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Effect of Chronic Stress and Sleep Deprivation on Both Flow-Mediated Dilation in the Brachial Artery and the Intracellular Magnesium Level in Humans

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Summary

Background: Chronic mental and physical stress has been suggested to be a trigger for cardiovascular events. In addition, a reduction in levels of intracellular magnesium has been reported to cause vasoconstriction while enhancing platelet-dependent thrombosis.

Hypothesis: The purpose of this study was to investigate whether chronic stress affects endothelial function and intracellular magnesium levels in humans.

Methods: Flow-mediated dilation (endothelium-dependent vasodilation) and sublingual nitroglycerin-induced dilation (0.3 mg, endothelium-independent vasodilation) were measured in the brachial artery in 30 healthy male college students, aged 22 ± 1 years, using high-resolution ultrasound both before and immediately after a 4-week final term examination period. Erythrocyte magnesium concentration was measured simultaneously. All students had chronic sleep deprivation for 4 weeks, during which sleep lasted < 80% of that on ordinary days; in addition, the students were under great stress to pass the examination. This condition was considered to be chronic stress.

Results: Chronic stress decreased flow-mediated dilation and erythrocyte magnesium concentration (from 7.4 ± 3.0 to 3.7 ± 2.3%, $p < 0.05$; from 5.7 ± 0.4 to 5.5 ± 0.4 mg/ml, $p < 0.05$, respectively). The change in flow-mediated dilation correlated significantly with that of the erythrocyte magnesium concentration ($r = 0.43$, $p < 0.05$), but not with nitroglycerin-induced dilation.

Conclusions: Chronic stress was found to attenuate endothelial function, which may also be associated with a reduction in the intracellular magnesium level in humans.

Key words: chronic stress, sleep deprivation, endothelial function, magnesium

Introduction

Prolonged mental stress and chronic fatigue are associated with sudden cardiac death and cardiovascular events.[1] Mental stress is reported to induce myocardial ischemia in patients with coronary artery disease (CAD).[2] Chronic fatigue frequently causes sudden cardiac death in both apparently healthy people and patients with heart disease.[3] Chronic sleep deprivation has been investigated as one of the models of chronic fatigue. It detrimentally influences various neurohumoral conditions as well as exercise capacity even in healthy subjects.[4] Chronic mental and physical stress have also been suggested to be a trigger for cardiovascular events.

The dynamics of magnesium level are related to coronary and systemic artery vasomotion and affect vascular smooth muscle tone. As a result, magnesium deficiency can cause coronary arterial spasms and lethal arrhythmia in patients with a history of myocardial infarction.[5] In some reports,[4, 6] chronic sleep deprivation has been reported to cause magnesium deficiency. Endothelial-dependent and -independent arterial vasomotion is also associated with the pathophysiology of cardiovascular disease.[7] Many atherosclerotic risk factors as well as cardiac diseases impair endothelial-dependent vasodilation.

Since both abnormal coronary and systemic vasomotion increase the number of cardiac vascular events, it is hypothesized that a combination of prolonged stress and chronic sleep deprivation may induce abnormal vasomotion, which includes endothelial-dependent and -independent vasodilation, due to magnesium deficiency. However, the effects of chronic mental and physical stress on vascular vasomotion and on intracellular magnesium levels have not yet been fully investigated in

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humans. The purpose of this study therefore was to investigate whether chronic stress, including sleep deprivation, affects endothelial function and intracellular magnesium levels in humans by measuring flow-mediated dilation (endothelium-dependent vasodilation) and sublingual nitroglycerin-induced dilation (endothelium-independent vasodilation) in the brachial artery in young normal subjects.

Methods

Study Population

The study population consisted of 30 healthy male college students ranging in age from 20 to 24 years (average 21.7 ± 1.1 years, mean ± standard deviation [SD]). None of the subjects smoked and none had other coronary risk factors including hypertension, diabetes mellitus, hyperlipidemia, or a family history of premature CAD. Written informed consent was obtained from each subject before the start of the study. All subjects refrained from drinking alcohol or any beverages containing caffeine throughout the entire study period. All evaluations were performed between 4:00 and 6:00 P.M.

