken into the laboratory for 4 days and underwent one-night SD and performed autonomic stress tests at 3 time points (Fig. 1). The subjects’ heart rate (HR) and BP were monitored during the autonomic tests using a Mindray BeneView T8 patient monitor (Shenzhen Mindray Bio-Medical Electronics Co. Ltd., Guangdong, China). The first set of stress tests was performed to monitor each subject’s habitual sleep night. After the stress tests, all subjects remained awake for 24 h. The second set of stress tests was performed in the morning within the same time interval (0900–1100) following the SD period. The third set of stress tests was performed in the morning following the recovery sleep night within the same time interval to prevent circadian influences.

Fasting venous blood samples (10 ml) were collected before the stress tests at approximately 0800. Serum samples were obtained and stored at −80°C until enzyme-linked immunosorbent assay (ELISA) tests. The subjects had a light breakfast 30 min before the stress tests. Following a 15-min rest, the stress tests were performed in the following sequence: the Valsalva maneuver, isometric hand grip, mental arithmetic, and cold pressor tests. The cold pressor test was performed always as the last test due to its sustained effects. No smoking and caffeine and alcohol consumption were allowed during the entire study period. Meals were provided at predetermined time points (lunch between 1300 and 1400 and dinner between 2030 and 2130). The subjects were occupied by activities such as reading books, watching TV, listening to music, and playing table games during their stay in the laboratory.

Autonomic stress tests
The Valsalva maneuver test was the first autonomic test in this setting. It is noninvasive, and due to its sensitivity, it is used to evaluate baroreceptor function and identify sympathetic adrenergic failure (15). The subjects rested for 15 min in the supine position with their heads elevated at 30°. Resting BP and HR were monitored and recorded. The subjects were instructed to take a normal inspiration and perform a forced expiratory effort against a closed airway for 15 sec. Airway closure was...
obtained using a mouthpiece. Then, the expiratory effort was suddenly released to restore normal respiration.

The isometric hand grip test was performed using a hydraulic hand dynamometer (12-0243; Baseline® Evaluation Instruments, Fabrication Enterprises, Inc., NY, US). The subjects were instructed not to perform Valsalva maneuver during handgrip and then hold a handgrip at one-third of the maximum contraction force for 3 min. The maximum handgrip force was measured during the initial assessment period. The subjects were verbally encouraged to continue when they started to reduce their effort before the test ended. BP and HR were recorded before and after the test.

The mental arithmetic test is based on performing serial subtraction (in this case, starting from 351 minus 7 and so on until zero). The purpose of this test is activating sympathetic nervous system. The subjects were encouraged when they hesitate or prematurely stop. Before and after the test, BP and HR were recorded.

The cold pressor test involved the immersion of the dominant hand into cold water (~4°C) for 90 sec. Buckets and ice batteries were prepared before the stress tests. During the test, the water temperature was checked using a digital thermometer. The activation of pain and temperature fibers leads to sympathetic activation, which in turn increases HR and BP. After 90 sec, we removed the subject’s hand from the ice water bucket and immediately measured their systolic and diastolic BPs.

**Ambulatory BP monitoring**

Each subject’s BP was measured and recorded every 30 min for the entire SD period using a noninvasive automatic ambulatory BP system (Bravo 24-HR ABP, Model 222-B; SunTech Medical, Inc. Morrisville, NC, US). The recorded parameters included mean systolic BP, mean diastolic BP, mean BP, and HR. The SD period was divided into two time intervals: daytime (0700–2330) and nighttime (2300–0700). Daytime and nighttime measurements were compared.

**Vascular inflammatory biomarkers**

Venous blood samples were collected from the antecubital vein before each autonomic stress test, and the serum samples were isolated and stored at −80°C until assay. The maximum storage time was three months. Serum ICAM-1, VCAM-1, and E-selectin assays were performed using commercial ELISA kits (ICAM-1: catalog no. YLA1554HU, Shanghai YL Biotech Co., Ltd., Shanghai, China; VCAM-1: catalog no. YLA1551HU, Shanghai YL Biotech Co., Ltd., Shanghai, China; E-selectin: catalog no. YLA1568HU, Shanghai YL Biotech Co., Ltd., Shanghai, China).

