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Evaluation of coronary endothelial dysfunction in healthy young smokers: Cold pressor test using [^{15}O]H_2O PET

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ABSTRACT

The purpose of this study was to investigate coronary endothelial dysfunction in young healthy smokers by measuring myocardial blood flow (MBF) using [^{15}O]H_2O-PET. The study population was 18 young male volunteers consisted of 9 smokers (age: 23.8±1.1 yr) and 9 non-smokers (age: 25.0±2.5 yr). The smokers had been smoking cigarettes for 6.6±2.5 pack years. Myocardial [^{15}O]H_2O-PET was performed at rest, during cold (5 °C) pressor stimulation and during adenosine infusion. Left ventricular (LV) input function and tissue time–activity curves were obtained by drawing region of interest (ROI) on the LV blood pool and myocardium images obtained by non-negative matrix factorization (NMF) of dynamic [^{15}O]H_2O-PET data, and MBF was calculated using these time–activity curves and single compartmental model. There were no significant difference in resting MBF between two groups (smokers: 1.43±0.41 and non-smokers: 1.37±0.41 ml/g/min; P = NS). However, during cold pressor stimulation, MBF in smokers was significantly lower than that in non-smokers (1.25±0.33 vs. 1.59±0.29 ml/g/min; P = 0.019). MBF changed to 90±24% of resting MBF in smokers and 122±28% in non-smokers. The difference in the ratio of cold pressor MBF to basal MBF between two groups was also significant (P = 0.024). During adenosine infusion, however, hyperemic MBF did not differ significantly between smokers and non-smokers (5.81±1.99 vs. 5.03±1.27 ml/g/min; P = NS). This study shows that [^{15}O]H_2O PET analysis can reveal that endothelial dysfunction occurs in even young smokers of about 6 pack years.

1. Introduction

The vascular endothelium regulates vascular smooth muscle tone by releasing vasoactive mediators, mainly nitric oxide (NO) synthesized by the enzyme NO synthase from the amino acid L-arginine (Vallance, 1992). It is known that a variety of risk factors for coronary artery disease (CAD) are related to the impairment of coronary endothelium-dependent vasodilator function (Egashira et al., 1993; Vita et al., 1990; Reddy et al., 1994). To evaluate the vasodilator function by coronary endothelium, coronary blood flow (CBF) after intracoronary infusion of acetylcholine or during cold pressor test (CPT) has been measured (Nabel et al., 1988; Zeiher et al., 1989) by invasive methods such as quantitative coronary angiography or coronary doppler ultrasonography. Recently, non-invasive positron emission tomography (PET) was introduced to measure myocardial blood flow (MBF) during CPT in patients with coronary artery obstructive disease (CAOD) or subjects with the risk factor of CAOD (Meeder et al., 1996; Campisi et al., 1998; Di Carli et al., 1999; Drzezga et al., 2000). Smoking is one of well-known risk factors for CAOD and known to impair endothelial-dependent coronary vasomotion (Nitenberg et al., 1993; Zeiher et al., 1995). It has been reported that endothelial-dependent vasodilator function is decreased in smokers when the MBF was measured using PET during the CPT (Iwado et al., 2002). This study was designed to compare MBF at rest, during CPT and during adenosine-induced hyperemia between young healthy smokers (with smoking history of about 6 pack years) and non-smokers using [^{15}O]H_2O PET and to demonstrate impaired endothelial-dependent coronary vasomotion in smokers.

2. Materials and methods

2.1. Study population

Study population of this study (Table 1) included 9 male young healthy smokers (23.8±2.9 year) and 9 male young healthy

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Table 1
Demographic data of study populations.