Brachial Arterial Endothelial Function Study

All studies were performed according to a previously reported method.[8] Briefly, all evaluations were conducted in a temperature-controlled room (25˚C) with the subject in a supine position. The electrocardiogram (ECG) was monitored continuously. Blood pressure was recorded from the left arm every 3 min with an automatic sphygmomanometer. The subject's right arm (the dominant arm) was comfortably immobilized in the extended position to allow for consistent access to the brachial artery for imaging purposes. The brachial artery diameter and flow velocity were imaged using a 7.5 MHz linear array transducer ultrasound system (Hewlett Packard, SONOS 1500, Andover, Mass., USA).

First, baseline two-dimensional images were obtained; then, pulsed-Doppler blood flow velocity was determined. Brachial arterial flow velocity was obtained using a Doppler signal at a 70˚ angle to the vessel, with the range gate (1.5 mm) in the center of the artery. After performing baseline measurements, a small-width blood pressure cuff was inflated on the most proximal portion of the forearm to occlusive pressure (systolic blood pressure + 30 mmHg) for 5 min in order to induce hyperemia. Next, the cuff was rapidly deflated. Immediately after deflation, pulsed-Doppler signals were recorded for 15 s. Two-dimensional images of the brachial artery were obtained for 60 s after cuff deflation. All images were recorded on super VHS videotape for later analysis.

The brachial artery blood flow at rest and during reactive hyperemia was determined by previously described methods.[9] The flow volume was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for angle and by heart rate) and the vessel cross-sectional area (πr^2), using public domain software (Hewlett Packard, SONOS 1500). The relative increase in blood flow at reactive hyperemia was calculated as the maximal flow recorded in the first 15 s after cuff deflation divided by the flow at baseline scan.

By playing back the recorded information on a videocassette recorder, a 10–20 mm segment of the brachial artery could be identified for analysis using the anatomic landmarks in each subject. To select the images reproducibly at the same point in the cardiac cycle, images at peak systole (maximum dilation, close to the end of the T wave on the ECG) were identified and the diameter of the brachial artery was digitized. A quantitative coronary angiography analysis computer (Kontron Elektronik, Cardio 500, Boston, Mass., USA) containing a digitizing board was used for these measurements. For each condition (baseline, reactive hyperemia at 60 s after cuff deflation), three separate images from three different cardiac cycles were digitized. The average segment diameter of these three images was determined. All of these measurements were performed in a blinded manner. To conduct a blind measurement of brachial artery endothelial function, the technician carrying out the study was not informed of the study protocol. In addition, study subjects were instructed not to inform the technician of the study protocol.

The percent diameter changes from baseline in response to hyperemia were calculated. The intra- and interobserver variabilities (coefficient of variation) for repeated measures of the diameter at baseline and reactive hyperemia in the brachial artery were < 2%, as previously reported.[10]

Biochemical and Intracellular Magnesium Measurements

Twenty ml of blood for an assay of catecholamine level, lipid profile, glucose level, and intracellular magnesium level were obtained from an antecubital vein within 1 h before performing the brachial artery endothelial function study and a blood flow velocity analysis. The blood was immediately transferred into chilled 10 ml polyethylene tubes containing ethylene diaminetetraacetic. The samples were centrifuged at 4˚C and 3,000 rpm for 10 min. Norepinephrine and epinephrine were then measured after absorption onto alumina at pH 6.5 using high-pressure liquid chromatography (pg/ml). The serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and glucose were measured using standard methods. The erythrocyte magnesium concentration was measured as intracellular magnesium level. Blood samples were heparinized in order to measure the erythrocyte magnesium concentration and were centrifuged at 3000 × G at 4˚C for 10 min. The erythrocyte magnesium concentration was determined by the atomic absorption method as previously reported,[6] and the value obtained was corrected for the number of erythrocytes; the magnesium concentration was expressed per 400 × 104/mm^3.

