SHORT COMMUNICATION

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LACK OF EFFECT OF AEROBIC PHYSICAL EXERCISE ON ENDOTHELIUM-DERIVED NITRIC OXIDE CONCENTRATIONS IN HEALTHY YOUNG SUBJECTS

KANAMI YAMAMOTO[1], TAKAAKI KONDO[1], AKIKO KIMATA[1], JUN UEYAMA[1], AYA SHIROTORI[2], YOSHIKO OKADA[2], DAISUKE SAKUI[3], MASAHIRO NAKASHIMA[3] and SUMIO YAMADA[3]

[1]*Program in Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine*
[2]*Department of Medical Technology, Nagoya University School of Health Sciences*
[3]*Program in Physical and Occupational Therapy, Nagoya University Graduate School of Medicine*

ABSTRACT

Previous experimental research showed an attenuated vasoconstrictor response after acute exercise. This study aims to determine the effect of aerobic physical exercise on the endothelium-derived circulating nitric oxide (NO) concentrations among healthy young persons. A total of 10 subjects (5 men and 5 women) aged 21–28 were examined for the difference in concentrations of serum and plasma NO metabolites (NO_x) before and after a single bout of aerobics, in which they performed cycling exercises at a constant workload corresponding to 90% of the subject-specific anaerobic threshold. A paired *t*-test resulted in no statistically significant differences between pre- and post-exercise concentrations of serum or plasma NO_x. It is suggested that the acute effect of aerobic exercise on the vasoconstrictor/vasodilator response is not mediated by an increase in the endothelial NO release.

Key Words: Nitric oxide, Vasodilation, Physical exercise, Anaerobic threshold

INTRODUCTION

The beneficial effects of engaging in moderate physical activity for the primary prevention of hypertension have been frequently reported.[1)] In a follow-up of normotensive persons with no history of cardiovascular disease, those who were highly fit were found to be at significantly lower risk for developing hypertension.[2)] Previous laboratory experiments have demonstrated that the peripheral vasodilatory responses induced by acetylcholine (Ach) were augmented by exercise training,[3)] and that these enhanced vasodilations were attributable to a greater release of nitric oxide (NO) from the arterial endothelium of trained animals.[4)] Moreover, an augmented Ach-induced NO release was suggested in the forearm of both hypertensive patients and normotensive subjects who completed 12 weeks of aerobic exercise training.[5)]

Along with these long-term influences of physical activity, the short-term effects of dynamic

Corresponding author: Takaaki Kondo, M.D., Ph.D.
Program in Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine, 1-1-20, Daiko-minami, Higashi-ku, Nagoya 461-8673, Japan
Phone & Fax: +81-52-719-1561 E-mail: taka@met.nagoya-u.ac.jp

exercise on blood pressure (BP) have also been vigorously investigated. One report showed that, in hypertensive subjects, dynamic exercise subjects caused a marked BP rise followed by a prompt post-exercise fall to BP levels significantly lower than their corresponding pre-exercise values, and this effect lasted throughout the 90-minute post-exercise period without persistent tachycardia accompanying hypotension.[6)]

Among a number of physiological roles played by endothelium-derived NO,[7)] the vasodilatory function is considered to be especially important in reducing BP.[8)] In the light of previous reports showing the effects of chronic exercise on the increase of NO release, there has been considerable speculation as to involvement of NO in the post-load BP reduction after acute exercise. This speculation requires confirmation. Attenuation of the α-adrenergic vasoconstrictor response after a single bout of acute exercise has been experimentally observed,[9)] suggesting an exercise-induced increase in NO release. However, the effect of acute exercise on endothelium-derived NO in humans has not been sufficiently investigated, and no significant difference was found in the NO production in saliva samples withdrawn prior to and those following a 10,000-m run in 24 healthy subjects.[10)]

The purpose of this study is to determine the effect of aerobic physical exercise on concentrations of circulating NO derived from endothelium among healthy young individuals.

