Impaired Endothelium-Dependent and -Independent Vasodilation in Young Female Athletes With Exercise-Associated Amenorrhea

To the Editor:

Estrogen has great roles in controlling vascular function.\(^1\) Especially, estrogen augments endothelium-dependent vasodilation by increasing the bioavailability of endothelium-derived nitric oxide.\(^2\) Not only does estrogen replacement improve endothelium-dependent vasodilation in postmenopausal women,\(^3\) but also endothelium-dependent vasodilation is enhanced by endogenous estradiol during the menstrual cycle in women.\(^4\) Exercise-associated amenorrhea (EAA) is observed in highly-trained female athletes.\(^5\) Although physical exercise training usually improves vascular function in subjects with cardiovascular risk factors,\(^6\) it is unknown whether such strenuous sport activity that causes amenorrhea affects vascular function.

Flow-mediated vasodilation (FMD) during reactive hyperemia can be quantitated as an index of endothelium-dependent vascular function.\(^7\) FMD was shown to be reduced in amenorrheic athletes,\(^8\) however it was unclear whether the abnormality of FMD was related to estrogen levels. Accordingly, we investigated vascular function in young highly trained athletes with and without EAA, and measured endogenous ovarian hormones to explore the underlying mechanisms of endothelial dysfunction in amenorrheic athletes. We also examined whether vascular function was restored by the recovery of the regular menstrual cycle after quitting competitive sport activity.

We enrolled 26 young female highly-trained volleyball players (mean age, 16.4 years) including athletes with a regular menstrual cycle (RMC, n=14) and those with EAA (n=12). Age-matched female non-athletes with a regular menstrual cycle were enrolled as controls (n=10). The players participated regularly in exercise training 6 days a week and all lived in their school dormitory. None of the subjects had cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes mellitus, and smoking. None of the subjects had primary amenorrhea. Blood sampling was performed on the day of the ultrasound study to measure biochemical parameters such as serum lipid profiles and ovarian hormones. In subjects with a regular menstrual cycle, all measurements were performed in the follicular phase or the luteal phase. The protocol was approved by the institutional ethic committee, and written informed consent was obtained from all subjects.

Vasodilatory responses of the right brachial artery were evaluated by measuring endothelium-dependent FMD and glyceryltrinitrate (GTN, an endothelium-independent dilator)-induced vasodilation using high-resolution ultrasonographic equipment with a 12-MHz transducer (Acuson Sequoia 512) as described previously.\(^8\) The pulse wave profile of blood flow was recorded, and then blood flow was calculated by multiplying the arterial cross-sectional area by the Doppler velocity. In this study, the intraobserver and interobserver variability (n=6) for measurements of baseline brachial artery diameter was 0.04±0.04 and 0.06±0.05 mm, respectively. In 7 athletes with EAA who had recovered regular menstrual cycle after quitting competitive sport activity, vascular reactivity and hormones were measured as described above. Data were presented as the mean value±SD. Multiple comparisons were performed by repeated-measures ANOVA. The relationship between two variables was evaluated by use of linear regression analysis. In follow-up data, statistical comparisons between the groups were performed by the paired Student t test. Differences were considered statistically significant at P<0.05.

In biochemical parameters, serum lipid profiles were similar among the three groups. Serum estradiol levels were similar between controls and RMC (65.8±37.7 and 73.0±48.0 pg/mL, P=NS), but they were significantly lower in EAA (32.1±19.9 pg/mL) than in RMC (P<0.05). Serum progesterone levels did not differ among the three groups. In hemodynamic parameters and endothelial function, percent increases in brachial artery blood flow during hyperemia and after GTN administration did not differ among the three groups. The brachial artery diameter at baseline was significantly larger in the athletes than in controls, but did not differ between RMC and EAA. Both FMD and GTN-induced vasodilation were similar between controls and RMC. In contrast, both FMD and GTN-induced vasodilation were significantly less in EAA than in controls, more predominantly for FMD (Figure A and B). FMD (r=0.50, P<0.005) and GTN-induced vasodilation (r=0.36, P<0.05) were significantly correlated with serum estradiol levels but not with serum progesterone levels.

