Decreased endothelium-dependent coronary vasomotion in healthy young smokers

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Abstract. Chronic cigarette smoking alters coronary vascular endothelial response. To determine whether altered response also occurs in young individuals without manifest coronary disease we quantified coronary blood flow at rest, following adenosine vasodilator stress and during the cold pressor test in healthy young smokers. Myocardial blood flow (MBF) was quantified by oxygen-15 labelled water positron emission tomography in 30 healthy men aged from 20 to 35 years (18 smokers and 12 non-smokers, aged 27.4±4.4 vs 26.3±3.3). The smokers had been smoking cigarettes for 9.4±4.9 pack-years. MBF was measured at rest, during intravenous adenosine triphosphate (ATP: 0.16 mg kg^{-1} min^{-1}) infusion (hyperaemic response), and during cold pressor test (CPT) (endothelial vasodilator response). Rest MBF and hyperaemic MBF did not differ significantly between the smokers and the non-smokers (rest: 0.86±0.11 vs 0.92±0.14 and ATP: 3.20±1.12 vs 3.69±0.76 ml g^{-1} min^{-1}; *P*=NS). Coronary flow reserve was similar between the two groups (smokers: 3.78±1.83; non-smokers: 4.03±0.68; *P*=NS). Although CPT induced a similar increase in rate-pressure product (RPP) in the smokers and the non-smokers (10,430±1,820 vs 9,236±1,356 beats min^{-1} $mmHg^{-1}$), CPT MBF corrected by RPP was significantly decreased in the smokers (0.65±0.12 ml g^{-1} min^{-1}) compared with the non-smokers (0.87±0.12 ml g^{-1} min^{-1}) ($P<0.05$). In addition, the ratio of CPT MBF to resting MBF was inversely correlated with pack-years ($r=-0.57$, $P=0.014$). Endothelium-dependent coronary artery vasodilator function is impaired in apparently healthy young smokers.

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Introduction

Cigarette smoke is known to contain approximately 4,000 chemical substances, including nicotine and carbon monoxide. Cigarette smoking is a risk factor for coronary artery disease and is known to alter coronary vasoreactivity [1, 2, 3, 4, 5], especially in individuals with a history of chronic cigarette smoking [6, 7, 8, 9]. The effect of smoking on endothelial function has been examined by measuring brachial artery dilation following administration of a vasodilator, and identifying diminished response [2, 3]. However, recent studies have identified a difference between the peripheral and the myocardial microcirculation [10], suggesting that myocardial vasomotor function should be evaluated directly to determine whether coronary endothelial function is normal. Positron emission tomography (PET) with oxygen-15 labelled water is a non-invasive method for quantification of myocardial blood flow (MBF) [11, 12, 13, 14, 15]. We used ^{15}O-labelled water to quantify myocardial perfusion on three occasions: at baseline, following infusion of adenosine triphosphate (ATP) and during the cold pressor test (CPT). ATP is a short-acting drug that mainly dilates coronary arterial smooth muscle. ATP-induced hyperaemia is useful to evaluate endothelium-independent coronary vasomotion [16, 17]. In contrast, the coronary response to CPT [18, 19] is useful to evaluate endothelium-dependent coronary vasomotion.

PET studies of myocardial perfusion suggest a reduction of both endothelial-independent and endothelial-de-

pendent coronary vasomotion in long-term smokers [20, 21, 22]. However, there are no reports showing the effects of smoking on coronary vasomotion in young smokers.

The aim of this study was to determine whether young men with no history of coronary disease have identifiable abnormalities of coronary reactivity.

Materials and methods

Study subjects. Thirty male volunteers (aged 20–35 years) were enrolled in this study. Eighteen were smokers with a mean (±SD) age of 27.4±4.4 years who had been smoking cigarettes for 9.1±4.5 years (range 2–15 years) and 9.4±4.9 pack-years (1 pack-year being defined as smoking of 20 cigarettes per day for 1 year or the equivalent). Twelve non-smokers with a mean age of 26.3±3.3 years served as controls. None of the study participants had a history of cardiovascular disease, hyperlipidaemia, hypertension or diabetes mellitus, and none were receiving any medication. All had normal electrocardiograms at rest and during stress test.