Study Protocol

The brachial artery endothelial function study as well as biochemical and intracellular magnesium measurements were performed under both normal and chronic stress conditions. A

normal condition was defined as a day after at least one week of sleeping well, and chronic stress was defined as a day immediately after a 4-week final term examination period. In chronic stress, all subjects had chronic sleep deprivation for 4 weeks when sleep lasted < 80% of that during ordinary periods; in addition, they were under mental duress due to anxiety about passing their term examinations. The sleep conditions were examined by questionnaire and were confirmed by an interview performed by one of the investigators.

Statistical Analysis

All data are presented as the means ± SD. The flow-mediated dilation data in the brachial artery were examined using Student's *t*-test. Correlations of the data between the results of brachial artery endothelial function study and other parameters were obtained by Pearson's product–moment correlation. Statistical significance was assumed if the null hypothesis was rejected at the p = 0.05 level.

Results

Under chronic stress, heart rate and blood pressure increased significantly compared with those under normal conditions (Table I). The mean values of resting and hyperemic brachial blood flow were not significantly different between chronic stress and a normal condition, nor was the relative brachial blood flow increase. The effects of chronic stress on brachial vasomotion, such as flow-mediated dilation and nitroglycerin-induced dilation, are shown in Table I and Figures 1 and 2. Chronic stress decreased flow-mediated dilation in the brachial artery in 29 of 30 subjects (Fig. 1), from 7.4 ± 3.0 to 3.7 ± 2.3%. In contrast, nitroglycerin-induced dilation was not influenced by chronic stress (Fig. 2). Plasma levels of epinephrine and norepinephrine also increased significantly under chronic stress. Intracellular magnesium measured as erythrocyte magnesium concentration decreased in 21 of 30 subjects, from 5.7 ± 0.4 to 5.5 ± 0.4 mg/ml, as shown in Table I and Figure 3. Chronic stress had no effect on serum levels of the lipid profile or glucose level (Table I). All 30 participants successfully passed their term examinations so that we could not evaluate a difference between those who passed and those who failed.

When we examined the relationship among flow-mediated dilation, hemodymanics, catecholamines, and intracellular magnesium concentration using Pearson's product-moment correlation, a significant correlation was obtained only between the changes in flow-mediated dilation and the changes in intracellular magnesium concentration, as shown in Figure 4. The absolute values in chronic stress or under normal condi-

TABLE I Summary of the results

	Chronic stress	Normal condition
Heart rate (beats/min)	60 ± 7[a]	53 ± 10
Systolic blood pressure (mmHg)	112 ± 10[a]	109 ± 8
Diastolic blood pressure (mmHg)	70 ± 9[a]	67 ± 8
Mean blood pressure (mmHg)	84 ± 8[a]	81 ± 6
Brachial artery diameter (mm)	4.17 ± 0.47	4.10 ± 0.54
Flow-mediated dilation, %	3.7 ± 2.3[a]	7.4 ± 3.0
Nitroglycerin-induced dilation, %	12.6 ± 5.3	13.7 ± 5.3
Norepinephrine level (pg/ml)	460 ± 144[a]	312 ± 110
Epinephrine level (pg/ml)	52 ± 25[a]	38 ± 17
RBC-magnesium level (mg/ml)	5.5 ± 0.4[a]	5.7 ± 0.4
Baseline blood flow (ml/min)	237 ± 150	197 ± 140
Reactive hyperemic blood flow (ml/min)	924 ± 301	703 ± 154
Relative increase of blood flow (× rest)	4.4 ± 2.3	4.1 ± 2.3
Total cholesterol (mg/dl)	174 ± 26	168 ± 23
Triglyceride (mg/ml)	136 ± 79	126 ± 76
HDL cholesterol (mg/ml)	58 ± 15	56 ± 14
Fasting blood glucose (mg/ml)	96 ± 7	94 ± 9

Mean ± standard deviation.
[a] p < 0.05 vs. normal condition.
Abbreviations: RBC-magnesium = intracellular magnesium levels in red blood cell, HDL = high-density lipoprotein.

