

Smoking as a Confounding Factor on the Influence of Cold Therapy on the Vascular Endothelium Function of Young Men

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Abstract. [Purpose] This study was designed to determine whether smoking affects endothelium function after cold therapy in young men. [Subjects] The final cohort included 27 healthy men (age, 20–21 years). Because an impact on vascular endothelium function was anticipated to be caused by smoking, the study enrolled 14 participants in a smoking group and 13 in a non-smoking group. [Methods] Vascular endothelial function was assessed by determining the reactive hyperemia index (RHI), using finger-tonometry. RHI was measured twice, at rest (baseline) and after a cold stimulus. The forearm was cooled with an ice bag for 10 min as the cold stimulus. Comparisons between the RHI at baseline and after cold treatment, and between the smoking and non-smoking groups, were performed using the paired and unpaired t-tests, respectively. [Results] There was a significant difference in baseline RHI values between the smoking and non-smoking groups, but there was no significant difference between the baseline and post-treatment RHI values in either group. [Conclusion] These results suggest that cigarette smoking damages the endothelial cells in young men with a short history of smoking. However, cold therapy did not have a significant impact on the RHI in either group.

Key words: Endothelium function, Cold therapy, Smoking

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INTRODUCTION

Recently, the aging of the Japanese population has become a serious social problem, which, in conjunction with a reduced birth rate, is leading to a decline in Japan's population. According to the Ministry of Internal Affairs and Communications, the population of Japan aged >65 years in 2011 was 29,750,000 people, accounting for 23.3% of the total population; and is expected to exceed 30 million people in 2012¹⁾. The total population of Japan has decreased for the first time since 1950, and is predicted to be <100 million by 2048²⁾.

With the aging of the population, the number of elderly people with vascular lesions is also increasing. Therefore, the number of elderly individuals in need of nursing care resulting from cerebrovascular disease is also increasing. In a survey conducted by the Ministry of Health, Labour and Welfare in 2011, cardiac disease and cerebrovascular disease ranked as the second and third most frequent causes of death in Japan. Cardiac and cerebrovascular disease caused by arteriosclerosis comprised approximately 30% of all causes of death³⁾. Furthermore, about 20% of the aged population requires long-term care because of cerebrovascular disease; about 4% require long-term care due to cardiac

disease, and about 3% due to diabetes mellitus. Therefore, preventing arteriosclerosis and extending the healthy life expectancy of the aged population is an important public health issue.

According to Dishman et al., arteriosclerosis begins as an injury to endothelial cells, which may include physical damage from lipoproteins, chemical damage from tobacco smoke, or high homocysteine levels⁴⁾. During atherosclerosis, endothelium injury is the primary stimulus leading to desquamation and smooth muscle cell migration and proliferation. Platelets adhere to the injured endothelium, releasing vasoconstrictive and thrombogenic substances, and lipids, primarily LDL cholesterol, accumulate in the lesions⁵⁾.

The endothelial cells comprise an endocrine organ that adjusts the constriction or dilatation of a blood vessel, blood coagulation, and vascular inflammation. Previous studies have reported that endothelium-derived relaxing factors (EDRF), including nitric oxide (NO), are produced by the cells lining the blood vessels^{6, 7)}. NO is a vasodepressor, and endothelium dysfunction resulting from arteriosclerosis impairs production of NO. The atherosclerotic process in coronary arteries begins in childhood⁴⁾, and it gradually advances with various diseases, typically becoming evident in middle age. Therefore, the prevention of arteriosclerosis requires lifestyle improvements beginning at a young age.

Vascular endothelium function measurement conventionally employs the flow-mediated dilatation (FMD) meth-

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od. The FMD method measures changes in vessel diameter following induced ischemic reactive hyperemia of an upper arm artery, based on an ultrasonic wave. Although the FMD method is safe and non-invasive, it has a number of limitations. These include the placement of the ultrasonic probe, which may easily cause data errors a poor inter-rater reliability, which hampers comparisons between investigating institutions and changes in vessel size, before and after avascularization, are minor, resulting in potential data errors. Recently, the measurement of the reactive hyperemia index (RHI) in a finger artery has become popular. This method has better inter-rater reliability because it takes automatic measurements, using a probe attached to a finger. Furthermore, because both the upper limbs are targeted, with 1 side as a control, the influence of the sympathetic nervous system can be minimized.

Physical therapists often use thermotherapy or cold therapy to reduce the pain of patients, including that of many cerebrovascular disease patients. Since the main cause of cerebrovascular disorders is arteriosclerosis, we hypothesized that cerebrovascular disease patients may have endothelial dysfunction. However, it was not clear whether thermotherapy or cold therapy affect vascular endothelium function. The decrease in vascular endothelium function caused by smoking has been clarified by various research studies, but those studies targeted middle-aged and elderly persons, and the influence of smoking on vascular endothelium function had not been established in younger persons. Therefore, we considered the influence of smoking as a confounding factor on vascular endothelium function among younger persons, and the purpose of this study was to determine whether smoking affects endothelium function after cold therapy in young men.

