International Journal of Cardiovascular Imaging

Altered coronary endothelial function in young smokers detected by magnetic resonance assessment of myocardial blood flow during the cold pressor test

--Manuscript Draft--

Manuscript Number:	CAIM-D-13-00592R1
Full Title:	Altered coronary endothelial function in young smokers detected by magnetic resonance assessment of myocardial blood flow during the cold pressor test
Article Type:	ASCI Special Issue
Keywords:	Coronary endothelial function; myocardial blood flow; cold pressor test; Magnetic resonance imaging; smoking
Corresponding Author:	YASUTAKA ICHIKAWA, M.D. Mie University Hospital Tsu, Mie JAPAN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Mie University Hospital
Corresponding Author's Secondary Institution:	
First Author:	YASUTAKA ICHIKAWA, M.D.
First Author Secondary Information:	
Order of Authors:	YASUTAKA ICHIKAWA, M.D.
	KAKUYA KITAGAWA, M.D.
	SHINGO KATO, M.D.
	KAORU DOHI, M.D.
	TADANORI HIRANO, M.D.
	MASAAKI ITO, M.D.
	HAJIME SAKUMA, M.D.
Order of Authors Secondary Information:	
Abstract:	Endothelial dysfunction is a key element in early atherogenesis. The purposes of this study were to evaluate the feasibility of magnetic resonance (MR) assessment of altered myocardial blood flow (MBF) in response to the cold pressor test (CPT), and to determine if coronary endothelial dysfunction in young smokers can be detected with this noninvasive approach. Fourteen healthy non-smokers (31 ± 6 years) and 12 smokers (34 ± 8 years) were studied. Breath-hold phase-contrast cine MR imaging (PC-MRI) of the coronary sinus (CS) were obtained at rest and during the CPT. MBF was measured as CS flow divided by LV mass and the rate pressure product. In non-smokers, MBF was 0.88 ± 0.19 ml/min/g at rest and significantly increased to 1.13 ± 0.26 ml/min/g during the CPT (P = 0.0001). In smokers, MBF was 0.94 ± 0.26 ml/min/g at rest and 0.96 ± 0.30 ml/min/g during the CPT (P = 0.73). ΔMBF (MBF during the CPT − MBF at rest) was significantly reduced in smokers compared with non-smokers (0.02 ± 0.20 ml/min/g vs. 0.26 ± 0.18 ml/min/g, P = 0.005). The intra-class correlation coefficient between measurements by two observers was 0.90 for ΔMBF. A significant reduction in MBF response to CPT was demonstrated in young smokers with PC-MRI at 1.5 tesla. This noninvasive method has great potential for assessment of coronary endothelial function.
Response to Reviewers:	Dear T-H Kim, Guest Editor We thank you for giving us an opportunity for publication of International Journal of Cardiovascular Imaging.

	As the reviewer suggested, we revised the final paragraph in the introduction section. The revision has been uploaded. We wish to express in advance our great appreciation for the time and consideration of the reviewers and editors regarding our revised manuscript. Sincerely, Yasutaka Ichikawa, M.D. Department of Radiology, Mie University Hospital E-mail: yasutaka@clin.medic.mie-u.ac.jp

Original research paper

Altered coronary endothelial function in young smokers detected by magnetic resonance assessment of myocardial blood flow during the cold pressor test

Yasutaka Ichikawa, MD[1], Kakuya Kitagawa, MD[2], Shingo Kato, MD[2], Kaoru Dohi, MD[3], Tadanori Hirano, MD[1], Masaaki Ito, MD[3], Hajime Sakuma, MD[2]

[1]Department of Radiology, Matsusaka Central Hospital, 102 Kobou, Kawai, Matsusaka, Mie, 515-8566 Japan

[2]Department of Radiology, and [3]Department of Cardiology, Mie University Hospital, 2-174 Edobashi, Tsu, Mie 514-8507, Japan

The authors report no conflicts of interest.

Total word account: 4626 words
Three tables and 6 figures
Brief title: Coronary sinus flow during the cold pressor test

Address for correspondence: Yasutaka Ichikawa, MD
Department of Radiology, Matsusaka Central Hospital
102 Kobou, Kawai, Matsusaka, Mie 515-8566, Japan
Phone: +81-598-21-5252 ; Fax: +81-59-232-8066
E-mail: yasutaka@clin.medic.mie-u.ac.jp

