Acute Effect of Whole-Body Periodic Acceleration on Brachial Flow-Mediated Vasodilatation Assessed by a Novel Semi-Automatic Vessel Chasing UNEXEF18G System

Bonpei Takase, MD[1], Hidemi Hattori, PhD[2], Yoshihiro Tanaka, MD[2], Akimi Uehata, MD[3], Masayoshi Nagata, MD[4], Masayuki Ishihara, PhD[2] and Masatoshi Fujita, MD[5]

[1]Department of Intensive Care Medicine, [2]Division of Biomedical Engineering, National Defense Medical College, National Defense Medical College Research Institute, Saitama, Japan
[3]Self Defense Forces Central Hospital, Tokyo, Japan
[4]Iruma Heart Hospital, Saitama, Japan
[5]Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan

Background: Repeated application of whole-body periodic acceleration (WBPA) upregulates endothelial nitric oxide synthase and improves brachial artery endothelial function (BAEF) as assessed by measurement of flow-mediated vasodilatation (FMD). However, the acute effect of a single application of WBPA on BAEF has not been fully characterized. In addition, although a novel semi-automatic vessel chasing system (UNEXEF18G) has now been developed in Japan, the direct comparison of UNEXEF18G with a conventional method for FMD measures has not been conducted even if UNEXEF18G has already been utilized in a relatively large scale study.
Methods: We have developed a novel semi-automatic vessel chasing system (UNEXEF18G) that can measure FMD on-line, identify time to peak vasodilatation (TPV), and determine the area under the vasodilatation curve (AUC). Thus, 45 min of WBPA was applied in 20 healthy volunteers (age, 34 ± 13 years), and BAEF was measured by UNEXEF18G before and after WBPA. Also, UNEXEF18G measured FMD was compared with those of a conventional FMD measurement method at rest in order to validate a novel UNEXEF18G measured FMD.
Results: Single WBPA resulted in a significant increase in FMD (from 6.4 ± 3.4 to 10.7 ± 4.3%, $p < 0.01$), a significant decrease in TPV and a significant increase in AUC. In the validation study for UNEXEF18G, Bland and Altman analysis showed that UNEXEF18G measured FMD was almost identical to those of the conventional method at rest.
Conclusion: These data suggest the usefulness of a new UNEXEF18G and that single application of WBPA results in acute improvement in BAEF in humans.

KEY WORDS: Cardiac rehabilitation · Endothelial function · Nitric oxide · Brachial artery.

INTRODUCTION

Despite significant advance in medical treatments, atherosclerotic disorders, such as stroke and acute coronary syndrome, are still leading causes of mortality in developed countries. The pathophysiology of these catastrophic events involves impaired endothelial function and increase inflammation and coagulation activity.[1)2)] Endogenous nitric oxide (NO) production, which mediated endothelial dependent vasodilatation, is a key mediator of these changes in endothelial function, inflammation and coagulation.[3-5)]

Repeated application of whole-body periodic acceleration (WBPA) upregulates NO synthase in animals and improves brachial artery endothelial function in humans, as measured by brachial artery flow-mediated vasodilatation (FMD).[6] Recent reports suggest that single application of WBPA can result in improved coronary microcirculation.[7] However, the acute effect of single application of WBPA on brachial artery endothelial function in humans has not been fully characterized.

We have developed a novel semi-automatic vessel chasing system (UNEXEF18G; UNEX Corporation, Nagoya, Japan) identify time to peak vasodilatation, and determine the area under the vasodilatation curve.[8)9)] UNEXEF18G can easily identify the time to peak vasodilatation as well as the area under the vasodilatation curve. The area under the vasodilatation curve is a more accurate assessment of brachial artery endothelial function than FMD alone and may be a good quantitative reflection of NO production. In addition, although a novel UNEXEF18G has now been developed in Japan,[9] the direct comparison of UNEXEF18G with a conventional method for FMD measures has not been conducted even if UNEXEF18G has already been utilized in a relatively large scale study.[8)9)]

Thus, the purpose of this study was to: 1) investigate the acute effect of single application of WBPA on brachial artery endothelial function in humans, and 2) to validate UNEXEF18G by comparing UNEXEF18G with conventional methods for measurement of brachial artery endothelial function. For this purpose, 1) we performed 45 min of WBPA in healthy volunteers, and 2) we compared the results of UNEXEF18G with that of conventional measures of brachial artery endothelial function at rest in same time in random conditions.