SUBJECTS AND METHODS

Subjects

A total of 10 subjects (5 men and 5 women) ranging 21–28 years in age were recruited for the study. A self-administered questionnaire, which covered a subject's present history of disease and such lifestyle characteristics as smoking status, drinking habit, and regular physical activity, revealed that all the subjects were in good health. Body mass index (BMI, kg/m^2) was calculated using the self-reported data on height and weight.

Physical exercise protocol

To determine the subject-specific anaerobic threshold (AT), all subjects performed an incremental exercise test of the ramp type on a mechanically braked cycloergonometer (ERGONOMICS 232C-XL, COMBI Wellness Corp.) at a bench height to facilitate the most effective pedaling. After a 3-minute warm-up at 20 W, the work rate was increased every minute by 20 W for men and 15 W for women. Throughout the test, the pedaling rate was set at 60 rpm. The subjects were monitored on the CM5 lead for electrocardiogram (ECG) and heart rate (HR), and were required to estimate their level of exhaustion every minute on Borg's Ratio of Perceived Exertion scale.[11)] The incremental workload was discontinued when one of the following criteria was met: abnormal ECG finding, HR of 220–age, or the maximum level of exhaustion on Borg's scale; a further 3-minute workload at 10 W followed for cooling down before completion of the exercise test. Breath-to-breath data on oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and minute ventilation ($\dot{V}E$) were obtained during the test by means of a system measuring expired gas (AE-280S, MINATO Medical Science Co., Ltd.). AT was quantified according to the V-slope method,[12)] which depends on graphic observations to identify the time point of a disproportionate $\dot{V}CO_2$ increase in relation to the linear VO_2 increase.[13)]

To familiarize our subjects with this exercise procedure, a rehearsal test was performed once a week before the AT determination. The subjects were instructed to avoid heavy exercise and drinking on the preceding day, have a restful overnight sleep, abstain from smoking on the day of the test, and fast during the 2-hour period before the test.

NO assay in serum and plasma

After a 1-week recovery period from the first test for AT determination, the subjects performed the second test, in which a constant workload corresponding to 90% of the predetermined AT level was applied for 20 minutes to each subject in combination with identical warm-up and cool-down procedures.

In the second test, venous blood samples were collected before the start of the test and immediately after its completion. Whole blood was drawn into two vacuum collection tubes each time, one plain and the other containing sodium EDTA as an anticoagulant, for the respective serum and plasma NO assays. Since NO is unstable and quickly auto-oxidized after production to stable metabolites, *i.e.*, the NO_x (nitrate plus nitrite), concentrations of NO_x in serum or plasma were instead determined using a commercial kit (Nitrate/Nitrite Colorimetric Assay Kit, CAT No. 780001, Cayman Chemical). Briefly, the subjects' samples were ultrafiltered (molecular cut-off of 10,000) at 6,000 × g for 60 minutes at 4°C. The ultrafiltrate was then incubated for 3 hours with nitrate reductase and its cofactor and allowed to react with Griess reagents for 20 minutes. Absorbance was measured at 540 nm with a microplate reader (Molecular Devices). The within-day and between-day coefficients of variation for this assay were less than 5% at a concentration of 50 μmol/l.[14)]

The subjects were instructed regarding the objectives and procedure of the study and gave written informed consent. The exercise tests for AT determination and blood sampling for NO assay were performed in May of 2007 in an air-conditioned laboratory kept at around 25°C. The Ethics Committee of the Nagoya University Graduate School of Medicine, Nagoya, Japan, approved the study protocol.

Statistical analysis

Since NO_x concentrations were found to be skewed in their distribution, they were normalized with a logarithmic transformation in advance of all analyses. The paired *t*-test was conducted to compare the NO_x concentrations in both serum and plasma before and after exercise. A gender-specific subanalysis was also conducted. All statistical analyses were performed using the SPSS statistical package for Windows version 11.0 (SPSS Inc.), and 2-sided p values of < 0.05 were considered statistically significant.

RESULTS

The male and female subjects averaged 23.0 ± 0.7 (mean ± SD) and 23.2 ± 2.9 years in age, respectively (Table 1). All had a BMI of $\leq$ 21.0 kg/m^2. While there were 2 smokers among the men, none of the women smoked. The range of determined 90% AT levels for a constant workload in the second bout test was 66–99 W in men and 56–75 W in women with a mean of 83.6 W and 65.9 W, respectively. Figure 1 showed serum and plasma NO_x concentrations of all the individuals in pre-exercise and post-exercise pairs.

