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Heart-Rate Response to Sympathetic Nervous Stimulation, Exercise, and Magnesium Concentration in Various Sleep Conditions

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The aim of this study was to clarify the mechanism of impaired exercise tolerance in chronic sleep-restricted conditions by investigating variables related to heart-rate (HR) response to sympathetic nervous stimulation. Sixteen healthy men (mean age 21.5 years) were tested in a control state, acute sleep-loss state, and chronic sleep-restricted state. Participants underwent cardiopulmonary exercise testing in each state. Their norepinephrine (NE) concentration was measured before and immediately after exercise. Intracellular magnesium (Mg) concentration was measured in a resting state. Exercise duration was shorter and the ratio of HR response to the percentage increase in NE was higher in the chronic sleep-restricted state than in the control state. Intracellular Mg gradually decreased from control to chronic sleep restriction. There was a negative correlation between peak exercise duration and the ratios of HR response to the rate of increase in NE. Intracellular Mg was positively correlated with the ratios of HR response to the increase in NE both in control and in acute sleep loss. The authors conclude that the impaired exercise tolerance in a chronic sleep-restricted state is caused by hypersensitivity of the HR response to sympathetic nervous stimulation, which showed a compensation for decreased intracellular Mg concentration.

Keywords: **s**leep deprivation, sympathetic nervous stimulation, exercise tolerance

In the field of competitive sports, athletes always take care of their physical condition, including fatigue or sleep status, and try to control it during training. It is very important to control it because it directly influences their performance. In our previous studies, oxygen uptake (VO_2) was decreased during exercise, as indicated by anaerobic threshold (AT) and peak VO_2, in a state of chronic sleep restriction in young healthy participants (Osada et al., 1993; Tanabe et al., 1998; Yamamoto et al., 1996). Our results suggest that magnesium (Mg) metabolism plays an important role in the low VO_2 observed with chronic sleep restriction; the intracellular Mg concentration decreased in this condition, and VO_2 after Mg

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administration was improved in comparison with that without Mg (Tanabe et al.; Yamamoto et al.). In a comparative study of a control state and chronic sleep-restricted state, changes in intracellular Mg levels were positively correlated with changes in flow-mediated dilation of the brachial artery (Takase, Akima, Uehata, Ohsuzu, & Kurita, 2004). Although it has been suggested that Mg concentration is associated with sympathetic nervous activity, which regulates norepinephrine (NE) secretion, the precise mechanism remains unclear. Moreover, the differences in hemodynamic response to sympathetic nervous stimulation between chronic sleep restriction and acute sleep loss have not been fully investigated. In particular, heart-rate (HR) response to sympathetic stimulation in chronic sleep-restricted states has not been investigated despite multiple studies of exercise after acute sleep loss, after 30–120 hr of sleeplessness (Bulbulian, Heaney, Leake, Sucec, & Sjoholm, 1996; Vondra et al., 1981), or after partial sleep loss for 1–3 days (Mougin et al., 1996; Reilly & Piercy, 1994).

Thus, the aim of this study was to clarify the mechanism of impaired exercise tolerance in chronic sleep-restricted conditions by investigating variables related to HR response to sympathetic nervous stimulation.

Participants and Methods

Participants

Sixteen healthy male medical-college students (mean age 21.5 ± 2.6 years) with no history of serious disease were enrolled in the study. Their height and weight were 170.8 ± 3.5 cm and 68.3 ± 9.5 kg, respectively. The participants' health status was determined on the basis of their medical history, routine physical examination, and resting electrocardiogram (ECG). Participants who had ECG or clinical abnormalities were excluded.

In accordance with the study protocol approved by the Committee on Human Investigation at our university, written informed consent was obtained from each participant before he entered into the study.

Methods

Experiments were conducted under three conditions: a control condition (usual amount of good sleep for at least 1 week), an acute sleep-loss state (partial sleep restriction for one night, sleep time less than 3 hr), and a chronic sleep-restricted state for term-end examinations (a day preceded by a month during which average sleep lasted <60% of usual). Each participant first completed a cardiopulmonary-exercise test (CPX) in the control state. Participants then performed the CPX in the chronic sleep-restricted state. Finally, they performed the CPX in the acute sleep-loss state. The acute sleep-loss state and chronic sleep-restricted state were separated by at least 2 weeks of ordinary sleep. All exercise tests were performed at the same time of day between 5 and 9 p.m. under similar conditions.