All subjects refrained from caffeine-containing beverages for at least 24 h before the PET examination. The smokers refrained from smoking for at least 4 h before the PET study. The purpose and potential risks of this study were explained to all subjects when obtaining written informed consent, and the study was approved by The Ethics Committee of Hokkaido University Graduate School of Medicine.

Production of ^{15}O-carbon monoxide and ^{15}O-labelled water. For the production of ^{15}O compounds, a low-energy deutron accelerator was used (cyclotron: CYPRIS-HM18, Sumitomo Heavy Industries, Tokyo, Japan). ^{15}O-labelled water was produced with a dialysis technique in a continuously working water module. Sterility and pyrogen tests were performed daily to verify the purity of the product. Gas chromatograph analysis was performed to verify the purity of the product before each study.

Image acquisition and processing. PET was performed using a whole-body scanner (Siemens/CTI ECAT/EXACT HR+) equipped with germanium-68/gallium-68 retractable line sources for transmission scans. All emissions and transmissions were reconstructed using a filtered back-projection. The full-width at half-maximum at the centre of the field of view was 4.7 mm. The optimal imaging position was determined by a 5-min rectilinear scan. A 6-min transmission scan was then acquired for the purpose of attenuation correction of all subsequent emission scans.

Blood volume images were produced in the following manner. The subject's nostrils were closed and the subject inhaled ^{15}O-carbon monoxide for 1 min (0.14% CO mixed with room air). After inhalation of the tracer, a period of 3 min was allowed for the CO to combine with haemoglobin before a 5-min static scan was started. During the 5-min scan period, venous blood samples were drawn every 2 min and radioactivity in the whole blood was measured with an automatic gamma counter (Fukuda Electric Company, Tokyo, Japan). The inhaled dose in the CO examination was 2,000 MBq.

^{15}O radioactivity returned to the background level 15 min after the blood volume scan. Then ^{15}O-water was infused into an antecubital vein as a slow (2 min) infusion. ^{15}O-labelled water was administered four times in the entire study. The administered dose of ^{15}O-water was 500 MBq min^{-1}. A 20-frame dynamic PET scan was performed for 6 min, consisting of 6×5 s, 6×15 s and 8×30 s.

The analysis of PET images was accomplished as described previously [11, 12, 14, 15, 24, 25]. MBF (ml g^{-1} min^{-1}) of the whole left ventricle was measured using ^{15}O-water as the flow tracer and the previously validated ^{15}O-water slow infusion technique. Measurement was performed at rest, during ATP infusion and during CPT. Approximately 15 min after the resting study (to allow for decay of the ^{15}O radioactivity), we repeated the MBF measurement during ATP-induced hyperaemia. ATP was infused for 9 min at 0.16 mg kg^{-1} min^{-1}, according to a standard protocol [16]. PET acquisition was started 3 min after the beginning of ATP infusion. Fifteen minutes later, the third ^{15}O-water PET scan was performed at rest under the same image acquisition sequence. Fifteen minutes later, CPT was started as follows: The subject's left foot was immersed in ice water. Fifty seconds later, the fourth ^{15}O-water PET scan was performed. CPT was continued for at least 4 min [26]. During the entire study the subject's movement was minimized by fastening a Velcro strap across the subject's chest. Blood pressure was recorded at 1-min intervals, and ECG was monitored continuously throughout the procedure at baseline and every minute during ATP-induced hyperaemia and CPT.

In addition, because MBF is determined by cardiac workload [27], resting MBF and MBF during CPT were corrected by the rate-pressure product [RPP (beats per minute times millimetres of mercury)], which is an index of myocardial oxygen consumption: corrected MBF = (MBF/RPP)×7,211 (7,211 is mean RPP of another eight subjects at rest). Because ATP uncouples MBF from cardiac work, the hyperaemic flows were not corrected by the RPP. Myocardial flow reserve was calculated as the ratio of MBF during hyperaemia to MBF at rest.