METHODS

STUDY POPULATION

The study population consisted of 20 healthy volunteers (12 men, 8 women; age, 34 ± 12 years). Twelve of these subjects had at least one of Framingham's coronary risk factors, including cigarette use (n = 6), hyperlipidemia (n = 2), family history of coronary artery disease (n = 2), and obesity with borderline diabetes mellitus (n = 1). None of the subjects were receiving any medications for their conditions.

All subjects were enrolled in Iruma Heart Hospital and all studies were conducted also in Iruma Heart Hospital. However, all analyses were performed in National Defense Medical College.

Fourteen non-smoking subjects (7 men, 7 women) from the above 20 subjects were also used for the UNEXEF18G validation study.

STUDY PROTOCOL

This was a random crossover study protocol (Fig. 1 and 2). All studies were done from 14:00 to 17:00 in a temperature-controlled room (25°C) with the subject in a fasting, resting,

and supine state. After at least 30 min of bed rest, subjects underwent 45-min of WBPA or 45 min of bed rest (control). WBPA was performed according to a protocol described previously;[6] in brief, periodic acceleration was applied with the motion platform (Fig. 2) at a frequency of 2-3 Hz with approximately ± 0.25 g for 45 min. Before and after the intervention (WBPA or bed rest), blood pressure was measured by Korotkoff's method with the patient in the supine position. CM5 lead electrocardiogram was continuously monitored, and heart rate was determined at the time of blood pressure measurements. Following their assigned intervention (WBPA or bed rest), patients crossed-over to receive the other intervention, and experimental parameters were measured in the same fashion (Fig. 1 and 2).

For the UNEXEF18G validation study, UNEXEF18G FMD measurement and conventional FMD measurement were performed in a random order, only at rest. Since the simultaneous measures of FMD are difficult because once 5-minutes occlusion-induced ischemia precludes the continuous measurement of FMD, the effect of WBPA on FMD were evaluated by UNEXEF18G system only. The validation of measurement system between UNEXEF18G and conventional method was performed at baseline only with random conditions. And two measures of FMD by different method were conducted about 30 min interval that is empirically confirmed to be enough time periods to cancel the effect of 5 min ischemia on FMD values.
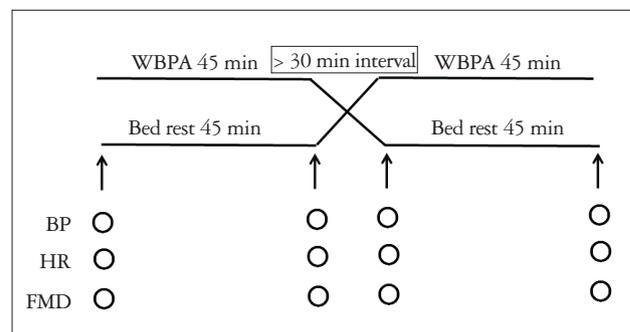

Fig. 1. Study protocol (random cross-over). WBPA: whole-body periodic acceleration, BP: blood pressure, HR: heart rate, FMD: flow-mediated vasodilatation.
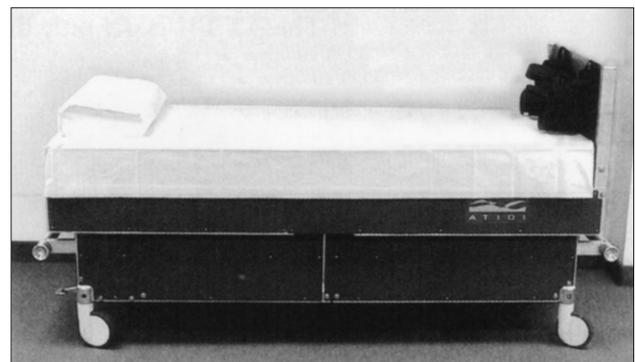

Fig. 2. Whole-body periodic acceleration platform (bed).

In addition, FMD values are easily changed by the measurement time during a day and the meals taken before the test, suggested by published FMD measurement standard,[1)2)] so that the comparison of two measurement methods was conducted only one point at rest with random measurement times of the day and conditions.