	Smokers	Non-smokers	*P*-Value
Number	9	9	NS
Age (yr)	23.8±1.1	25.0±2.5	NS
Height (cm)	176.0±5.9	174.2±7.7	NS
Weight (kg)	72.7±7.6	72.3±11.3	NS
Body mass index (kg/m^2)	23.4±1.8	23.8±2.9	NS
Smoking history (Pack years)	6.6±2.5		NS

NS: statistically not significant.

non-smokers (25.0±2.5 year). All smokers had smoked over 10 cigarettes per day for more than 5 years (6.6±2.5 pack year). None of the study participants had a history of cardiovascular disease and any risk factor such as hypertension, diabetes mellitus, hyperlipidemia, or family history of CAOD. None were receiving any medication.

All participants refrained from intake of caffeine-containing food or beverages for at least 24 h before the study. The smokers abstained from smoking for at least 4 h before the PET study. This study was approved by the Institutional Review Board in the hospital and all participating subjects signed statements of informed consent.

2.2. Imaging protocol

PET was performed using a whole-body scanner (Siemen/CTI ECAT 47; Knoxville, USA) equipped with germanium-68 line sources for transmission scans. Myocardial [^{15}O]H_2O PET was performed at rest, during cold pressor stimulation and adenosine infusion. At first resting dynamic PET scanning (12×5, 9×10, and 3×30 s) was started simultaneously with the bolus injection of [^{15}O]H_2O into the antecubital vein of the subject. After the resting scan, one hand of the participant was submerged in ice water (5 °C) from 60 s before the PET scan to 60 s after the start of the scan. Finally, adenosine of 0.14 mg/kg/min was continuously infused for 7 min, [^{15}O]H_2O was injected 3 min after the start of adenosine infusion. PET acquisition was accomplished for 4 min. Pulse rate, blood pressure, and EKG were monitored throughout the experiment in all subjects.

2.3. Image analysis

To obtain left ventricular (LV) blood pool and myocardium image from the dynamic PET data, non-negative matrix factorization (NMF) method (Paatero and Tapper, 1997; Lee and Seung, 1999; Lee et al., 2001) was used since the method has the theoretical advantages vs. the conventional factor analysis.

LV input function and tissue time–activity curves were obtained by drawing region of interest (ROI) on the LV blood pool and myocardium images, and MBF was calculated using these time–activity curves and single compartmental model in which the correction terms for partial volume and spillover effects were incorporated (Fig. 1).

Coefficient of variation was calculated in total 54 images—three times per person—from 18 subjects to evaluate the reproducibility of our algorithm. Calculated coefficient of variation was 9.8%. Calculated MBF was standardized with rate–pressure product (RPP).

2.4. Statistical analysis

Student's *t*-test for the demographic characteristics and the Wilcoxon signed-rank sum test for MBF data in two groups were performed. The data were expressed as means±standard error. MBF was corrected by the rate–pressure product at rest, during CPT, and during adenosine-induced hyperemia: The standardized MBF = {MBF × 10,000}/{pulse rate × mean systolic pressure}. Data were analyzed using the SPSS 11.0. Statistical significance was set at $P<0.05$.

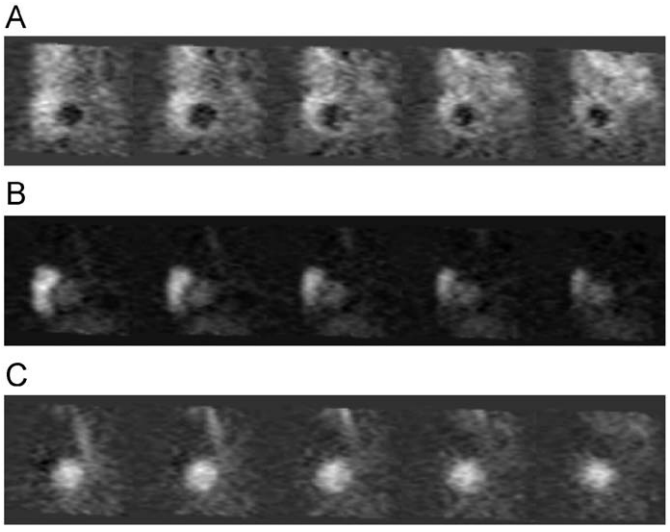

Fig. 1. Factor images using NMF methods: (A) myocardium, (B) right ventricle and (C) left ventricle.