Serum lipid measurements. Total serum cholesterol levels <200 mg/dl were considered normal; cholesterol levels between 200 and 239 mg/dl were defined as borderline; and levels >240 mg/dl were considered elevated. High-density lipoprotein (HDL) cholesterol ≥35 mg/dl was defined as normal. Low-density lipoprotein (LDL) cholesterol values <130 mg/dl were considered normal; values between 130 and 159 mg/dl were considered borderline; and values ≥160 mg/dl were considered elevated [28].

Statistical analysis. All data are presented as mean±SD. The Mann-Whitney *U* test was used to compare any pair of mean group values. Haemodynamic measurements and MBF at rest were compared with those during ATP-induced hyperaemia and during CPT using the Wilcoxon signed rank test. All *P* values less than 0.05 were considered to indicate statistical significance.

Results

The clinical characteristics of the 30 patients are shown in Table 1. There were no significant differences between the two groups. All procedures were well tolerated apart from common side-effects caused by ATP, such as flushing and feeling of tightness in the chest.

Haemodynamic findings

The procedure was well tolerated by all volunteers. Table 2 shows the heart rate, blood pressure and RPP at

Table 1. Clinical characteristics of the 30 subjects

	Smokers	Non-smokers	*P* values
No.	18	12	
Age (years)	27.4±4.4	26.3±3.3	NS
Total cholesterol (mg/dl)	182±30	163±44	NS
LDL cholesterol (mg/dl)	99±27.4	84±41	NS
HDL cholesterol (mg/dl)	52±18	62±10	NS
Height (cm)	170±6.3	173±5.5	NS
Weight (kg)	70±11	69±11	NS
Body mass index (kg/m^2)	24.0±3.7	23.0±3.0	NS
Smoking (years)	9.1±4.5	–	
Pack-years	9.4±4.9	–	

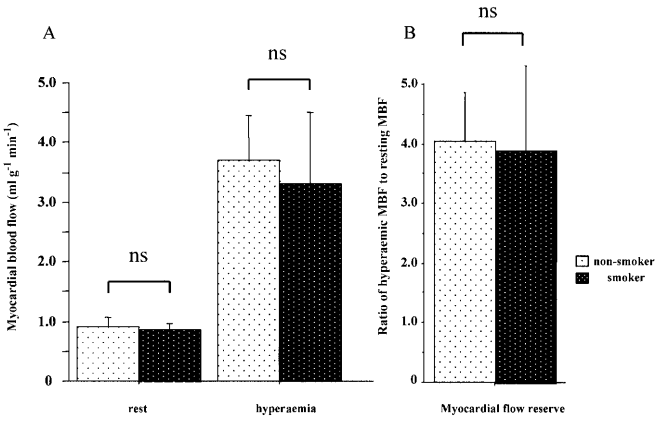

Fig. 1. A Absolute MBF corrected by RPP at rest and during the hyperaemic state in smokers (*right columns*) and non-smokers (*left columns*). The MBF response to hyperaemia in smokers was not significantly different from that in non-smokers. **B** Ratio of hyperaemic MBF to resting MBF (myocardial flow reserve) in smokers (*right column*) and non-smokers (*left column*). No significant difference in myocardial flow reserve was observed between the two groups

baseline, during hyperaemia and during CPT. In the smokers, systolic BP (137±15.5 mmHg), mean BP (97±12.7 mmHg) and diastolic BP (77±11.8 mmHg) during CPT were significantly higher than those in the non-smokers (128±7.2, 88±6.1 and 69±6.8 mmHg, respectively). However, there was no significant difference in RPP during CPT between the smokers and the non-smokers (10,430±1,820 vs 9,236±1,356 beats min^{-1} mmHg^{-1}; *P*=NS).