Ultrasound FMD measurements in the brachial artery by UNEXEF18G

Except validation studies, all ultrasound studies were performed from 14:00 to 17:00 in a temperature-controlled room (25°C) with the subject in a fasting, resting, and supine state. Heavy meals, including a high-fat diet and caffeine-contain-
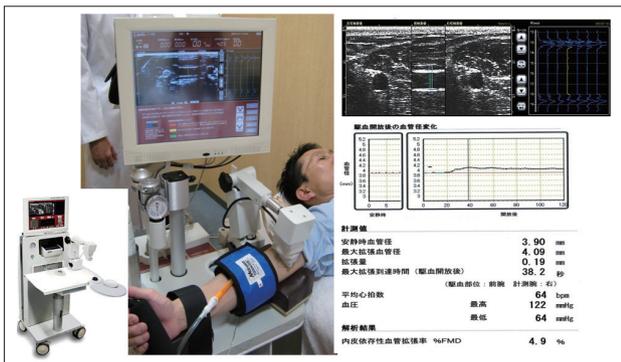

Fig. 3. Novel equipment for measurement of FMD (endothelial function; UNEXEF18G; UNEX, Nagoya, Japan; Semi-automatic). Right middle to lower portion; Japanese letters were contained because of simply showing original view of panel. FMD: flow-mediated vasodilatation.
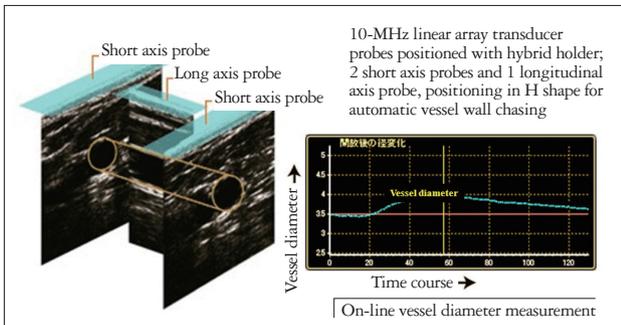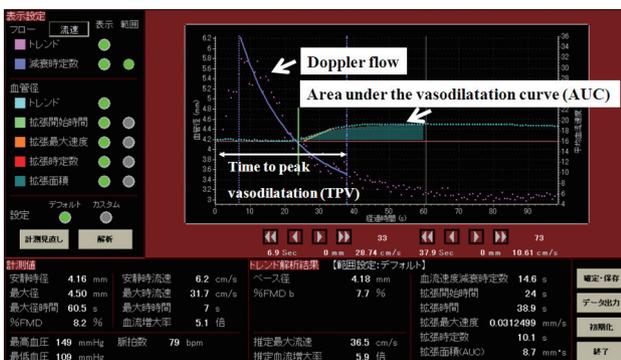

Fig. 4. UNEXEF18G probe.

Fig. 5. UNEXEF18G analysis software. Japanese letters were contained because of simply showing original view of panel.

ing beverages, were prohibited beginning the night before the study. Patients were not allowed to have lunch on the day of ultrasound study. Blood pressure (BP) and heart rate were recorded from the left arm every 3 minutes with an automatic sphygmomanometer (Nihon Korin, BP-203, Tokyo, Japan) during the ultrasound procedure. Vasodilatation responses of the brachial artery were determined by the ultrasound technique using a semi-automatic device (UNEXEF18G; UNEX, Nagoya, Japan) (Fig. 3-5). Briefly, the diameter of the brachial artery was measured from B-mode ultrasound images using a 10-MHz linear array transducer. Then, a BP cuff was inflated to 50 mmHg above the systolic BP over the proximal portion of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring the brachial artery diameter. The changes in the diastolic diameter were continuously recorded. Then, FMD was determined as the maximum change in diameter after cuff release normalized to the baseline diameter (% of baseline diameter). The novel UNEXEF18G equipment consisted of three ultrasound probes with an appropriately flexible stabilizing arm that supports and fixes the ultrasound probes to the human arm. There were two H-shaped ultrasound probes and one longitudinal ultrasound probe (Fig. 4), which enabled the technician to easily track target brachial artery images during the FMD study. In addition, this machine has specially designed software that can measure FMD on-line, identify time to peak vasodilatation, and determine the area under the vasodilatation curve (Fig. 5). In details, as shown in right upper portion of Fig. 3, UNEXEF18G identifies both the intima media and the main body of brachial artery vessel walls of both near and far wall of the brachial artery as A-mode ultrasound signals. The signals of either intima media or the main body of brachial artery vessel walls can be re-selected, if necessary, by operators. These signals are automatically chasing and being recorded continuously to obtain the maximum vasodilatation of brachial artery after the reactive hyperemia. By using manual techniques, the interpreters or the operators can modify or re-selected the appropriate measuring points on either the intima media or the main signals of the brachial artery vessel walls on both near and far wall images. All measurements are automatically performed by sophisticated software installed in UNEXEF18G in order to indentify the changes in the vessel diameter, the values of FMD, the time to peak vasodilatation and the area under the vasodilatation curve.