SUBJECTS AND METHODS

The participants in this study were 30 healthy men (age range, 20–21 years). The mean (SD) history of smoking and the number of cigarettes smoked per day were 2 (1) years and 13.2 (4.1) cigarettes in the smoking group, respectively. After excluding participants with unmeasurable pulse waves, there were 14 evaluable subjects in the smoking group and 13 in the non-smoking group. This research complied with the terms of the Declaration of Helsinki. The research protocol was explained to the participants, who provided their informed consent, prior to the commencement of the study. Moreover, the prior explanation of the research included explanations about freedom of research participation, protection of personal data, and freedom to withdraw from the experiment after provision of consent. Explanations to the participants were given verbally and in writing, and the participants signed a letter of consent.

This study was an interventional study that investigated the function of the vascular endothelium, before and after cold therapy. In order to check the influence of other factors, we measured body composition, including body weight (BW), body mass index (BMI), percent of body fat (%BF), and percent skeletal muscle (%SM), using an HBF-362 (Omron Healthcare, Kyoto, Japan). Physical therapists interviewed the subjects to record their activities during the

preceding 24 h, and their levels of physical activity (PA) were calculated on the basis of the position and intensity of physical activity (PIPA)^{8, 9}. To standardize the PA of the subjects, the PA was divided by BW to calculate the amount of physical activity per kg of BW.

Vascular endothelium function, before and after intervention, was compared, as were the outcomes between the smoking and non-smoking groups. Vascular endothelial function was assessed by determining the RHI by finger tonometry using the endo-PAT 2000 (Itamar Medical, Caesarea, Israel). When a blood vessel is released from an ischemic state, it expands because of the NO secreted by the vascular endothelium¹⁰. We measured the pulse wave using the endo-PAT2000 for 5 min before and after compression of the upper arm. The RHI was calculated by the comparison before and after compression, and by comparing both upper arms. As a cold stimulus, the target side forearm was cooled with an ice bag for 10 min. RHI was measured twice, at rest (baseline RHI) and after cold stimulus (after RHI).

The baseline RHI and that after cold therapy were compared using the paired t-test. The smoking and non-smoking groups were compared using the unpaired t-test. Odds ratios were calculated based on the percentages of the individuals in the smoking and non-smoking groups with abnormal baseline RHI who had a normal RHI after cold stimulation. Statistical significance was chosen as the 5% level. All statistical analyses were performed using SPSS, version 19.0, software (IBM, Armonk, NY, USA).

RESULTS

We evaluated and verified the normality of the data using the Shapiro-Wilk test. Therefore, all statistical analyses employed parametric tests.

The physical characteristics of the participants are shown in Table 1. There were no statistically significant differences between the smoking and non-smoking groups of men, based on their height, BW, or BMI. Similarly, Table 2 shows that there were no significant differences between two groups in %BF, %SM, PA, or PA/BW.

The mean (SD) value of %BF was 13.50% (5.20%) in the smoking group and 12.88% (5.18%) in the non-smoking group, whereas the mean %SM was 36.49% (1.51%) in the smoking group and 36.95% (1.90%) in the non-smoking group. The PA was 2396.56 kcal (674.82 kcal) in the smoking group and 1864.79 kcal (330.06 kcal) in the non-smoking group. The mean (SD) PA/BW value was 34.60 kcal/kg (9.43 kcal/kg) in the smoking group and 31.21 kcal/kg (3.96 kcal/kg) in the non-smoking group. There were no significant differences in these measurements between the 2 groups.

The mean (SD) value of the baseline RHI was 1.73 (0.32) in the smoking group and 2.09 (0.60) in the non-smoking group, whereas the post-cold exposure RHI was 1.93 (0.80) in the smoking group and 1.79 (0.41) in the non-smoking group (Table 2). The baseline RHI values were significantly different ($p=0.027$) between the 2 groups; there were no significant differences between the baseline and post-intervention values in both groups. An abnormal RHI value is considered to be ≤ 1.67 and 6 persons in the smoking group

Table 1. Participant characteristics

	Mean (SD)	
	Smoking group	Non-smoking group
Age (years)	20 (1)	20 (1)
Body height (cm)	175.6 (4.7)	171.6 (3.7)
Body weight (kg)	67.8 (9.2)	59.8 (6.6)
BMI (kg/m ²)	21.9 (2.4)	20.3 (2.7)

BMI, body mass index

and 3 persons in the non-smoking group had abnormal RHI values. The odds ratio of an unusual relationship between the vascular endothelium function in the smoking group to the non-smoking group was 25.7. There were 2 persons who returned to normal, following cold therapy, among the individuals with unusual baseline RHI values in each group. The odds ratio for a return to normal RHI, after cold therapy administered to stimulation of smokers compared to non-smokers was 4.0.