Both chronic and partial sleep restriction were caused by office work, preparing for an examination, not for entertainment or leisure activity. All participants slept at home during the examination period and were not permitted to drink alcohol or take hypnotic drugs or sedatives. Before CPX under each condition, they

were interviewed by physicians to determine how long they had slept the night before. During the chronic sleep-restricted state, they logged their sleep time themselves.

CPX

Symptom-limited CPX with a ramp protocol on a cycle ergometer (CORIVAL 400, Lode B.V., Groningen, Holland) was performed. After a 4-min rest on the cycle ergometer, exercise began with a 4-min warm-up (20 W) followed by an increase in load (1 W per 3 s). During exercise testing, participants underwent 12-lead ECG monitoring via a stress-test system (ML-5000, Fukuda-Denshi Co., Tokyo, Japan), and HR, ST- and T-wave change, and arrhythmias were identified and recorded. Blood pressure was measured at 1-min intervals with a cuff (STBP-780 COLIN Co., Aichi, Japan). The exercise test was terminated by discontinuation criteria of the American Heart Association (Fletcher et al., 2001), and VO_2, carbon dioxide output (VCO_2), and minute ventilation (VE) were measured throughout CPX with an RM-300 respiromonitor and an MG-360 gas analyzer (Minato Medical Science Co., Osaka, Japan). The measurement system for CPX was calibrated before the start of each study. Expired gas was sampled with a breath-by-breath technique, and AT was determined by conventional criteria (Wasserman, Hansen, Sue, Casaburi, & Whipp, 1999): VE/VO_2 increased after holding constant or decreasing, whereas VE/VCO_2 remained constant or decreased, and the gas-exchange ratio started to increase steeply. Exercise durations to reach AT and peak VO_2 also were recorded. We calculated ΔHR as peak value minus resting value of HR.

Measurement of Plasma NE Concentration

To measure the plasma NE concentration in nonworking regions of the body, venous blood was drawn from an 18-gauge cannula inserted into the antecubital vein and immediately aspirated into a polypropylene tube containing EDTA. The sampled blood was cooled on ice immediately and centrifuged at 3,000 rpm for 10 min at 4 °C to separate the plasma, which was maintained in frozen storage at –70 °C until analysis. Plasma NE concentrations at rest and immediately after exercise in each condition were analyzed by high-performance liquid chromatography assay. The increase (peak – rest = ΔNE) and rate of increase ([peak – rest]/rest = %ΔNE) in NE concentration were calculated.

Measurement of Erythrocyte Mg Concentration

Venous blood was obtained from the catheter with the participant in a resting state, and samples of heparinized blood for measuring the erythrocyte Mg concentration were centrifuged at 3,000 *g* at 4 °C for 10 min. After removal of plasma and the buffy coat, the erythrocyte sediment was washed three times with 9% NaCl solution and centrifuged at 4 °C at 3,000 *g* for 10 min. After removal of the supernatant, 9% NaCl solution was added until the total sample volume reached 4 ml; this volume was divided equally into two samples. One sample was used to count erythrocytes. The other sample was centrifuged at 3,000 *g* at 4 °C for 10 min. After removal of the supernatant fluid, distilled water was added until the

total sample volume reached 2 ml to hemolyze the erythrocytes. Erythrocyte Mg concentration was measured by the atomic absorption method, and the value obtained was corrected for the number of erythrocytes; the Mg concentration was expressed per 400×10^4 mm^{-3}.

Calculation

To evaluate the HR response to sympathetic nervous stimulation by exercise, ΔHR/ΔNE and ΔHR/%ΔNE were calculated. These parameters were previously reported by Colucci et al. (1989) and modified by our coworker (Samejima et al., 2003).

Statistical Analysis

All data are expressed as $M \pm SD$. Comparisons between the three sleep conditions were made by a two-way analysis of variance followed by Dunnett's post hoc tests. Correlation between variables was determined by calculating Pearson's correlation coefficient. A p value of <.05 was considered significant.

Results

The sleep time of participants was 7.56 ± 0.79 hr in the control state and 4.59 ± 0.95 hr for 34.8 ± 9.9 days in the chronic sleep-deprived state. The sleep time 1 day before CPX in the chronic sleep-deprived state was 4.28 ± 1.38 hr. The sleep time in the temporary sleep-deprived state was checked before CPX to confirm that participants had slept less than 3 hr.