Ultrasound FMD measurements in the brachial artery using the conventional method

All studies were performed according to a previously reported method.[10)11)] 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, MA, 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-degree 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 + 50 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 sec. Two-dimensional images of the brachial artery were obtained for 60 sec 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.[11] The flow volume was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for the angle and 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 sec 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 reproducibly select the images at the same point in the cardiac cycle, images at peak diastole (maximum dilation, close to the R 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, MA, USA) containing a digitizing board was used for these measurements. For each condition (baseline, reactive hyperemia at 60 sec after cuff de-

flation), 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 performing 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.

Comparison of calculations with the UNEXEF18G and the conventional method in our laboratory showed that the intra- and inter-observer variabilities (coefficient of variation) for repeated measures of diameter before and after reactive hyperemia in the brachial artery were < 3%.[10)11]

STATISTICAL ANALYSIS

Data are expressed as the mean ± SD. The paired Student's t-test was used to compare data before and after each treatment. To examine the repeatability of FMD measurements by the UNEXEF18G or the conventional method, we used the method proposed by Bland and Altman,[12] which analyses the plot of the differences in the parameter values between the two FMD measurements against their means. A coefficient of repeatability was then computed as twice the standard deviation of the differences.[13] Differences or statistical values were considered significant at $p < 0.05$. Even if the sample size was small, the histogram of each sample were not skewed (data not shown) so that we presumed each samples in this study are drawn from normally distributed data. Parametric statistical methods were subsequently utilized.

RESULTS

The effects of either 45-min of WBPA or 45-min of bed rest on hemodynamics and FMD parameters are summarized in Table 1. There was a significant decrease in heart rate following 45-min of WBPA when compared with that following 45-min of control bed rest. Three parameters of FMD significantly changed in response to 45-min of WBPA but not to 45-min of bed rest. Specifically, 45-min of WBPA resulted in

Table 1. Ultrasound study measurements of flow-mediated vasodilatation in the brachial artery, and effect of WBPA or bed rest

	Control phase (n = 20)		WBPA phase (n =20)	
	Pre procedure	Post procedure	Pre procedure	Post procedure
Systolic BP (mmHg)	112 ± 11	110 ± 11	112 ± 11	111 ± 10
Diastolic BP (mmHg)	69 ± 9	65 ± 7	68 ± 9	66 ± 8
Heart rate (beats/min)	66 ± 10	63 ± 9	66 ± 10	61 ± 8*
Brachial artery diameter at baseline (mm)	3.80 ± 0.45	3.81 ± 0.44	3.83 ± 0.46	3.71 ± 0.48
FMD (%)	6.60 ± 3.46	7.52 ± 2.98	6.43 ± 3.44	10.67 ± 4.34*
Time to PD (s)	66 ± 23	60 ± 25	67 ± 22	49 ± 21*
AU-FMD (s.%)	5.2 ± 4.1	7.1 ± 6.9	4.5 ± 4.2	10.2 ± 11.6*

Data are mean ± SD. *$p < 0.05$ vs. pre procedure. WBPA: whole-body periodic acceleration, BP: blood pressure, FMD: flow-mediated vasodilatation, PD: peak dilation, AU-FMD: area under the FMD, s.%: second times percent

a significant increase in FMD, a significant decrease in time to peak vasodilatation and a significant increase in the area under the vasodilatation curve (Table 1).