**Statistical analysis**

Descriptive variables are presented as means ± standard deviations and percent values where appropriate. To compare BP and HR responses to the stress tests among the three time points, we calculated the percent change (i.e., posttest value minus pretest value divided by pretest value) and then compared the percent change values using the Friedman test, along with the post-hoc Wilcoxon signed-rank test. We assessed the relationship between vascular inflammatory biomarkers and resting BP levels using the Spearman correlation test. An additional correlation analysis of the vascular inflammatory markers with BP responses to the stress tests on the three days was performed. *P* values of <0.05 were used to denote statistical significance.

**Results**

The baseline characteristics of the study sample are given in Table 1. Complete blood count and echocardiographic evaluation revealed that all subjects were within normal limits in terms of hematological parameters and cardiac function. No subject had anemia or systolic/diastolic BP dysfunction. The PSQI scores revealed that all subjects had no sleep impairments.

The BP changes in response to the autonomic tests at three time points are given in Figure 1. After a regular sleep night,
systolic and diastolic BPs increased in response to the Valsalva maneuver test. This increase was abolished by one-night SD and recovered after recovery sleep. When we compared the percent change values among the three days, we found a significant difference in percent change values of systolic BP ($p<0.05$), but diastolic BP failed to reach significance level ($p>0.05$). This finding suggested that autonomic regulation of the heart was affected by an acute short-term sleep loss. In the isometric handgrip test, one-night SD worsened the systolic and diastolic BP responses. Significant differences in percent change values of both systolic ($p<0.01$) and diastolic ($p<0.05$) BPs were found. Surprisingly, the mental arithmetic test led to slight changes in systolic and diastolic BPs, however, no significant differences were observed among the baseline, SD, and recovery days ($p>0.05$ for both). Finally, the cold pressor test led to increases in systolic and diastolic BPs at baseline, and this increase was intensified on the SD day and returned to baseline values after recovery sleep. Comparisons of the percent change values showed a significant difference in diastolic BP ($p<0.02$), whereas systolic BP changes failed to reach statistical significance. Taken together, these results suggest that acute short-term SD leads to alterations in cardiac autonomic regulation, and physical stress (i.e., isometric handgrip and cold) was more powerful than mental stress in affecting BP in the presence of total sleep loss.

Figure 2 shows HR changes in response to the autonomic tests at three time points (baseline, SD, and recovery sleep). Valsalva maneuver decreased HR, whereas isometric handgrip and mental arithmetic stress increased HR.

However, comparison of the mean percent change values among the three time points revealed that the HR response to the stress tests was not affected under the conditions of one-night SD. In other words, HR responses to the stress tests were comparable among the habitual sleep, SD, and recovery sleep days ($p>0.05$ for all stress tests).

Ambulatory BP monitoring showed that the mean systolic and diastolic BPs were 121.1±8.5 mm Hg and 72.8±6.3 mm Hg, respectively, between 0700 and 2300 and 120.3±9.6 mm Hg and 74.1±6.1 mm Hg between 2300 and 0700 during the SD day ($p>0.05$ for both). These results revealed that nighttime systolic and diastolic BP values remained high as in daytime values, resembling a non-dipping pattern.

The results of the vascular inflammatory biomarkers and resting BP measurements are given in Table 2. The ICAM-1 level remained the same following one-night SD and after recovery sleep. Serum VCAM-1 levels remained comparable between the baseline and SD days but showed a slight increase after recovery sleep.

On day 3 (recovery sleep), a significant difference in VCAM-1 levels was found between the baseline and SD days (Table 2). E-selectin levels showed a similar pattern with VCAM-1. E-selectin levels remained comparable between the baseline and SD days but showed a significant increase after recovery sleep. The mean serum E-selectin level on the recovery sleep day was significantly higher than those on the baseline ($p<0.021$) and SD ($p<0.005$) days. Correlation analysis showed that resting BP levels were not related to the vascular inflammatory biomarkers ($p>0.05$ for all). No significant correlation was found between the vascular inflammatory biomarkers and systolic and diastolic BP responses to the stress tests ($p>0.05$ for all).

| Table 1. General characteristics of the study sample |
|-----------------------------------------------|
| **Variables**                                      | **N (%) or mean ± SD** |
| Number of subjects                                | 10                      |
| Age (years)                                       | 21.6±1.2                |
| Sex (male)                                        | 10 (100)                |
| BMI (kg/m²)                                       | 22.9±3.4                |
| PSQI score                                        | 5.2±1.3                 |
| Current smoker                                    | 0 (0)                   |
| Hemoglobin (g/dL)                                 | 14.6±1.4                |
| Hematocrit (%)                                    | 42.8±3.9                |
| SBP (mm Hg)                                       | 129.7±9.5               |
| DBP (mm Hg)                                       | 74.6±8.0                |
| LVEF (%)                                          | 60.0±5.0                |
| EDV (mL)                                          | 99.1±23.3               |
| ESV (mL)                                          | 39.1±11.2               |
| LVDD (mm)                                         | 47.9±2.7                |
| LVSD (mm)                                         | 31.2±2.5                |
| IVST (mm)                                         | 9.3±1.2                 |

