Accepted Manuscript

Title Page

Type of manuscript: Regular Articles

Title: Effects of repetition exercise training on vascular endothelial function in healthy men

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ABSTRACT

High-intensity interval training improves the vascular endothelial function better than moderate-intensity continuous training. However, few studies have examined the effects of repetition training consisting of high-intensity exercise, followed by complete rest, on arterial function. We performed this study to investigate the effects of repetition training on the vascular endothelial function determined by flow-mediated vasodilation. Twenty healthy male participants were randomized and stratified into two training groups, one group performed moderate-intensity continuous exercise (n = 10) while another performed repetition exercise (n = 10), both 3 times a week for 6 weeks. Before and after
each training protocol, the vascular endothelial function of the right brachial artery was assessed by the flow-mediated vasodilation. In moderate-intensity continuous training group, flow-mediated vasodilation has changed 9.92 ± 2.45% to 10.58 ± 2.93% (ns). In repetition training group, flow-mediated vasodilation has changed 7.26 ± 1.46% to 8.58 ± 1.22% (p < 0.05). In repetition training, it showed more increase on the vascular endothelial function compared to continuous training. These results suggest the possible effect of repetition training for the prevention of cardiovascular disease.

**Key Words**

repetition training, vascular endothelium, exercise, vasodilation.
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要約：インターバルトレーニングは、持続的トレーニングよりも血管内皮機能を改善することが明らかにされている。一方、高強度運動および完全休息で構成されるレペティショントレーニングが、血管内皮機能に及ぼす効果については十分に検討されていない。本研究は、レペティショントレーニングが血管内皮機能に及ぼす影響について検討することを目的とした。被験者は、運動習慣のない健康な成人男性20名であり、持続的トレーニング群（10名）またはレペティショントレーニング群（10名）に無作為割り当てられた。トレーニング前後に右上腕動脈の血流依存性血管拡張反応を測定した。持続的トレーニング群は9.92±2.45から10.58±2.93%に変化した（n.s.）。レペティショントレーニング群は7.26±1.46%から8.58±1.22%に有意な変化が認められた（p<0.05）。レペティショントレーニングは、持続的トレーニングと比較して、血管内皮機能を改善させ、心血管疾患の予防に寄与できる可能性が示唆された。
Exercise training has been reported to prevent hypertension and arteriosclerosis, and moderate-intensity continuous training (CT) has been recommended to improve arterial stiffness and vascular endothelial function\textsuperscript{1–4}. High intensity interval training (IT) has also been reported to significantly improve pulse-wave velocity (PWV) and blood pressure compared with CT\textsuperscript{5} and significantly increase vascular endothelial function\textsuperscript{6–8}. On the other hand, the effect of repetition training (RT), which repeats high-intensity exercise and complete rest, on vascular endothelial function has not been clarified.

There are two previous studies evaluating the acute effects of repetition exercise on vascular endothelial function in healthy men\textsuperscript{9,10}. The repetition exercise in the previous studies consisted of cycling exercise at maximal oxygen uptake (\( VO_2\text{max} \)) and complete rest, but the time ratio between high-intensity exercise and complete rest is different\textsuperscript{9,10}. According to the study, repetition exercise, which consisted of 20 × 20-sec cycling intervals at 100\% \( VO_2\text{max} \) separated by 40-sec recovery periods at complete rest increased flow-mediated vasodilation (FMD)\textsuperscript{10}. Another studies reported that high-intensity
Exercise training was more effective in reducing cardiometabolic risk (e.g., endurance capacity \(^{11,12}\), insulin sensitivity \(^{13,14}\), blood pressure \(^{15,16}\) and weight/visceral fat loss \(^{11,17}\)) than CT. Previous studies have revealed a strong relationship between vascular function and cardiometabolic risk. Namely, a significant correlation between FMD and \(\text{VO}_{2\text{max}}\) has been evidenced \(^{18}\). It has also been demonstrated that obesity is associated with endothelial dysfunction \(^{19,20}\), and that weight loss is associated with improved FMD \(^{21}\). Therefore, RT may improve vascular endothelial function compared to CT.

The characteristic of RT is that one can perform high-intensity exercise in shorter time than interval exercise, as RT involves repeated exercise with complete rest in between; however, the effect of RT on vascular endothelial function has not been clarified. The RT program in the previous study \(^{10}\) includes a complete rest, so the actual exercise time is about 7 minutes, and is classified as low-volume IT (i.e., <15 min of high-intensity efforts in total during the session) \(^{22,23}\). It also implicated the possibility to lower the load intensity for respiratory and circulatory function compared to that of CT \(^{10}\). AS IT has been reported to be a safe option for high-risk patients \(^{23}\) and in the cardiac
rehabilitation\textsuperscript{24}, RT can also be applied in clinical situations such as rehabilitation. By confirming the effect of RT on vascular endothelial function, it can be proposed as a new effective exercise program to prevent cardiovascular disease that is safe and requires only a short period of time. Thus, this study aimed to examine the effects of RT on endothelial function.