Myocardial blood flow and flow reserve

Table 3 shows the results of flow measurements. There was no significant difference in resting MBF between the smokers and the non-smokers (0.86±0.11 vs 0.92±0.14 ml g^{-1} min^{-1}; *P*=NS) (Fig. 1A). There was no significant difference in ATP-induced hyperaemic MBF

Table 2. The heart rate, blood pressure and MBF at rest, during hyperaemia and during CPT in the smokers and the non-smokers

	Smokers						Non-smokers					
	Syst. BP (mmHg)	Mean BP (mmHg)	Diast. BP (mmHg)	HR (beat/min)	RPP	Non-corrected MBF (ml g^{-1} min^{-1})	Corrected MBF (ml g^{-1} min^{-1})	Syst. BP (mmHg)	Mean BP (mmHg)	Diast. BP (mmHg)	HR (beat/min)	RPP	Non-corrected MBF (ml g^{-1} min^{-1})	Corrected MBF (ml g^{-1} min^{-1})
Rest	106±7.4	70±6.1	52±6.6	63±7.9	6,717±917	0.79±0.14	0.86±0.11	103±5.2	68±3.6	51±4.4	59±8.0	6,073±750	0.80±0.14	0.92±0.14
Hyperaemia	106±11.6	70±9.0	52±8.5	83± 111.5***	8,974± 2,003***	3.20± 1.12***		101±11.3	66±8.4	48±7.7	82± 14.6***	8,369± 1,785***	3.69± 0.76***	
CPT	137± 15.5*,***	97± 12.7*,***	77± 11.8*,***	76± 10.3***	10,430± 1,820***	0.95± 0.21*,***	0.65± 0.12*,***	128± 7.2***	88± 6.1***	69± 6.8***	72± 8.8***	9,236± 1,356***	1.12± 0.28***	0.87±0.12

*P<0.05 vs non-smokers; **P<0.05 vs rest; ***P<0.01 vs rest

Table 3. Coronary vascular resistance and MBF ratiosin the smokers and non-smokers

	Smokers (*n*=18)	Non-smokers (n=12)	*P* value
Coronary vascular resistance (mmHg g^{-1} min^{-1} ml^{-1})			
Rest	90.8±19.1	88.8±16.0	NS
Hyperaemia	26.0±11.6	18.6±4.7	NS
CPT	106.9±30.2	82.1±16.8	<0.05
Ratio of stress MBF to resting MBF			
Hyperaemic MBF/resting MBF	3.78±1.83	4.03±0.68	NS
CPT MBF/resting MBF	0.77±0.14	0.96±0.19	<0.05

between the smokers and the non-smokers (3.20±1.12 vs 3.69±0.76 ml g^{-1} min^{-1}; *P*=NS) (Fig. 1A). Myocardial flow reserve showed no significant difference between the smokers and the non-smokers (3.78±1.83 vs 4.03±0.68; *P*=NS) (Fig. 1B). In addition, in the study of smokers, myocardial flow reserve did not correlate with pack-years (*r*=0.29; *P*=NS). Coronary vascular resistance was calculated as the ratio of mean arterial blood pressure (mmHg) to MBF (mm g^{-1} min^{-1}). Coronary vascular resistance at rest and during hyperaemia was not significantly different between the smokers and the non-smokers (rest: 90.8±19.1 vs 88.8±16.0 mmHg g^{-1} min^{-1} ml^{-1}, hyperaemia: 26.0±11.6 vs 18.6±4.7 mmHg g^{-1} min^{-1} ml^{-1}; *P*=NS).

Myocardial blood flow at rest and during cold pressor test

To account for interindividual differences in the flow response to cold, MBF was corrected by the RPP at rest and during CPT in both groups. There were no significant differences between resting MBF in the first measurement (at the beginning) and in the second measurement (between the ATP stress test and CPT) in either the smokers (0.86±0.11 vs 0.84±0.11 ml g^{-1} min^{-1}; *P*=NS) or the non-smokers (0.92±0.14 vs 0.91±0.09 ml g^{-1} min^{-1}; *P*=NS). MBF corrected by RPP during CPT was significantly lower in the smokers than in the non-smokers (0.65±0.12 vs 0.87±0.12 ml g^{-1} min^{-1}; *P*<0.01) (Fig. 2A). The ratio of CPT-MBF to resting MBF was significantly lower in the smokers than in the non-smokers (0.77±0.14 vs 0.96±0.19; *P*<0.05) (Fig. 2B). In addition, in the study of smokers the ratio of CPT-MBF to resting MBF was inversely correlated with pack-years (*r*=–0.57; *P*=0.014) (Fig. 3), but not significantly correlated with smoking years (*r*=–0.38; *P*=NS). Coronary vascular resistance during CPT was significantly higher in the smokers than in the non-smokers (106.9±30.2 vs 82.1±16.8 mmHg g^{-1} min^{-1} ml^{-1}; *P*<0.05).