For all participants, the exercise test was terminated when leg fatigue or shortness of breath occurred. No participant experienced ischemic ST- and T-wave change or severe arrhythmia. HR both at rest and at AT in the acute sleep-loss condition was lower than that in the control state ($p < .05$). Peak HR and ΔHR did not differ statistically between conditions. The mean AT was significantly lower in both the acute sleep-loss and chronic sleep-restricted states than in the control state ($p < .01$ for both). Although peak VO_2 was lower in both the acute sleep-loss and chronic sleep-restricted states than in the control state, there was no statistical difference between states. The periods of ramp exercise needed to reach AT and peak exercise were significantly shorter ($p < .05$ and $p < .01$, respectively) in the chronic sleep-restricted state than in the control state (see Table 1).

Plasma NE concentrations in the resting state and after peak exercise did not differ between conditions. The %ΔNE was significantly lower in the chronic sleep-restricted state (10.43 ± 5.23) than in the acute sleep-loss and control states (18.94 ± 12.23 and 19.25 ± 9.97, respectively). The intracellular Mg concentration decreased gradually from the control to the chronic sleep-deprived state (1.76 ± 0.33, 1.28 ± 0.23, and 1.14 ± 0.27 mg/dl, respectively; see Table 2).

ΔHR/%ΔNE was significantly higher ($p < .05$) in the chronic sleep-restricted state than in the acute sleep-loss state. ΔHR/ΔNE tended to be higher in the chronic sleep-restricted state than in the control state ($p = .08$).

In total amount of three conditions, both ΔHR/ΔNE and ΔHR/%ΔNE had weak negative correlations with peak exercise time ($r = –.29$, $p < .05$, and $r = –.32$, $p < .05$, respectively; see Figure 1).

Table 1 Cardiopulmonary-Exercise-Testing Parameters in Each Sleep Condition

	Control	Acute sleep loss	Chronic sleep restriction
Heart rate (beats/min)			
rest	79.6 ± 8.7	73.4 ± 8.4*	79.4 ± 10.6†
anaerobic threshold	123.1 ± 15.6	113.9 ± 15.6*	122.3 ± 14.5
peak	190.8 ± 10.1	187.7 ± 11.7	190.8 ± 10.4
ΔHR	111.2 ± 8.6	114.3 ± 13.0	111.3 ± 9.9
VO_2 (ml · min^{-1} · kg^{-1})			
rest	3.6 ± 0.5	3.4 ± 0.5	3.7 ± 0.4
warm-up	8.9 ± 1.1	8.7 ± 1.0	9.3 ± 1.0††
anaerobic threshold	17.3 ± 2.5	14.7 ± 2.5**	15.7 ± 1.9**
peak	42.3 ± 5.6	40.4 ± 6.3	40.3 ± 5.2
Exercise duration (s)			
anaerobic threshold	222.1 ± 44.2	180.9 ± 39.1**	193.3 ± 45.8*
peak	699.3 ± 72.7	677.0 ± 77.7	626.0 ± 56.7**†

Note. Δ = peak – rest.

*$p < .05$ vs. control, **$p < .01$ vs. control. †$p < .05$ vs. acute sleep loss, ††$p < .01$ vs. acute sleep loss.

Table 2 Serum NE, Intracellular Mg, and HR Response to Sympathetic Nervous Stimulation in Each Sleep Condition

	Control	Acute sleep loss	Chronic sleep restriction
NE (pg/ml)			
rest	300.5 ± 200.5	240.9 ± 103.0	315.1 ± 159.3
peak	4765.5 ± 1814.8	4553.7 ± 2293.9	3333.6 ± 1504.3
ΔNE	4054.8 ± 1841.6	3838.1 ± 2240.3	2948.1 ± 1336.4†
%ΔNE	18.94 ± 12.23	19.25 ± 9.97	10.43 ± 5.23†
RBC-Mg (mg/dl)	1.76 ± 0.33	1.28 ± 0.23*	1.14 ± 0.27**
ΔHR/%ΔNE	8.64 ± 5.58	7.23 ± 3.18	13.34 ± 6.84†
ΔHR/ΔNE	1.03 ± 0.20	1.13 ± 0.16	1.28 ± 0.36

Note. Δ = peak – rest; NE = norepinephrine; RBC = red blood cell; Mg = magnesium; HR = heart rate.