BMI - body mass index; DBP - diastolic blood pressure; EDV - end-diastolic volume; ESV - end-systolic volume; IVST - interventricular septum thickness; LVDD - left ventricular systolic diameter; LVEF - left ventricular ejection fraction; LVSD - left ventricular diastolic diameter; PSQI - Pittsburgh Sleep Quality Index; SBP - systolic blood pressure; N - number; SD - standard deviation.

| Table 2. Vascular inflammatory biomarker values and resting blood pressure measurements at baseline (Day 1), after one-night sleep deprivation (Day 2), and after recovery sleep (Day 3). All blood samples were collected in the morning between 0800 and 0900 |
|-----------------------------------------------|
| **Variables**                                      | **Day 1** | **Day 2** | **Day 3** |
| ICAM-1 (ng/L)                                     | 1250±1010 | 1022±254  | 992±483    |
| VCAM-1 (ng/mL)                                    | 14.6±4.7  | 14.5±5.9  | 16.6±5.6   |
| E-selectin (ng/L)                                 | 326±109   | 331±109   | 375±129    |
| SBP (mm Hg)                                       | 126.9±8.9 | 120.2±8.1 | 116.1±10.4 |
| DBP (mm Hg)                                       | 76.8±7.5  | 70.5±5.2  | 68.8±6.6   |
| HR (bpm)                                          | 89.0±18.7 | 81.7±14.7 | 89.7±16.6  |

All numerical values in the table are ELISA measurements (mean ± standard deviation). The Friedman tests for comparison of the three days: $P=0.368$, $P=0.045$, $P<0.004$, $P=0.002$, $P=0.001$, $P=0.033$.

Post-hoc Wilcoxon tests: $P=0.028$ vs. Day 1, $P=0.022$ vs. Day 2, $P=0.021$ vs. Day 1, $P=0.005$ vs. Day 2, $P=0.001$ vs. Day 1, $P=0.008$ vs. Day 1, $P=0.007$ vs. Day 1, $P=0.008$ vs. Day 1, $P=0.036$ vs. Day 1, $P=0.047$ vs. Day 2.

ICAM-1 - intracellular adhesion molecule; VCAM-1 - vascular cell adhesion molecule; SD - sleep deprivation.
The main finding of this study is that one-night (24-h) total SD resulted in alterations in BP responses to cardiac autonomic stress tests in healthy men, although resting systolic and diastolic BPs were unaffected. Furthermore, serum levels of vascular inflammatory biomarkers, that is, ICAM-1, VCAM-1, and E-selectin, were not associated with the altered BP responses. Several experimental studies involving healthy subjects have shown significant increase in BP after nights where sleep was completely deprived (16, 17). However, other studies have reported that one-night SD was insufficient to induce BP alterations (12, 18) unless additional stress conditions were provided (19). The results of this study conform to the findings of previous studies that one-night SD may not be sufficient to increase BP under resting conditions (12, 18, 19), and we extend these studies by demonstrating that autonomic stress tests are sufficient and effective to sift out the BP dysregulation induced by sleep loss. This study introduces several new findings. First, one-night SD may lead to subtle changes in BP-regulating mechanisms. These changes may be unapparent under resting conditions. However, challenging the regulatory mechanisms using stress tests may reveal these BP changes. Second, the effects of physical and mental stresses on cardiac autonomic regulation may differ. Finally, the vascular inflammatory biomarkers ICAM-1, VCAM-1, and E-selectin were not associated with SD-induced alterations in BP responses to stress tests.

To date, no study has compared the systolic and diastolic BP responses to Valsalva maneuver between control sleep and SD. The Valsalva maneuver test is a sensitive indirect clinical test to identify milder forms of sympathetic impairment (20). A direct measurement of sympathetic activity requires some invasive techniques such as muscle sympathetic nerve activity, which remains mainly as a research tool rather than a clinical application. During the maneuver, intrathoracic and intra-abdominal pressure changes activate several cardiovascular reflex mechanisms including the arterial baroreflex, which regulates BP. Ogawa et al. (16) have demonstrated that 24-h SD tends to blunt arterial baroreflex sensitivity. Accordingly, the findings of this study showed the lack of BP response to Valsalva maneuver after SD, which reappeared after recovery sleep.