Serum lipid profiles

Total cholesterol, LDL cholesterol, HDL cholesterol and body mass index did not differ between the smokers and

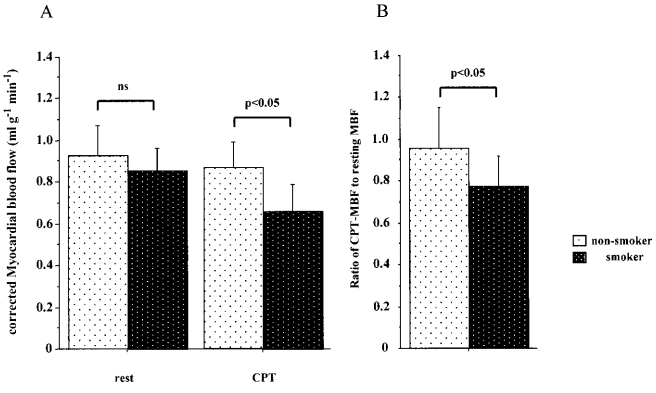

Fig. 2. A Absolute MBF corrected by RPP at rest and during CPT in smokers (*right columns*) and non-smokers (*left columns*). MBF response to CPT was significantly reduced in smokers compared with non-smokers. **B** Ratio of CPT-MBF to resting MBF in the smokers (*right column*) and the non-smokers (*left column*). Ratio of CPT-MBF to resting MBF was significantly reduced in smokers compared with non-smokers

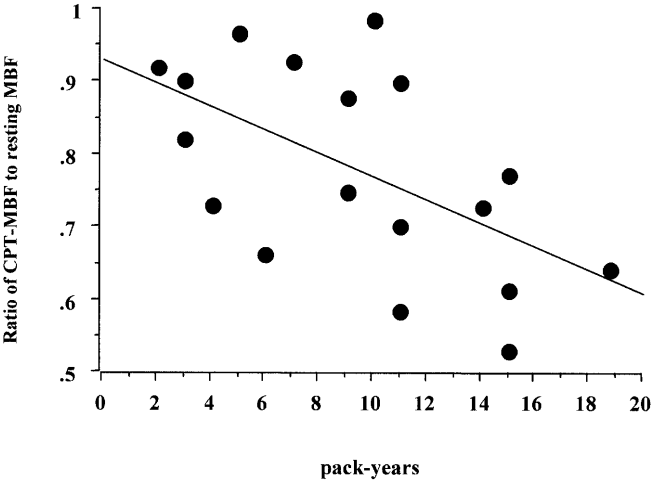

Fig. 3. Correlation between the ratio of CPT-MBF to resting MBF and pack-years in smokers. An inverse correlation was observed (*r*=–0.57; *P*=0.014)

the non-smokers (Table 1). None of the non-smokers had elevated total cholesterol levels [10 were normal (<200) and two were borderline (200–240)], none had decreased HDL cholesterol levels and two had borderline LDL cholesterol levels. Among the smokers, none had elevated total cholesterol values (11 were normal and 7 had borderline values), none had decreased HDL cholesterol values and two had borderline LDL cholesterol levels.

Discussion

This study demonstrates that smoking for about 9 years causes coronary artery endothelial dysfunction even in young healthy adults. These findings suggest that neither increased age nor smoking for >9 years is a prerequisite for coronary endothelial dysfunction as demonstrated by CPT [20, 21].

Effect of smoking on coronary artery

The mechanisms of smoking-associated endothelial damage have not been established, but a number of factors may contribute to the impairment of the functional integrity of the endothelium. Nicotine has been reported to produce structural damage in endothelial cells [7, 9, 29]. Smokers appear to be particularly susceptible to the activity of oxygen free radicals (which are often increased in smokers) [30]. Smoking-generated free radicals and abnormal lipids may exert an additive effect in reducing coronary endothelial vasodilator function. The free radicals in cigarette smoke may also initiate and/or accelerate secondary processes, including depletion of anti- oxidants (such as vitamin C, vitamin E or L-arginine) [31, 32] and activation of phagocyte-platelet-endothelial cell interactions.