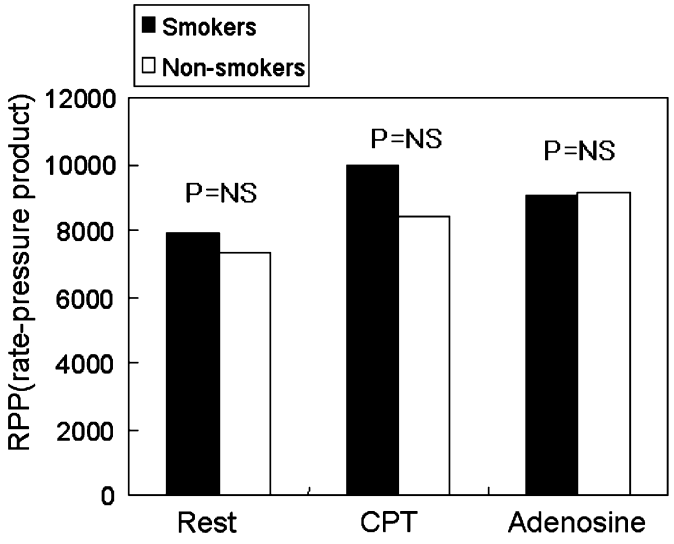

Fig. 2. Rate–pressure product (RPP) at rest, during cold pressor stimulation, and during adenosine infusion in smokers and non-smokers.

3. Results

3.1. Hemodynamic findings

In the smokers, pulse rate during CPT was significantly higher than that in the non-smokers ($P<0.02$). However, there was no significant difference in RPP at rest, during cold pressor test and during adenosine-induced hyperemia between the smokers and the non-smokers (Fig. 2 and Table 2).

Table 2
Systolic blood pressure (SBP), pulse rate (PR) and rate–pressure product (RPP) in the smokers and the non-smokers.

Variables	Smokers	Non-smokers	*P*-Value
SBP (mmHg)			
Rest	124±13	122±16	NS
CPT	133±17	136±20	NS
Adenosine	116±14	122±17	NS
PR (bpm)			
Rest	64±9	60±7	NS
CPT	75±10	63±9	<0.02
Adenosine	78±12	75±12	NS
RPP (bpm × mmHg)			
Rest	7933±1391	7341±985	NS
CPT	9967±1996	8456±68	NS
Adenosine	9021±70	9232±2029	NS

bpm = beat per minute.NS = statistically not significant.

Table 3
Myocardial blood flow (MBF) at rest, during CPT and during adenosine-induced hyperemia in the smokers and the non-smokers.

Variables	Smokers	Non-smokers	*P*-Value
MBF (ml/g/min)			
Rest	1.43±0.41	1.37±0.41	NS
CPT	1.25±0.33	1.59±0.29	0.019
Adenosine	5.81±1.99	5.03±1.27	NS
MBF ratio to MBF at rest (%)			
CPT	90.4±23.5	122.1±28.2	0.024
Adenosine	417.5±120.7	390.2±144.4	NS

NS = statistically not significant.
MBF ratio to MBF at rest (%) = MBF at stress × 100/MBF at rest.

3.2. Myocardial blood flow and flow reserve

Table 3 shows the results of the MBF measurements. Smokers had a slightly increased resting myocardial perfusion (1.43±0.41 vs. 1.37±0.41 ml/min/g), but the difference was not statistically significant. However, there was significant difference in MBF during CPT between two groups ($P = 0.019$, Fig. 3); MBF of the smokers was significantly lower than non-smokers (1.25±0.33 and 1.59±0.29 ml/min/g, respectively). Endothelial-dependent MBF reserve, the ratio of MBF during CPT to resting MBF, was higher in non-smokers than smokers (122±28% vs. 90±24%, Fig. 4) and their difference was statistically significant ($P = 0.024$). On the contrary, MBF during adenosine infusion were similar between two groups. MBF of non-smokers and smokers were 5.03±1.27 (ratio to resting MBF = 390±144%) and 5.81±1.99 ml/min/g (418±121%).