\textbf{Materials and Methods}

\textit{Participants and informed consent.} Twenty-one healthy men were randomized into two exercise intervention groups (RT and CT groups). One participant was withdrawn from the study due to personal reason. The RT group (n=10) participated in a 20-min RT program, while the CT groups (n=10) performed the CT. The anthropometric variables before the intervention were homogeneous between the training groups, and no change in body mass was observed in each group before or after the intervention. (RT group, average age 23.4 ± 4.8 years, average height 174.2 ± 5.2 cm, average body mass 67.0 ± 7.1 kg to 67.6 ± 7.2 kg; CT group, average age 23.7 ± 2.9 years, average height 172.5 ± 5.2 cm, average body mass 69.1 ± 12.3 kg to 68.8 ± 13.3 kg).
All the participants were normotensive, non-obese, and free of chronic disease as assessed via their medical histories, physical examination, and complete blood chemistry and hematological evaluation. And the participants were nonsmokers, did not have regular exercise habits. The study was conducted in accordance with the principles laid down in the declaration of Helsinki and twenty-one healthy men gave their written informed consent to participate in this study, which was performed with approval of the Ethics committee of Tokushima University (#152).

**Experimental design.** Participants performed a maximal exercise load test to confirm eligibility with a bicycle ergometer on the first evaluation day. The physical activity of participants were assessed by the short form of international physical activity questionnaire-short form (IPAQ-SF) before and after the intervention and confirmed to be categories 1 or 2, and it did not change before and after the intervention. Participants were instructed to refrain from exercising other than their allocated training regimen, and all exercises were supervised during the 6-week training period. Similarly, Participants were instructed by an officially registered dietitian to keep their diet as before the
intervention and managed their diet during the whole training period. Considering improvement in physical strength accompanied by the training, a maximum exercise load test has been done again 3 weeks after the start of the training, and the exercise load intensity for each participant was relatively adjusted. The final vascular function test was done 3-5 day after the last training session. All experiments were performed in a laboratory with the settings controlled at a room temperature of 24–26°C and humidity of 50%–70%. All exercises were performed on an electromagnetic cycle ergometer (232C Model, Combi Co., Ltd., Japan).

Training protocol. The training program used an exercise protocol that was confirmed to enhance vascular endothelial function in acute exercise study 10). In the RT group, the participants performed 20 × 20-sec cycling intervals at 100% \( \dot{V}O_2\text{max} \) separated by 40-sec recovery periods at complete rest. In the CT group, the participants performed continuous exercise at 50% \( \dot{V}O_2\text{max} \) intensity. The time duration from the start to the end of each session was 20 min for both groups, and the pedal rotation speed was set at 60 rotations.
Each training frequency and duration were 3 day/week and 6 weeks, respectively. **Brachial artery endothelial function.** The participants were instructed to fast for at least 3 h and to abstain from caffeine for at least 12 h prior to the measurements. The participants were asked to rest in the supine position for 5 min, which was followed by the upper arm systolic/diastolic blood pressure (SBP/DBP) measurement on their left arm by the standard sphygmomanometer. An occlusion cuff was placed around the right forearm and two ECG leads were attached to the wrists for measuring heart rate (HR). Subsequently, the FMD was measured using UNEX EF (UNEX CO. Ltd., Japan), an ultrasound instrument specialized for FMD measurement. A high-resolution ultrasound device with a 10-MHz linear artery transducer was coupled with a computer assisted analysis software (UNEXEF18G, UNEX Co, Nagoya, Japan) that used an automated edge detection system for monitoring the brachial artery (BA) diameter. BA was scanned longitudinally 5–10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces was manually determined, the transducer was held at the
same point throughout the scan by a novel stereotactic probe holder (UNEXEF18G, UNEX Co, Nagoya, Japan) to ensure continuous recordings of the image. The depth and gain were set to optimize images of the arterial lumen wall interface. In this system, the diastolic diameter of the brachial artery was determined semi-automatically and the diastolic diameter of the brachial artery beat was synchronized with the electrocardiographic R-waves and tracked automatically. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. The baseline diameter of brachial artery was recorded for 30 sec, and the occlusion cuff of the right forearm was then inflated to 50 mmHg over the systolic blood pressure value for 5 min. Changes in the diastolic diameter were recorded continuously until 3 min after cuff deflation. FMD was calculated as the percent change in peak brachial artery diameter during reactive hyperemia from the baseline value. FMD was calculated as the percentage change in diameter from the baseline value (Di_{base}) before cuff release to the peak value after cuff release (Di_{peak}). The detailed prescription for measurement is provided in another
The participants were observed at the same time of the day before and after the exercise intervention. Post-training measurement has been done 3-5 days after the last bout of exercise. FMD calculated as:

\[
FMD (\%) = \frac{D_{\text{peak}} - D_{\text{base}}}{D_{\text{base}}} \times 100
\]

All tests were performed by the same examiner simultaneously. To determine intersession reliability of the measurements of \(D_{\text{base}}\) and FMD, the measurement was performed three times in 10 healthy young men. The intraclass correlation coefficient \([\text{ICC} (1.1)]\) for the measurements of \(D_{\text{base}}\) and FMD was 0.97 and 0.90, respectively.