No statistically significant differences in the means of serum NO_x concentrations were detected in the pre- *vs.* post-exercise comparison (Table 2). Comparison of plasma NO_x concentrations also failed to yield any statistically significant differences. Moreover, in the gender-specific subanalysis, neither men nor women showed significant differences between before and after exercise in either serum or plasma NO_x concentrations.

Table 1 Basic characteristics of 10 subjects.

	Men (n = 5)	Women (n = 5)
	Mean ± SD (Min, Max)	Mean ± SD (Min, Max)
Age (y)	23.0 ± 0.7 (22, 24)	23.2 ± 2.9 (21, 28)
Body mass index (kg/m^2)	20.2 ± 0.6 (19.5, 21.0)	19.4 ± 0.9 (18.5, 20.6)
90% AT[a)] workload (Watt)	83.6 ± 14.1 (66, 99)	65.9 ± 8.0 (56, 75)
	Frequency	
Smoking habit		
Yes	2	0
No	3	5
Drinking habit		
Yes	4	4
No	1	1
Regular physical activity		
Yes	2	2
No	3	3

[a)] AT, Anaerobic threshold

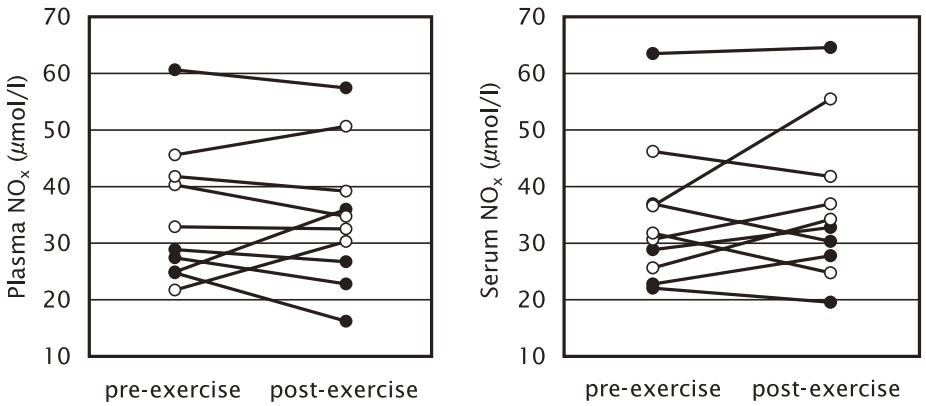

Fig. 1 Paired NOx concentrations in plasma (left panel) and serum (right panel). Solid circles indicate men, and open circles women.

Table 2 Paired t-test for comparison of NO_x concentrations[a)] before and after exercise (Mean ± SD)

	Total (n = 10)	Men (n = 5)	Women (n = 5)
Plasma			
Pre-exercise	3.50 ± 0.33	3.44 ± 0.38	3.56 ± 0.30
Post-exercise	3.49 ± 0.37	p = 0.84	3.37 ± 0.48	p = 0.59	3.61 ± 0.20	p = 0.63
Serum			
Pre-exercise	3.49 ± 0.33	3.47 ± 0.43	3.51 ± 0.22
Post-exercise	3.55 ± 0.36	p = 0.44	3.47 ± 0.44	p = 0.95	3.62 ± 0.29	p = 0.43

[a)] Log-transformed

DISCUSSION

The strength of this study is the use of the V-slope method for AT quantification, which has been considered the gold standard.[13] Since gender, habitual exerciser, or other factors influenced AT, it varies widely from individual to individual. Precise AT determination is thus thought to be critical to maintain the constant aerobic workload under a specifically controlled metabolic condition.