3.3. Correlation with smoking history

There was no significant correlation between smoking history (pack year) and CPT-induced decrease in MBF reserve in smokers.

4. Discussion

It has long been well known that cold exposure frequently induces angina attack. It was proved that sympathetic stimulation such as cold exposure dilates normal coronary artery but

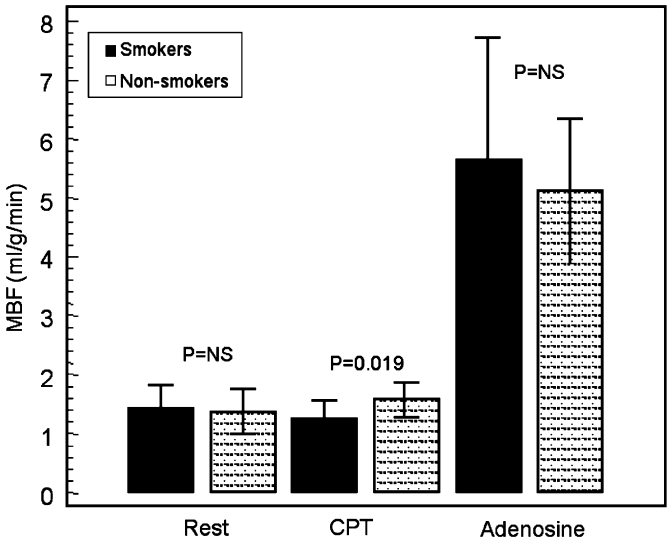

Fig. 3. Comparison of global MBF between smokers (left black columns) and non-smokers (right columns) at rest, after cold pressor stimulation and during adenosine infusion.

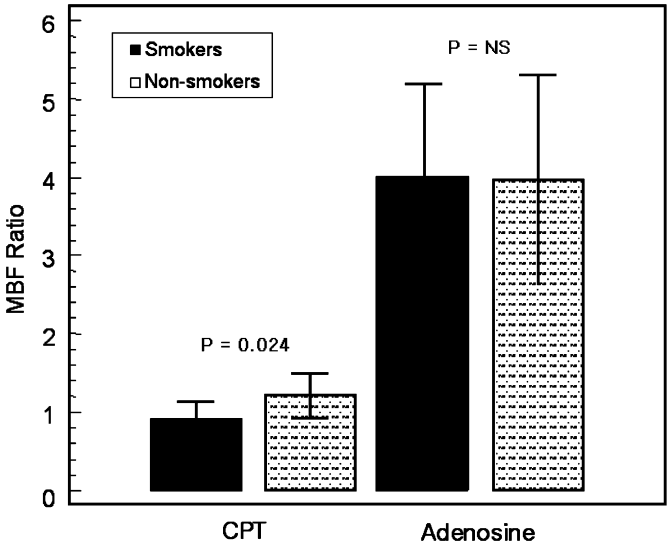

Fig. 4. Ratio of MBF to resting MBF after cold pressor stimulation and during adenosine infusion (that is, myocardial flow reserve) in smokers (left black columns) and non-smokers (right columns).

paradoxically constricts abnormal arteries in individuals with risk factors for coronary artery disease (Nabel et al., 1988; Zeiher et al., 1989). CPT is an established tool to assess endothelial function noninvasively and a lot of myocardial perfusion studies have been performed by CPT. In normal coronary artery, CPT stimulates NO release from endothelium results in vasodilation and MBF is increased. In coronary artery with endothelial dysfunction, on the other hand, NO release is reduced and coronary artery does not dilate or even constricts in response to CPT.