As shown in Fig. 6 and 7, 17 of 20 subjects experienced an increase in FMD and a decrease in the time to peak vasodilatation in response to WBPA. The three other subjects experienced a decrease in FMD and an increase in time to peak vasodilatation. Changes in the area under the vasodilatation curve and the shortening of the time to peak vasodilatation have similar clinical significance to increased FMD values (Table 1). Two of these subjects did not have any risk factors for atherosclerosis, and the remaining patient was a smoker. However, the decrement in FMD and the increment in the time to peak vasodilatation in response to WBPA were relatively small. No unique clinical characteristics were identified among these subjects.

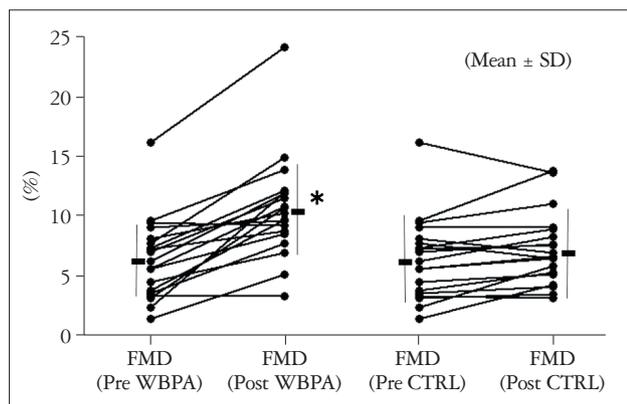The repeatability of the UNEXEF18G measurements, as

Fig. 6. Effect of 45-min of WBPA or bed rest on FMD. *$p < 0.05$ vs. Pre. WBPA: whole-body periodic acceleration, FMD: flow-mediated vasodilatation, Pre WBPA: before 45 min of whole-body periodic acceleration, Post WBPA: after 45 min of whole-body periodic acceleration, Pre CTRL: 45 min of bed rest, Post CTRL: after 45 min of bed rest, Pre: before.

Fig. 7. Effect of 45 min of WBPA or 45 min of bed rest on time to PD. *$p < 0.05$ vs. Pre. WBPA: whole-body periodic acceleration, PD: peak vasodilatation, TPD: time to peak vasodilatation, Pre WBPA: before 45 min of whole-body periodic acceleration, Post WBPA: after 45 min of whole-body periodic acceleration, Pre CTRL: 45 min of bed rest, Post CTRL: after 45 min of bed rest, Pre: before.
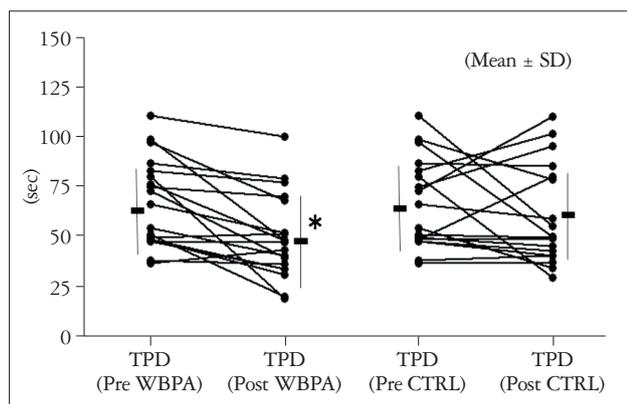
assessed by Bland and Altman's method as well as by calculation of the correlation coefficient, deemed to be adequate, indicating that UNEXEF18G could replace the conventional method for measuring FMD (Fig. 8). UNEXEF18G was easy to use. Parameters other than FMD, such as the time to peak and the area under the vasodilatation curve, were adequately measured by the UNEXEF18G. In addition, online detection of the diameter changes after reactive hyperemia enabled easy and precise detection of the peak brachial vasodilatation easily (Fig. 3-5).