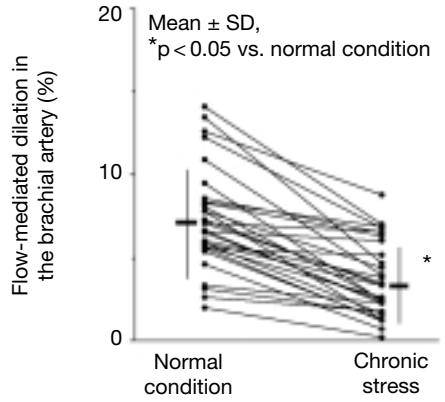

FIG. 1 Changes in flow-mediated dilation in the brachial artery. SD = standard deviation.

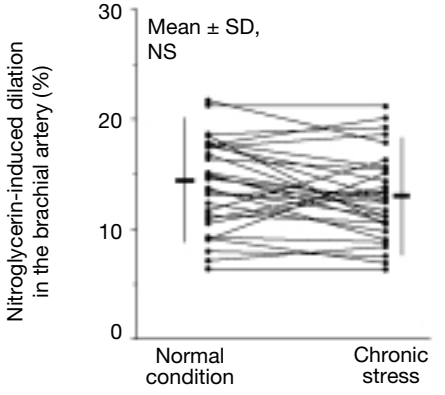

FIG. 2 Changes in nitroglycerin-mediated dilation in the brachial artery. SD = standard deviation, NS = not significant.

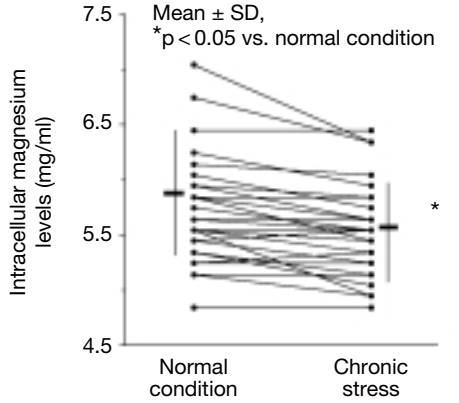

FIG. 3 Changes in intracellular magnesium levels. SD = standard deviation.

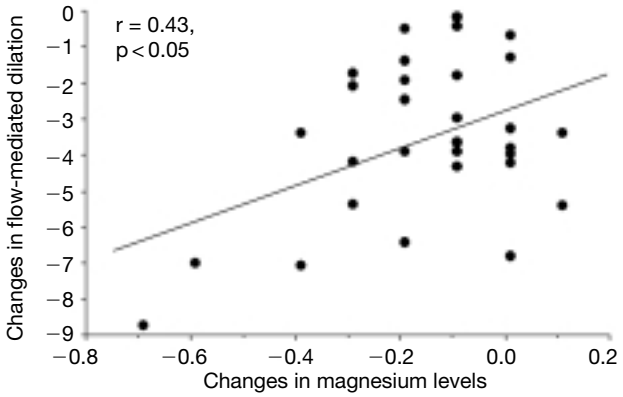

FIG. 4 Correlation between changes in magnesium levels and flow-mediated dilation.

tions showed no significant relationship to any parameters examined in this study.

Discussion

The present study demonstrated that chronic stress defined as sleep deprivation and mental stress due to studying for term examinations impairs flow-mediated dilation in the brachial artery (endothelium-dependent vasodilation) and decreases the intracellular magnesium level. In addition, a moderate but significant correlation was observed between the changes in flow-mediated dilation and magnesium levels. Nitroglycerin-induced dilation in the brachial artery was not influenced by chronic stress, and therefore chronic stress was found to cause a transient endothelial dysfunction in the brachial artery in the young healthy male subjects. Chronic stress defined in this study significantly changed the hemodynamics and plasma levels of catecholamines; however, flow-mediated dilation in the brachial artery showed no significant correlation with these parameters.