Endothelial dysfunction is known to be impaired in subjects with various risk factors for CAOD such as smoking, hypertension, hyperlipidemia, obesity and diabetes mellitus. We investigated endothelial dysfunction caused by smoking, using $[^{15}O]H_2O$ PET. MBF can be measured by PET scan using $[^{13}N]NH_3$ or $[^{15}O]H_2O$. However, $[^{13}N]NH_3$ is not linearly proportional to blood flow and metabolized in a small amount. $[^{15}O]H_2O$ is an ideal radiotracer to measure MBF because it is freely diffusible and metabolically inert.

One of the major advantages of NMF used in our study is that the NMF cost function uses Poisson statistics as a noise model. This is more appropriate for gamma camera images than the Gaussian model because the gamma camera images really represent some sort of photon counts. In addition, NMF provides a good simple learning rule, which is guaranteed to converge monotonically without the need for setting any adjustable parameters such as a learning rate, in contrast to the tricky optimization in factor analysis (Lee et al., 2001).

Our results are concordant with the previous studies about smoking-induced endothelial dysfunction considering that only MBF reserve during CPT is reduced but MBF reserve during adenosine infusion preserved. Up to now, several studies using PET (Iwado et al., 2002) have reported that endothelial-dependent MBF reserve is decreased in smokers. In most studies, $[^{13}N]NH_3$ has been used and, to our knowledge, only Iwado et al. (2002) evaluate endothelial-dependent MBF reserve using $[^{15}O]H_2O$ PET in young smokers. They demonstrated that coronary endothelial function was reduced in smokers with a smoking history of 9.4 ± 4.9 pack years. We showed that endothelial dysfunction is present even in young smokers with a smoking history of only 6.6 ± 2.5 pack years, which is consistent with the recent reports for impaired endothelial function in healthy young adults by passive smoking (Woo et al., 2000) and even in the children (Kallio et al., 2007).

In our study, baseline MBF was 1.37 ± 0.41 ml/min/g, which is slightly higher than other studies (0.6–1.2 ml/min/g). This may be due to the underestimation of the initial peak of LV input function by the dead time loss of count. Slow injection of $[^{15}O]H_2O$ to reduce the dead time loss lead to the failure in segmentation of the cardiac components. However, there would be no problem in the evaluation of the ratio of MBF during CPT to the baseline because our result in non-smokers ($22\pm28\%$) is similar to those in the previous studies (Meeder et al., 1996b; Campisi et al., 1998).

No correlation was found between smoking history (pack year) and decrease in endothelium-dependent MBF reserve. This may be due to inaccurate history taking of smoking in pack years, but more likely due to difference in susceptibility to endothelial injury by smoking and other factors such as exercise, nutrition, genetic factor and so on.

Recently, it was reported that NO synthase activity is decreased in coronary endothelium exposed to the smoker's serum (Barua et al., 2003). It suggests that substances absorbed into blood by smoking may influence NO synthase activity in the endothelium.

Clinically, endothelial vasodilatory function is important in the aspect of prognosis and prevention of ischemic heart disease. Several articles about the close relation of endothelial dysfunction with future cardiac events were published (Suwaidi et al., 2000; Halcox et al., 2002; Gokce et al., 2002; Quyyumi, 2003). In addition, it was reported that endothelial dysfunction can be recovered by exercise and administration of L-arginine or antioxidants. Thus, non-invasive accurate measurement of endothelial function is getting more important position. Doppler ultrasonography of brachial artery is widely used for the assessment of endothelial function, but is not a direct method to measure coronary endothelial function.

Therefore, non-invasive methods to evaluate endothelial function, such as $[^{15}O]H_2O$ PET may be a valuable tool. More investigations for endothelial function in relation to prognosis and prevention of ischemic heart disease should be done in future.

5. Conclusion

In this study, we demonstrated that MBF after cold pressor stimulation is significantly lower in young smokers compared with non-smokers by $[^{15}O]H_2O$ PET. The results indicate that even about 6 years of smoking could induce endothelial dysfunction.

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