We originally hypothesized that the concentrations of circulating NO_x, reflecting the post-exercise increase in NO release from the endothelium, increase immediately after the test. This hypothesis was advanced in connection with a previous report suggesting an attenuated vaso-constrictor response in Sprague-Dawley rats after acute exercise.[9] Contrary to our expectations, however, our subjects showed no acute effects of aerobic exercise on either their serum or plasma NO_x concentrations. This result agrees with the previous study by Gonzales *et al.* indicating a lack of any significant pre- to post-exercise variation in NO production.[10] Nevertheless, how the endothelium-derived NO release was reflected in saliva samples has been called into question.

The result of the present study suggested that the presumed acute post-exercise augmentation of vasodilation is not mediated by an increase in the NO release from the endothelium. However, our subjects were not examined for exercise-induced changes in vasculocirculatory markers such as BP, which might be correlated with NO_x concentrations. In line with the conclusion of Bennett *et al.*, we considered that the exercise-induced BP response was less prominent in normotensive subjects due to their greater ability to cope with interventions likely to cause a BP reduction.[6] One explanation of the unexpected results of our study is that the workload was metabolically insufficient to affect the endothelial function due to the high coping capability of our healthy young subjects. Similar studies with the inclusion of generationally or medically diversified populations are warranted to draw more definitive conclusions.

REFERENCES

1) Roberts CK, Barnard RJ. Effects of exercise and diet on chronic disease. *J Appl Physiol*, 2005; 98: 3–30.
2) Blair SN, Goodyear NN, Gibbons LW, Cooper KH. Physical fitness and incidence of hypertension in healthy normotensive men and women. *JAMA*, 1984; 252: 487–490.
3) Wang J, Wolin MS, Hintze TH. Chronic exercise enhances endothelium-mediated dilation of epicardial coronary artery in conscious dogs. *Circ Res*, 1993; 73: 829–838.
4) Delp MD, McAllister RM, Laughlin MH. Exercise training alters endothelium-dependent vasoreactivity of rat abdominal aorta. *J Appl Physiol*, 1993; 75: 1354–1363.
5) Higashi Y, Sasaki S, Kurisu S, Yoshimizu A, Sasaki N, Matsuura H, Kajiyama G, Oshima T. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation*, 1999; 100: 1194–1202.
6) Bennett T, Wilcox RG, Macdonald IA. Post-exercise reduction of blood pressure in hypertensive men is not due to acute impairment of baroreflex function. *Clin Sci*, 1984; 67: 97–103.
7) Triggle CR, Howarth A, Cheng ZJ, Ding H. Twenty-five years since the discovery of endothelium-derived relaxing factor (EDRF): does a dysfunctional endothelium contribute to the development of type 2 diabetes? *Can J Physiol Pharmacol*, 2005; 83: 681–700.
8) Kimura T, Yokoyama T, Matsumura Y, Yoshiike N, Date C, Muramatsu M, Tanaka H. NOS3 genotype-dependent correlation between blood pressure and physical activity. *Hypertension*, 2003; 41: 355–360.
9) Patil RD, DiCarlo SE, Collins HL. Acute exercise enhances nitric oxide modulation of vascular response to phenylephrine. *Am J Physiol*, 1993; 265: H1184–H1188.
10) Gonzalez D, Marquina R, Rondon N, Rodriguez-Malaver A, Reyes R. Effects of aerobic exercise on nitric oxide, uric acid, antioxidant activity and oxidative stress in human saliva. *Med Sci Sports Exerc*, 2007; 39: S354.

11) Borg G. Psychophysical scaling with applications in physical work and the perception of exertion. *Scand J Work Environ Health*, 1990; 16: 55–58.
12) Wassermann K, McIlroy MB. Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *Am J Cardiol*, 1964; 14: 844–852.
13) Higa MN, Silva E, Neves VF, Catai AM, Gallo Jr L, Silva de Sa MF. Comparison of anaerobic threshold determined by visual and mathematical methods in healthy women. *Braz J Med Biol Res*, 2007; 40: 501–508.
14) Kitaichi K, Wang L, Takagi K, Iwase M, Shibata E, Nadai M, Hasegawa T. Decreased antipyrine clearance following endotoxin administration: in vivo evidence of the role of nitric oxide. *Antimicrob Agents Chemother*, 1999; 43: 2697–2701.