DISCUSSION

The present study demonstrated that single application of WBPA improved brachial artery endothelial function in humans and that the UNEXEF18G, a novel apparatus for measurement of FMD, was valid for clinical use in healthy subjects when compared with a conventional method. WBPA resulted in a significant increase in FMD, a significant decrease in time to peak brachial artery vasodilatation, and a significant increase in area under the vasodilatation curve. The area under the vasodilatation curve and the time to peak brachial artery vasodilatation have recently been proposed as new indices for the assessment of brachial artery endothelial function.[14-16] The clinical application of FMD as a marker of endothelial function was described more than two decades ago,[17] and clinical guidelines for the use of FMD have been published.[18] However, the wide intra- and inter-variability of FMD measurement between difference institutions and investigators has limited its utility in the clinical assessment of endothelial function.[18] Recently, a novel apparatus (UNEXEF18G) has been developed for measurement of brachial FMD and has been used widely in Japan for the clinical assessment of endothelial function.[8)9)] The present study directly compared the UNEXEF18G to conventional methods for the measurement of brachial FMD. Our group has been involved in brachial FMD studies since the early phase, and one of our investigators (A.U.) was involved in development of FMD measurement methods[19] and underwent training in the US for the measurement of FMD more than 20 years ago.[20] Thus, our conventional technique for measuring FMD is considered to be the standard. However, the present study showed that the UNEXEF18G produced adequate measurement of endothelial function in humans, as demonstrated by calculation of the correlation coefficient and by Bland and Altman plotting.

The present study demonstrated that single application of WBPA resulted in acute improvement in brachial endothelial function in normal healthy volunteers with Framingham risk factors for atherosclerotic disease. This observation is consistent with data from previous reports, in which repeated application of WBPA produced beneficial effects in normal subjects and cardiovascular patients.[6] However, those previous reports did not clearly characterize the acute effect of WBPA on FMD. In one report, the acute effect of WBPA resulted in
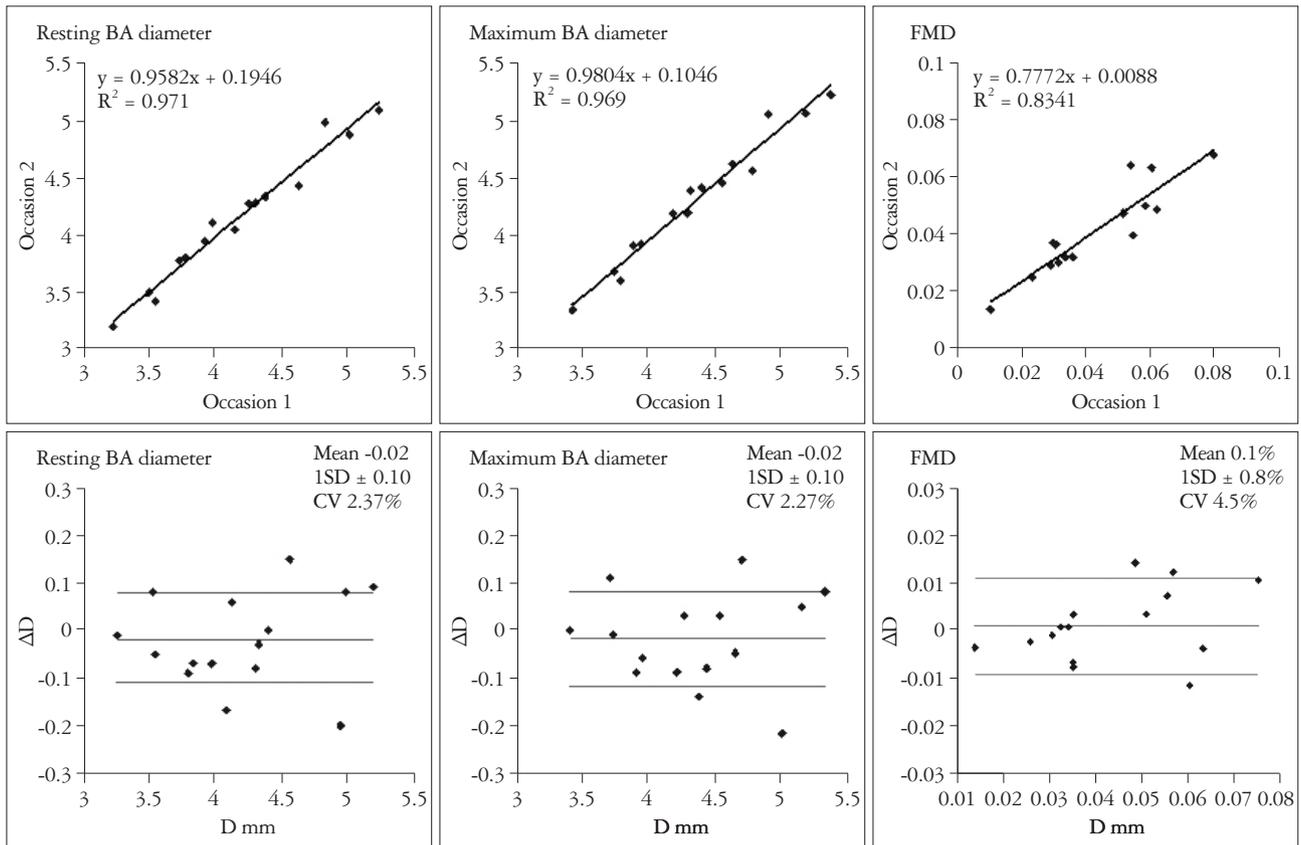
Fig. 8. Reproducibility of UNEXEF18G for measurement of FMD (14 control subjects). FMD: flow-mediated vasodilatation, BA: brachial artery, D: diameter.