**Maximal test.** Participants performed a maximum load test on an electromagnetic cycle ergometer starting with a 40 Watts load after 3 minutes of rest and gradually increasing by 20 Watts every minute. Participants were instructed to maintain pedaling cadence at 60 revolutions per minute. We presumed maximal oxygen uptake in case at least two of the following criteria met: (i) levelling off of \(\dot{V}O_2\) with an increase in work stage; (ii) measured \(HR_{\text{max}} \geq\) age-predicted \(HR_{\text{max}} (210 - 0.8 \times \text{age})\); (iii) respiratory exchange ratio \(\geq 1.20\); and (iv) ratings of perceived exertion of 19 or 20. For the measurement of
VO$_{2\text{max}}$, the oxygen concentration, carbon dioxide concentration and ventilation volume were analyzed every 30 s using an automatic breath gas analyzer (AR-1 Type-3, ARCO SYSTEM inc., Kashiwa, Japan). HR was measured on an HR monitor (Polar RS100, Polar Electro, Inc., Kempele, Finland).

**Statistical analysis.** Data distribution was confirmed using the Shapiro-Wilk test. To examine the effects of training, we took the two-way analysis of group factors (CT, RT) and time (pre/post training) variant repeatedly. Analyses were performed to test for interaction and main effects. When interactions and main effects were observed, multiple comparison tests were performed by Bonferroni's method. The Mann-Whitney U test was used to compare changes in FMD ($\Delta$FMD) before and after each training. Data were analyzed with SPSS ver.25.0 (IBM Corp., Armonk, NY, USA). All measurements were described as mean $\pm$ standard deviation, and significance level was set at 5%.

**Results**

*Brachial artery function before and after each training.* All the participants in the training group completed the 6-week, 3-times-a-week training program. No significant
differences were observed in terms of VO$_{2\text{max}}$ (47.1 ± 9.3 to 46.1 ± 10.7 ml·kg$^{-1}$·min$^{-1}$ in the RT group, 47.7 ± 10.1 to 48.9 ± 11.2 ml·kg$^{-1}$·min$^{-1}$ in the CT group). Table 1 presents the changes in vessel function before and after each training. No significant differences were observed in the SBP, DBP, and HR. No significant interaction on the Di$_{\text{base}}$ and Di$_{\text{peak}}$ was observed (Table 1). On the other hand, a significant main effect of time on Di$_{\text{base}}$ (F = 5.03, P = 0.038) and Di$_{\text{peak}}$ (F = 8.83, P = 0.008) was observed. According to the post hoc analysis, both Di$_{\text{base}}$ and Di$_{\text{peak}}$ have significantly increased after the RT. However, no significant changes were found after the CT. Fig 1 represents the changes in the FMD throughout each training. Compared with the pre-training value (7.26 ± 1.46%), the FMD was significantly increased at post-training (8.58 ± 1.22%, p<0.01) after the RT. However, no significant changes were found throughout the CT (9.92 ± 2.45 and 10.58 ± 2.93%, p=0.13). There wasn't any difference in ΔFMD between the RT and the CT groups (1.1 ± 0.3% versus 0.7 ± 1.2%, p=0.058).

**Discussion**
In this study, we analyzed the effects of RT on vascular endothelial function and made a comparison to the ones of CT. The FMD values in the RT group significantly increased after training compared with before training. The effect of repetition training on endothelial function has not been reported. This new finding highlights the effect of the improving vascular endothelial function.