*$p < .05$ vs. control, **$p < .01$ vs. control. †$p < .05$ vs. acute sleep loss.

Although ΔHR/ΔNE in control and acute sleep loss had significantly positive correlations with intracellular Mg concentrations ($r = .67$, $p < .01$, and $r = .63$, $p < .05$ respectively), no significant correlation was observed between these in chronic sleep restriction.

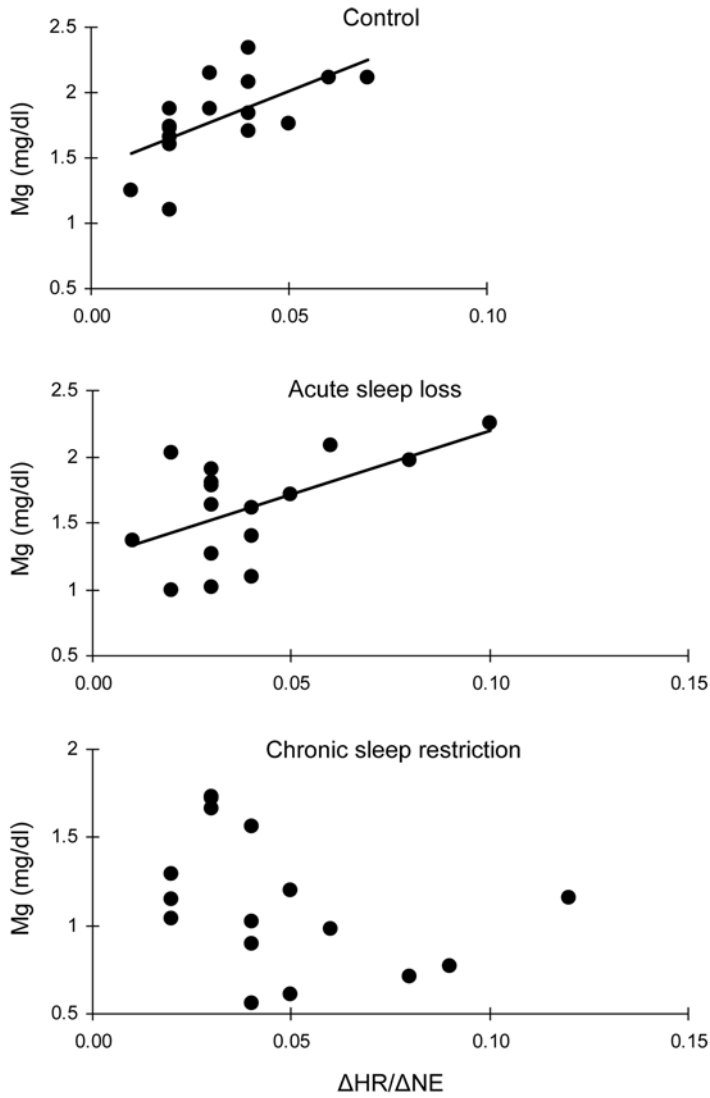

Figure 1 — Correlation between ΔHR/ΔNE and intracellular magnesium concentration. Mg = magnesium; ΔHR/ΔNE = change in heart rate divided by change in norepinephrine.

Discussion

Our coworker (Osada, 1994) previously reported that effects of acute sleep loss and chronic sleep restriction on functional capacity and stress-hormone secretion differed. He reported that although both peak VO2 and %ΔNE decreased with

chronic sleep restriction in young healthy volunteers, there were no significant differences in these variables between the control state and the acute sleep-loss state. He concluded that the lack of increase in stress hormone might be a main cause of reduced functional capacity in the chronic sleep-restricted state and that a compensatory mechanism might be at work in acute sleep loss. The same finding that %ΔNE was high but not significantly so was obtained in the acute sleep-loss condition in the current study. NE has been reported not to increase with 50 hr of acute sleep loss (Martin & Chen, 1984) or with partial sleep restriction caused by early awakening or delayed bedtime (Mougin et al., 2001). These findings, as well as those of the current study, indicate only minor alterations in the NE responses to exercise after partial sleep restriction.