Sleep deprivation has been reported to decrease the intracellular magnesium level in humans,[4,6] and mental stress has also recently been reported to cause transient endothelial dysfunction in the brachial artery in humans;[11] however, the relationship between intracellular magnesium levels and endothelial function has not yet been fully investigated. Magnesium is considered to be a physiologic calcium antagonist and has been reported to decrease vascular tone.[12,13] Magnesium deficiency induces arterial constriction and is a possible cause of myocardial damage in an experimental model.[14,15] Especially, a reduction of magnesium concentration has been demonstrated to cause vasoconstriction while also enhancing platelet-dependent thrombosis.[16,17] Decreased intracellular magnesium levels might also counteract nitric oxide. Generally, an increased intracellular magnesium level has been shown to be related to adenosine triphosphate (ATP) production while also showing a protective effect on myocardial ischemia.[12,18] An experimental study showed that hypomagnesemia impaired nitric oxide release from the vascular endothelium[15] while it augmented oxidative stress.[19] In addition, both experimental and human studies revealed that mental and psychosocial stress decrease endothelial function.[11,20,21] According to Tanabe *et al.*'s reports,[4,6] the decreased levels of intracellular magnesium in this study were identical to those between temporary and persistent sleep deprivative conditions; they are thus considered to be a reflection of sleep deprivation in our study protocol.

Mental stress and sleep deprivation are known to be related to augmented sympathetic activity. Increased blood pressure, heart rate, and catecholamine levels suggest an increase in sympathetic activity during stress conditions and sleep deprivation. However, endothelium-dependent vasodilation is not related to these parameters. In addition, the resting brachial diameters and the relative increase in brachial blood flow both at chronic stress and under normal conditions were not significantly different. The reason that the resting brachial diameter under chronic stress conditions tended to be larger than that under normal conditions might be simply the measurement variability. Even if the imaging study of the brachial artery had been performed with caution to detect the same portion of the artery using the anatomic landmarks, the variation could occur if the study had been conducted on a different day. From a statistical point of view, mean brachial artery diameter under chronic stress conditions did not differ from that under normal conditions. The relative change such as flow-mediated dilation is more important. In addition, although the sympathetic activity influences endothelial nitric oxide production,[7] the intracellular magnesium levels may be more important than the sympathetic activity for endothelium-dependent vasodilation in our study protocol.

Since nitric oxide is a potent vasodilator and an inhibitor of platelet function, and also has an anti-atherosclerotic effect, hypomagnesemia may promote vasoconstriction and thrombosis and subsequently be related to cardiovascular events. Mental stress is also known to cause myocardial ischemia and rupturing of atherosclerotic plaque, thus resulting in cardiovascular events.[1,2,11] Our findings in this study may be important.

Study Limitations

In our study protocol, the definition of chronic stress was qualitative and not quantitative and, as a result, the status of mental stress is considered to be different from earlier mental stress studies. However, as described above, the decreased intracellular levels of magnesium are considered to be due to sleep deprivation. In addition, studying for term examinations is one of the normal types of stress in ordinary daily life. Our model may be useful in clinical practice. Another limitation of our study is that mental stress is associated with immunologic factors,[22] and we did not evaluate these changes. However, Ghiadoni *et al.*[11] reported that mental stress impaired transient endothelial dysfunction but had no significant effect on inflammatory cytokin levels. As a result, neither mental stress- nor fatigue-induced endothelial dysfunction may thus be closely associated with these factors.

Conclusions

Chronic stress attenuates endothelial function, which is possibly associated with a reduction in intracellular magnesium levels. Our findings in this study may provide a novel insight into the role of chronic stress in the development of cardiovascular events.

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