Vascular endothelial cells produce nitric oxide (NO), a potent vasodilator, and endothelin-1 (ET-1), which has potent proliferative activity on vascular smooth muscle cells. It has been clarified that NO and ET-1 concentrations changes by exercise intervention. In a study of IT using a treadmill for 8 weeks on prehypertensive young people, FMD and nitrogen oxides (NOx) levels significantly increased, and ET-1 level and blood pressure decreased. It has been reported that a 6-week intervention of IT with cycling on older hypertensive subjects who experienced medical treatment developed a decrease in ET-1 and an increase in NO. The scientific literature typically refers to IT as intense aerobic-based interventions. IT is characterised as high-intensity efforts, usually between 80 and 100% VO2max or predicted maximum HR.
interspersed with light recovery exercise or no exercise between intervals\textsuperscript{23}). Furthermore, IT allows greater physiological stimulus and adaptation than CT for cardiorespiratory fitness and other cardiometabolic processes\textsuperscript{31}). The repetition exercises performed in this study are classified as IT with no exercise between interval. It has been reported that the acute effects of repetition exercise increased FMD, which may have been influenced by NO concentration\textsuperscript{10}). Therefore, it is possible that the significant improvement in FMD after RT in this study was due to an increase in blood NO concentration and a decrease in ET-1 concentration.

Although the type of exercise is different from this study, several studies have reported that FMD improves in the early stages of training (2 to 6 weeks) and arterial structure gradually improves throughout the training period\textsuperscript{32–34}). First, regarding FMD, in 8 weeks of handgrip exercise training of healthy adult men, the FMD change value improved from week 2 to week 6 compared with that of before the intervention and then returned the baseline value at week 8\textsuperscript{32}). Following 6 weeks of low-intensity resistance training with blood flow restriction in healthy adult men, popliteal artery FMD improved at 2nd and
the 4th week compared to that of before intervention and resumed the baseline value at
6th week 33). Further, following endurance training consisting of running and cycling
exercises in healthy adult men, the rate of change in brachial and popliteal artery FMD
improved at the 2nd and the 6th week compared with that of before intervention and then
resumed the baseline value at 8th week 34). In this study, FMD improved with the 6-week
RT. Next, regarding structural changes in vessels, the peak of reactive hyperemic blood
flow through the brachial artery 32), the maximum diameter of the popliteal artery 33), and
the maximum brachial/ popliteal vasodilator capacity 34), which are all the indicators of
remodeling of arterial vascular structure, gradually increased during the intervention
period. This study also found increases in brachial arterial peak diameter with the RT.
However, previous studies of 8-12 weeks of continuous training in middle-aged and older
people did not improve carotid intima-media thickness (IMT) 35,36). Similarly, Carotid
IMT did not improve in the previous 12-week IT study of middle-aged patients 37).
Therefore, it is possible that the IMT, which is the main vascular structure index, did not
change during the intervention period of this study.
Our study has some limitations. First, it would be ideal to determine the effects of training on the vascular endothelial function of participants with different physical fitness levels and disease states and also of older people as our participants were limited to healthy young adult men. Furthermore, although this study was compared with CT, it also needs to be compared with interventions by other forms of exercise such as IT interspersed with light recovery exercise. We considered the effects of NO and ET-1 on the improvement of FMD, but we did not perform biochemical tests. By measuring them, it might be possible to discern the mechanism of improvement affected by training. No significant increase in $\text{VO}_2\text{max}$ was observed in both groups after training. Therefore, it is difficult to improve endurance capacity with the RT of this study, and it is considered that the exercise program was specialized in improving vascular endothelial function.

Conclusion

In this study, we compared RT with CT to clarify the effect of RT on vascular endothelial function. The results show that the improvement in vascular endothelial
function was observed only in the RT group. It suggests that RT might be effective for
the prevention of cardiovascular disease.

Acknowledgements

The authors wish to thank Tomohiro Mori and Tatsuya Ikeda for their skillful assistance with data collection for this study.

Conflicts of Interests

The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

Authors Contributions

YT and HM conceived and designed the research. YT, YH, JD, AA and MI conducted the experiments. YT, and KD analyzed the data. YT, HM, and KD wrote the manuscript.

References


**Table 1.** Changes in vessel function before and after each training.

<table>
<thead>
<tr>
<th></th>
<th>RT (n=10)</th>
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<th>Group × Time Interaction</th>
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<td>Pre</td>
<td>Post</td>
<td>P Value</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>SBP (mmHg)</td>
<td>114.4 ± 7.9</td>
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<td>DBP (mmHg)</td>
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<td>HR (beats·min⁻¹)</td>
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<td>Dibase (mm)</td>
<td>3.80 ± 0.54</td>
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<td>Dipeak (mm)</td>
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<td>4.31 ± 0.65</td>
<td></td>
<td>3.97 ± 0.48</td>
<td>4.01 ± 0.47</td>
</tr>
</tbody>
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Values are mean ± SD. RT: repetition exercise training, CT: continuous exercise training, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, Dibase: brachial artery baseline diameter, Dipeak: brachial artery peak diameter.
Fig. 1 Changes in FMD before and after each training.

Values are mean ± standard deviation. RT: repetition exercise training, CT: continuous exercise training, FMD: flow-mediated vasodilation. *: post hoc significantly different from pre (p<0.01). #: post hoc significantly different between the RT and CT group (p<0.01).