During the follow-up periods of 2.5±0.9 months, serum levels of estradiol after the recovery of a regular menstrual cycle significantly returned to the control levels (from 30.7±16.2 to 48.8±15.6 pg/mL, P<0.05). Serum levels of progesterone did not change (from 0.53±0.49 to 1.07±1.12 ng/mL, P=NS). Both FMD and GTN-induced vasodilation after the recovery of a regular menstrual cycle were significantly restored (Figure C and D).

In general, FMD is expressed as percent change in the post-stimulus diameter compared with the baseline diameter. However, it is well known that a larger baseline diameter yields a smaller measure of percent change, whereas smaller arteries appear to dilate relatively more than do larger arteries.\(^7\) In this study, because the baseline diameter of brachial artery was larger in athletes than in controls, the vascular reactivity was assessed using absolute change in the diameter.

Exercise training augments blood flow and shear stress, resulting in enhanced FMD with an increased nitric oxide production.\(^9\) In this study, however, FMD was similar between controls and athletes with normal menstrual cycle, thus indicating that exercise itself does not alter endothelial function in young healthy athletes. The most likely explanation is that in young females with normal endothelial function, exercise training may not further augment endothelial function. In this study, the impaired endothelial function in EAA was not attributable to the risk factors of atherosclerosis. Furthermore, the impaired endothelial function in EAA was not attributable to exercise training per se because the athlete groups were trained at a similar frequency and intensity of exercise.

Flow-mediated vasodilation (A) and GTN-induced vasodilation (B) in controls, athletes with RMC, and athletes with EAA, and those (C and D) before and after the recovery of a regular menstrual cycle. Note that endothelium-dependent and -independent vasodilation were impaired in athletes with EAA and were improved after the recovery of the regular menstrual cycle. *P<0.001 vs controls; †P<0.01 vs RMC.
In this study, both FMD- and GTN-induced vasodilation in EAA were less than those in controls, indicating impairment of endothelium-dependent and -independent vasodilation. The ratio of FMD- to GTN-induced vasodilation was greater in RMC than in EAA. These findings indicate that in amenorrheic athletes endothelium-dependent vasodilation may be dominantly impaired compared with endothelium-independent vasodilation. To further examine the underlying mechanisms of impaired vascular function in amenorrheic athletes, we measured serum concentrations of endogenous ovarian hormones. In our study, lower levels of serum estradiol were noted in EAA, and the estradiol levels were correlated with vascular function. These findings suggest the impaired vascular function in amenorrheic athletes is attributable to the hypoestrogenic state. This was further supported by the findings of restored vascular function associated with increased serum estrogen levels after quitting strenuous sport activity.

Because the impairment of endothelial function is an initial step in the development of atherosclerosis, further studies may be needed to investigate whether strenuous training in association with amenorrhea would predispose to higher cardiovascular events in their late stage of life.

In conclusion, the present study provides the first demonstration that strenuous exercise training that relates amenorrhea may impair endothelium-dependent and -independent vasodilation by hypoestrogenic state. However, quitting competitive sports activity can quickly restore vascular functions by increasing levels of estrogen.

Noriko Yoshida  
Institute of Health and Sports Sciences  
Kurume University, Japan

Hisao Ikeda  
Department of Internal Medicine III  
Kurume University School of Medicine, Japan

Kenzo Sugi  
Division of Cardiology  
Sugi Hospital, Omuta, Japan

Tsumotu Imaizumi  
Department of Internal Medicine III  
Kurume University School of Medicine, Japan

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Noriko Yoshida, Hisao Ikeda, Kenzo Sugi and Tsutomu Imaizumi

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