Effects of smoking on hyperaemic flow

In this study, there was no difference in the coronary vasodilator response of smokers and non-smokers to ATP infusion, confirming the results of Czernin et al. [22] On the other hand, two other studies demonstrated a decrease in myocardial flow reserve in response to vasodilators in long-term smokers [20, 31]. However, these subjects were older smokers and myocardial flow reserve was within normal limits and only relatively decreased compared with the non-smokers. The present study excluded patients with other risk factors such as hyperlipidaemia, hypertension and diabetes mellitus, which may explain why hyperaemic MBF was not significantly reduced.

Effect of smoking on endothelium-dependent coronary vasomotion

Several mechanisms might account for the smoking-induced alteration. The CPT induces sympathetic release of noradrenaline and adrenaline. Both α- and β-adrenergic activity neurally mediates effects in the coronary vascular bed [18]. Sympathetic nerve activation dilates normal coronary arteries by several mechanisms, whereas in mild atheromatous vessels there is epicardial vasoconstriction and reduction in coronary flow [18]. Therefore, the CPT has been proposed as a non-invasive method to probe endothelium-dependent coronary vasomotion. The reflex sympathetic stimulation of cold pressor resulted in a significant increase in MBF. MBF is determined by cardiac workload [27]. The vascular response to this stress in myocardium is directly related to the increase in myocardial work induced by this stimulation. The present study showed no significant difference in RPP during CPT between the smokers and the non-smokers, but MBF during CPT was significantly lower in smokers than in non-smokers. Among smokers, MBF corrected by RPP was significantly lower during CPT than at rest. This means that MBF was not sufficiently increased for increased myocardial work. These data reflect decreased endothelium-dependent coronary vasomotion and may indicate the beginning of atherosclerosis in the smokers. Furthermore, such a decrease may be related to pack-years, indicating a significant relationship between the severity of endothelial dysfunction and smoking history. We also evaluated the relationship between years of smoking history and response to CPT, but no significant relationship was observed. The fact that pack-years was significantly related to endothelial dysfunction means that not only the length of smoking history but also the amount of smoking has an important effect on endothelial function.

This study showed that blood pressure during CPT was higher in the smokers than in the non-smokers. Smoking releases catecholamines from local adrenergic nerve terminals and the adrenal medulla, resulting in a variety of cardiovascular changes, including increased heart rate and blood pressure, cutaneous vasoconstriction and increased muscle blood flow [5, 33]. It has been demonstrated that cigarette smoking is a major risk factor for coronary spasm [4, 34]. Smokers suffer from activation of the sympathetic nervous system each time they smoke a cigarette. Such stress may lead to an abnormal reaction of the sympathetic nerves and may have been responsible for the abnormal response to CPT observed among the smokers.

Technical considerations

The aim of this study was to clarify the effect of smoking on endothelium in young adults. Because oestrogen

might affect vasomotor function and thus response to CPT, female subjects were not included in the study population.

In this study ATP stress test and CPT were performed on the same day. We demonstrated no significant differences between the resting MBF in the first measurement (at the beginning) and in the second measurement (between the ATP stress test and CPT). The interval between the ATP stress test and CPT was considered sufficient to enable recovery from ATP stress In addition, our measurements of MBF were quite reproducible.

This study did not measure catecholamine levels during CPT. Since there were individual differences in CPT response, as a marker of adrenergic sympathetic stimulation, MBF during CPT was corrected by RPP.

A close correlation has previously been demonstrated between serum lipid levels and endothelial dysfunction [35]. In the present study on young healthy subjects, however, total cholesterol, LDL cholesterol and HDL cholesterol levels did not differ significantly between the smokers and the non-smokers. Thus, the observed differences in flow-dependent coronary dilation cannot be attributed to different lipoprotein profiles between the smokers and the non-smokers.

Conclusion

This study shows diminished endothelium-dependent coronary artery vasodilator function in healthy young smokers despite normal endothelium-independent vasodilator function. Thus smoking may carry a high risk for development of atherosclerosis in coronary arteries even in healthy young adults.

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