A limited number of studies have conducted cardiac autonomic stress tests in the SD setting (12, 21, 22). Kato et al. (11) have found comparable mean BP responses to the isometric handgrip, mental stress, and cold pressor tests between the normal sleep and SD days, and they suggested that SD does not potentiate cardiovascular responses to stressful stimuli. Similar results were reported by Yang et al. (21), who suggested that 24-h SD does not affect BP reactivity to mental stress and cold pressor tests. In contrast, Franzen et al. (22) have administered acute psychological stress involving social-evaluative threats to their subjects and found that a night of SD amplifies BP reactivity. In the former two studies (11, 21), physical (i.e., cold or handgrip) and mental (i.e., serial subtraction) stimuli were used, whereas the latter study (22) used Stroop color-word naming interference and speech tasks. In this study, we used both physical (handgrip and cold) and mental (serial subtraction) stimuli and found that SD affected BP responses to physical stress, but not to mental stress. Collectively, these studies suggest that the nature of stressful stimuli influences the BP response.

Potential mechanisms underlying the association of SD with BP regulation may include autonomic imbalance and endothelial dysfunction. Short-term acute SD studies have shown increased sympathetic and decreased parasympathetic cardiac modulation in healthy humans (23). Moreover, these adverse effects of SD on cardiac autonomic balance may be evident as early as 12 h of SD without progressive impairment (23). Autonomic nervous system activity shifts from the sympathetic side to the parasympathetic side, leading to BP dips by an average of 10%–20% during sleep (3). Short sleep duration or sleep loss may be associated with reduced nocturnal dipping (4). Concordantly, the findings in this study provided evidence for a non-dipping pattern of BP during the SD period.

Another interesting finding of this study was the differential BP and HR response patterns. It is evident that the HR response did not change with SD, whereas the BP response changed (Fig. 1 and 2). This may be explained by different regulatory brainstem mechanisms for HR and BP. Distinct brainstem centers exist, that is, vasomotor center for regulating BP and cardio-accelerator center for regulating HR. The sensitivity of these centers to the effects of SD may vary. Thus, the response patterns of HR and BP to SD may vary. The second explanation may involve peripheral vascular alterations in response to SD. SD might change vascular responses, which in turn may lead to BP changes without affecting HR. This was the main idea when we decided to measure vascular adhesion molecules in this study.

The role of endothelium in BP regulation is well-known. Cell adhesion molecules are strong biomarkers of vascular inflammation and endothelial dysfunction. Recently, Ciobanu et al. (13) have
demonstrated a positive association of ICAM-1 with daytime systolic, daytime diastolic, and 24-h diastolic BP variability and an association of VCAM-1 with daytime systolic BP variability. Two studies have investigated the effects of SD on cell adhesion molecules and have shown that 40-h total SD induces a significant increase in ICAM-1 and E-selectin levels, whereas VCAM-1 levels remain comparable (18, 24). In addition, Sauvet et al. (18) have concluded that total SD causes vascular dysfunction before the increase in sympathetic activity and systolic BP. Taken together, cell adhesion molecules may be a link between SD and impaired BP reactivity. Therefore, we measured the ICAM-1, VCAM-1, and E-selectin levels at three time points representing normal sleep, SD, and recovery sleep (Table 2). Our results did not support the hypothesis that ICAM-1, VCAM-1, and E-selectin are associated with resting BP measurements during acute SD.

Study limitations
This study has several limitations. First, BP measurements during the stress tests were not continuous. We measured systolic and diastolic BPs before and after each stress test. Continuous monitoring of BP would provide a better response profile. Second, we recorded each subject’s ambulatory BP only on the SD day. Three-day monitoring would give a chance to compare the SD day with the baseline and recovery sleep days. However, ambulatory BP monitoring during SD provided important data on BP changes as a result of SD. Third, the sample size is relatively small, which is not surprising in such a labor-intensive preclinical study. Finally, the study included only male subjects, and one should be careful when extrapolating these results to females.

Conclusion
In conclusion, one-night total SD resulted in alterations in BP responses to cardiac autonomic stress tests in healthy men without affecting resting systolic and diastolic BPs. Collectively, the findings in this study highlight the importance of sleep management to combat hypertension.

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