improvements in coronary flow, as assessed by cardiac ultrasound,[7] which is consistent with observations from the present study. Further, the present study showed that single application of WBPA resulted in an acute decrease in the time to peak vasodilatation, which may reflect improved endothelial function. A previous study reported that decreased time to peak vasodilatation in the brachial artery could be used as a marker of brachial endothelial function.[16] In terms of the effect of WBPA on area under vasodilatation curve, father study is needed to clarify the clinical significance of this finding. Fortunately, the UNEXEF18G apparatus can automatically calculate the area under the FMD, and UNEXEF18G is likely to be employed in more than 400 institutions in Japan by the end of 2012 (data from UNEX, Nagoya, Japan).

The observation that WBPA may improve endothelial function suggests that in may improve cardiovascular outcome by increasing production of NO. Thus, both single and repeated application of WBPA may have clinical utility in patients with cardiovascular disease. The present study used the forearm occlusion method (rather than upper arm) for measurements of FMD. Thus, the FMD value likely reflects the production of NO in the brachial artery.[18] NO is a direct vasodilator and has anti-atherosclerotic,[21-24] anti-inflammatory and anti-tumorigenic actions.[25] In addition, FMD or shear stress resulted in production of NO by the brachial arterial endothelium as well

as increases prostacyclin and tissue plasminogen activator, which also affect vasomotor tone, fibrinolysis and coagulation.[3-5)26] All of these effects are closely related to the pathogenesis of atherosclerosis.

In addition, WBPA significantly decreased resting values of heart rate as shown in Table 1. As we have presented,[27] WBPA increases high frequency power of heart rate variability. We presume WBPA can possibly increase cardiac vagal autonomic activity and subsequently decreased heart rate following 45-min of WBPA compared with control arm.

This study has several limitations. First, the number of the subjects was small, and the study was conducted and analyzed in only two medical centers, including one academic medical school hospital. Although the control and crossover design of this study enhances the validity of our results despite the small subject numbers, this study should be duplicated with a multi-center randomized clinical trial design. Second, we assessed the acute effect of WBPA only in normal healthy subjects, even if most of the subjects had Framingham risk factors for atherosclerotic disease. Thus, future study should determine the acute effects of WBPA in patients with established cardiovascular disease.

In conclusion, these data suggest that single application of WBPA results in acute improvement in brachial artery endothelial function in humans and that the novel UNEXEF18G

apparatus is valid for clinical measurement of FMD.

REFERENCES

1. Philpott AC, Lonn E, Title LM, Verma S, Buithieu J, Charbonneau F, Anderson TJ. *Comparison of new measures of vascular function to flow mediated dilatation as a measure of cardiovascular risk factors. Am J Cardiol 2009;103:1610-5.*

2. Reriani MK, Lerman LO, Lerman A. *Endothelial function as a functional expression of cardiovascular risk factors. Biomark Med 2010;4:351-60.*

3. Hambrecht R, Adams V, Erbs S, Linke A, Kränkel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, Schuler G. *Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. Circulation 2003;107:3152-8.*

4. Kang JL, Park W, Pack IS, Lee HS, Kim MJ, Lim CM, Koh Y. *Inhaled nitric oxide attenuates acute lung injury via inhibition of nuclear factor-kappa B and inflammation. J Appl Physiol 2002;92:795-801.*