DISCUSSION

In this study, significant differences were not observed in the PA per BMI, %BF, %SM, and BW between the smoking and non-smoking groups, indicating similar metabolic rates, based on energy consumption under similar conditions.

A blood vessel is comprised of an outer membrane, tunica media, and intima, and the vascular endothelium provides the lining of the vessel. Various vasodilators, including NO, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF), as well as vasoconstrictors, are released from the endothelium¹¹. Generation of NO from the endothelium occurs due to endothelial shear stress caused by blood flow. As NO is secreted from the endothelial cells, expansion of the blood vessel occurs, through NO stimulation of the vascular smooth muscle cells of the tunica media. When arteriosclerosis develops, the vasodepressor effect of NO decreases because its production is reduced as a result of oxidization by low-density lipoprotein. Ischemic reactive hyperemia increases the shear stress and causes vessel dilatation by promoting additional NO secretion.

This study revealed there was a significant difference in the baseline RHI values between the smoking and non-smoking groups. The odds ratio suggests that the risk of vascular endothelial dysfunction caused by smoking is high. Levick reported that smoking affects the vascular endothelium by stimulating nicotinic receptors on post-ganglionic neurons, to increase sympathetic vasoconstrictor activity; by activating leukocytes to generate free radicals in oxidative bursts that react with NO to form harmful peroxynitrite and reduce the level of beneficial NO; and by increasing the sympathetic activity causing further vasoconstriction¹². Barua et al. reported that human umbilical vein endothelial cells exposed to smokers' serum decreased nitric oxide (NO) production and endothelial nitric oxide synthase (eNOS) activity in the presence of increased eNOS expression^{13, 14}. Bard et al. also suggested that second-hand

Table 2. Subject measurements taken during the study

	Mean (SD)	
	Smoking group	Non-smoking group
%BF (%)	13.50 (5.20)	12.88 (5.18)
%SM (%)	36.49 (1.51)	36.95 (1.97)
PA (kcal)	2,396.56 (674.82)	1,864.79 (330.06)
PA/BW (kcal/kg)	34.60 (9.43)	31.21 (3.96)
Baseline RHI	1.73 (0.32)	2.09 (0.60)*
Post-therapy RHI	1.93 (0.80)	1.79 (0.41)

%BF, percent body fat; %SM, percent skeletal muscle; PA, physical activity; PA/BW, physical activity normalized to kg of body weight; RHI, reactive hyperemia index.*p<0.05

smoke increases blood pressure and the progress of arteriosclerosis in non-smoking adults¹⁵. In the present study, the subjects were young and had comparatively short histories of smoking. Regardless, the results suggest that endothelium dysfunction was already evident; however, the blood pressures of both groups were not different. There was little evidence of the influence of tobacco-based NO changes to blood pressure, but early vascular endothelium vessel dilatation was suspected.

In the results of this study, a significant difference between baseline RHI and post-intervention RHI was not evident, but there was a trend suggesting that the RHI decreased in the non-smoking group, and increased in the smoking group. If the arterioles of the skin are warmed, they extend; if they are cooled, they contract. This study demonstrated the vasodilation of the fingertip artery caused by a cold stimulus applied to the forearm. We believe that the fingertip vessels contract as a result of poor blood flow, caused by vasoconstriction in the forearm. However, we could not document a vascular response in the smoking group. In the smoking group, the vessels became dilated after cold stimulus, suggesting that flow reduction caused by a cold stimulus does not arise in smokers. In subjects with abnormal baseline RHI, many of those who did not return to normal after cold therapy were smokers, and the odds ratio was 4.0. This suggests the possibility that vascular endothelium function is lower in the smokers.

This study was a cross-sectional study, and although we researched vessel responses after cold stimulus, we could not identify the cause of this response. A basic study may be necessary to elucidate the cause of this response. Moreover, this study did not show that the influence of a cold stimulus applied to young subjects is conducive to arteriosclerosis. We need to clarify the changes in the function of the vascular endothelium under continuous stimulation. Similarly, research is needed to investigate the results of thermotherapy as this technique is frequently used to treat patients with cerebrovascular disease. A limitation of this study was that cooling by cold therapy occurred only once, and in order to show equal cooling, we should have measured the cutaneous temperature. In addition, there were few samples in this study, and they may not extrapolate well. Further research is needed to increase the number of cases studied.

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