In our previous study (Yamamoto et al., 1996), %ΔNE in the chronic sleep-restricted state with Mg administration was equal to that in the control state, whereas %ΔNE was significantly lower in the chronic sleep-restricted state without Mg administration. We speculated that the repeated stress of chronic sleep restriction induced a shift in intracellular Mg to the extracellular space and finally to the urine. In addition, NE concentrations at rest, below AT, and at peak exercise were significantly higher in chronic sleep restriction with Mg administration than without Mg. Decreased intracellular Mg induces decreased NE secretion and low exercise tolerance even in young healthy participants. It has been reported that the NE concentration increased after Mg administration in patients who suffered disabling primary Raynaud's phenomenon and in control participants (Leppert et al., 1994). Those investigators suggested that the increased plasma NE concentration could be a result of either increased release or altered clearance. In the current study, the intracellular Mg concentration was low even in the acute sleep-loss state, which seemed to induce relatively low-grade stress in comparison with the control state.

Other investigators have reported inverse effects of Mg to catecholamine. It was reported that cardiac NE release was not increased after intravenous Mg infusion during handgrip stress in cardiac patients (Ohtsuka, Oyake, Seo, Eda, & Yamaguchi, 2002) and also that preoperative oral Mg supplementation led to NE reduction after coronary artery bypass surgery (Pasternak et al., 2006). Moreover, investigators showed that Mg infusion inhibited NE release by blocking n-type calcium channels at peripheral sympathetic nerve endings in spontaneous hypertensive rats, decreasing blood pressure independently during electrical spinal stimulation (Shimosawa, Takano, Ando, & Fujita, 2004). It is speculated that intracellular Mg had an effect of normalization or stabilization on catecholamine secretion, not only for NE increase but also for suppression when NE is not needed, which was also demonstrated in this study.

Plasma growth-hormone and NE values at rest, during submaximal or maximal exercise, and in recovery are reportedly the same after acute sleep loss as in a control state (Mougin et al., 2001). Investigators concluded that only minor alterations occur in the hormonal responses to exercise after acute sleep loss. Chronic sleep restriction might lead to chronic fatigue and decreased NE production after long-lasting sympathetic nerve stimulation. Moreover, plasma NE concentration, as well as intracellular Mg concentration, might decrease with chronic sleep restriction. This might be one of the mechanisms underlying low oxygen uptake during exercise in the chronic sleep-restricted state, because the %ΔNE in partici-

pants under chronic sleep restriction with Mg administration was significantly higher than in participants without Mg administration (Yamamoto et al., 1996).

It has been reported that the HR response to sympathetic nervous activity decreases and that the magnitude of decrease correlates with exercise intolerance in patients with chronic heart failure (CHF; Colucci et al., 1989; Samejima et al., 2003). In contrast, in the current study, increased HR response to sympathetic nervous stimulation correlated with exercise intolerance. The discrepancy between CHF patients and normal participants with chronic sleep restriction can be explained, in a part, by the difference in cardiac function, degree of injury in postsynaptic adrenal fibers, or cytokine levels. Postsynaptic β-adrenergic desensitization in the sinoatrial node is reportedly the main mechanism of chronotropic incompetence in patients with CHF (Colucci et al.). Inversely, it was speculated that β-adrenergic sensitization in the sinoatrial node in chronic sleep restriction occurred as a result of the same HR response to low NE concentration during exercise. In the chronic sleep-restricted state, efficiency of the HR increase would be good because ΔHR was almost the same in the chronic state as in the other two conditions, although low %ΔNE was observed. In the chronic sleep-restricted state, chronic stress resulting from sleep restriction led to the termination of NE production by low intracellular Mg but also to hypersensitive HR response to decreased NE. The HR response to NE secretion was inappropriately increased in the chronic sleep-restricted state, and this might be a mechanism of compensating for chronic stress. The HR response to NE in healthy participants also might be attenuated if chronic sleep restriction lasts much longer.

There were limitations to the study. The degree of fatigue caused by chronic sleep restriction might have differed between participants because sleep time in this study was reported by the participants themselves, and there were no lifestyle restrictions other than those against alcohol ingestion and hypnotic drug use. In addition, other stresses, especially psychic stress, would be added to that of chronic sleep restriction because the participants were in an extraordinary, end-term-examination period. Therefore, conditions could have varied somewhat because it was impossible to standardize participants' eating, sleeping, and working habits in their own homes during the observation period.

Conclusion

We conclude that impaired exercise tolerance in the chronic sleep-restricted state is caused by hypersensitivity of the HR response to sympathetic nervous stimulation, which showed a compensation for decreased intracellular Mg concentration.

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