5. Pipili-Synetos E, Papageorgiou A, Sakkoula E, Sotiropoulou G, Fotsis T, Karakiulakis G, Maragoudakis ME. *Inhibition of angiogenesis, tumour growth and metastasis by the NO-releasing vasodilators, isosorbide mononitrate and dinitrate. Br J Pharmacol 1995;116:1829-34.*

6. Sackner MA, Gummels E, Adams JA. *Nitric oxide is released into circulation with whole-body, periodic acceleration. Chest 2005;127:30-9.*

7. Fukuda S, Shimada K, Kawasaki T, Kono Y, Jissho S, Taguchi H, Maeda K, Yoshiyama M, Fujita M, Yoshikawa J. *"Passive exercise" using whole body periodic acceleration: effects on coronary microcirculation. Am Heart J 2010;159:620-6.*

8. Tomiyama H, Matsumoto C, Yamada J, Teramoto T, Abe K, Ohta H, Kiso Y, Kawauchi T, Yamashina A. *The relationships of cardiovascular disease risk factors to flow-mediated dilatation in Japanese subjects free of cardiovascular disease. Hypertens Res 2008;31:2019-25.*

9. Tomiyama H, Kohro T, Higashi Y, Takase B, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H, Yamashina A. *A multicenter study design to assess the clinical usefulness of semi-automatic measurement of flow-mediated vasodilatation of the brachial artery. Int Heart J 2012;53: 170-5.*

10. Hamabe A, Takase B, Uehata A, Kurita A, Ohsuzu F, Tamai S. *Impaired endothelium-dependent vasodilation in the brachial artery in variant angina pectoris and the effect of intravenous administration of vitamin C. Am J Cardiol 2001;87:1154-9.*

11. Takase B, Akima T, Uehata A, Ohsuzu F, Kurita A. *Effect of chronic stress and sleep deprivation on both flow-mediated dilation in the brachial artery and the intracellular magnesium level in humans. Clin Cardiol 2004; 27:223-7.*

12. Bland JM, Altman DG. *Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-10.*

13. Bland JM, Altman DG. *Agreed statistics: measurement method comparison. Anesthesiology 2012;116:182-5.*

14. Padilla J, Johnson BD, Newcomer SC, Wilhite DP, Mickleborough TD, Fly AD, Mather KJ, Wallace JP. *Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. Cardiovasc Ultrasound 2008;6:44.*

15. Peretz A, Leotta DF, Sullivan JH, Trenga CA, Sands FN, Aulet MR, Paun M, Gill EA, Kaufman JD. *Flow mediated dilation of the brachial artery: an investigation of methods requiring further standardization. BMC Cardiovasc Disord 2007;7:11.*

16. Black MA, Cable NT, Thijssen DH, Green DJ. *Importance of measuring the time course of flow-mediated dilatation in humans. Hypertension 2008;51:203-10.*

17. Celermajer DS, Sorensen K, Ryalls M, Robinson J, Thomas O, Leonard JV, Deanfield JE. *Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. J Am Coll Cardiol 1993;22:854-8.*

18. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ. *Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011;300:H2-12.*

19. Uehata A, Lieberman EH, Gerhard MD, Anderson TJ, Ganz P, Polak JF, Creager MA, Yeung AC. *Noninvasive assessment of endothelium-dependent flow-mediated dilation of the brachial artery. Vasc Med 1997;2: 87-92.*

20. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, et al. *Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235-41.*

21. Green DJ, Jones H, Thijssen D, Cable NT, Atkinson G. *Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension 2011;57:363-9.*

22. Sessa WC. *Regulation of endothelial derived nitric oxide in health and disease. Mem Inst Oswaldo Cruz 2005;100 Suppl 1:15-8.*

23. Förstermann U, Münzel T. *Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 2006;113:1708-14.*

24. Jones SP, Bolli R. *The ubiquitous role of nitric oxide in cardioprotection. J Mol Cell Cardiol 2006;40:16-23.*

25. Belge C, Massion PB, Pelat M, Balligand JL. *Nitric oxide and the heart: update on new paradigms. Ann N Y Acad Sci 2005;1047:173-82.*

26. Nathan C. *Points of control in inflammation. Nature 2002;420:846-52.*

27. Takase B, Nagata M, Hujita M. *Acute effect of whole-body periodic acceleration on cardiac autonomic function and brachial endothelial function. J Arrhythm 2012;28(Supplement):546.*