Abstract Endothelial dysfunction is a key element in early atherogenesis. The purposes of this study were to evaluate the feasibility of magnetic resonance (MR) assessment of altered myocardial blood flow (MBF) in response to the cold pressor test (CPT), and to determine if coronary endothelial dysfunction in young smokers can be detected with this noninvasive approach. Fourteen healthy non-smokers (31 ± 6 years) and 12 smokers (34 ± 8 years) were studied. Breath-hold phase-contrast cine MR imaging (PC-MRI) of the coronary sinus (CS) were obtained at rest and during the CPT. MBF was measured as CS flow divided by LV mass and the rate pressure product. In non-smokers, MBF was 0.88 ± 0.19 ml/min/g at rest and significantly increased to 1.13 ± 0.26 ml/min/g during the CPT (P = 0.0001). In smokers, MBF was 0.94 ± 0.26 ml/min/g at rest and 0.96 ± 0.30 ml/min/g during the CPT (P = 0.73). ΔMBF (MBF during the CPT − MBF at rest) was significantly reduced in smokers compared with non-smokers (0.02 ± 0.20 ml/min/g vs. 0.26 ± 0.18 ml/min/g, P = 0.005). The intra-class correlation coefficient between measurements by two observers was 0.90 for ΔMBF. A significant reduction in MBF response to CPT was demonstrated in young smokers with PC-MRI at 1.5 tesla. This noninvasive method has great potential for assessment of coronary endothelial function.

Keywords Coronary endothelial function · Myocardial blood flow · Cold pressor test · Magnetic resonance imaging · Smoking

Introduction

The endothelium plays an important role in antiatherosclerotic and antithrombotic events in vessels [1-2]. Patients with coronary endothelial dysfunction have a higher cardiovascular event rate than those with normal coronary vasomotion [3-4]. Several mechanisms contribute to impairment of endothelial function [5-6]; for example, chronic smoking may cause endothelial dysfunction in subjects without epicardial coronary artery stenosis [7-9].

The cold pressor test (CPT) has been used as one of several approaches to evaluate endothelial function [10-13]. In healthy subjects, the vasodilatory effect of the CPT is mediated through a central sympathetic response which stimulates the release of nitric oxide from the coronary endothelium [11]. Prior studies have shown that a vasoconstrictive response to the CPT is observed in angiographically normal subjects with cardiovascular risk factors [14-15], and that such a response is an independent predictor of future cardiac events [15]. Thus, a flow response to CPT might be a sensitive measure of coronary endothelial dysfunction in the early stages of atherosclerosis.

Phase-contrast cine magnetic resonance imaging (PC-MRI) of coronary sinus (CS) blood flow can provide a noninvasive estimate of total myocardial blood flow (MBF) [16], because the CS drains over 96% of the myocardial venous blood from the left ventricle (LV) [17]. Quantification of CS flow using PC-MRI in response to vasodilator pharmacological stress has been used to assess coronary flow reserve in patients with hypertrophic cardiomyopathy, heart failure and dilated cardiomyopathy [18-20]. In a more recent study by Maroules et al. [21], they investigated coronary flow response to CPT in asymptomatic women using spiral velocity encoded MRI at 3

tesla. However, the spiral velocity encoded sequence at 3 tesla is a research pulse sequence that is not available in clinical cardiac MRI studies. In addition, altered MBF response to CPT in subjects with abnormal endothelial function compared with control subjects due to smoking or atherosclerosis has not been investigated.

The purposes of this study were to evaluate the feasibility and intra- and inter-observer variabilities of measuring MBF alteration during the CPT by using a 1.5 tesla MR system and a PC-MRI sequence that are widely available, and to determine if impaired coronary endothelial dysfunction in smokers can be detected with this noninvasive approach.

Methods

Study population

The study population consisted of 14 healthy male non-smokers (mean age, 30.9 ± 5.9 years) and 12 healthy male smokers (mean age, 33.8 ± 7.8 years) who had been smoking ≥ 5 years (range, 5–20 years) and a total of 10.6 ± 8.0 pack-years (1 pack-year is defined as smoking of 20 cigarettes per day for 1 year or the equivalent). All subjects were male and non-obese (body mass index < 27 kg/m^2), and did not have hypertension, diabetes or a family history of vascular disease. They were clinically well and taking no regular cardiovascular medication or antioxidant vitamin supplementation. Before MRI examination, all subjects abstained from consuming caffeine and alcohol and from smoking for at least 12 hours, and from consuming flavonoid-containing food for at least 24 hours. This study was approved by the local Institutional Review Board, and all participants provided written informed consent.

MRI acquisition

MRI was acquired at 1.5 tesla with 5-channel cardiac coils (Achieva, Nova-dual gradient; Philips Medical Systems, Best, The Netherlands). After placement of vector-electrocardiographic monitoring leads, subjects underwent imaging in the supine position. For cardiac orientation, scout images were acquired in three orthogonal planes. After acquiring vertical long axis and horizontal long axis cine images of the LV using a steady-state free precession sequence, short-axis cine images of the LV were acquired from apex to base with suspended shallow breath-holds (repetition time = 3.2 ms, echo time = 1.6 ms, flip angle = 55°, field of view = 350 × 350 mm, reconstruction matrix = 256 × 256, reconstruction pixel size = 1.37 × 1.37 mm, slice thickness = 10 mm, and phases per cardiac cycle = 20).

To identify the location of the CS, axial plane breath-hold cine MRI was obtained through the atrioventricular groove (repetition time = 2.9 ms, echo time = 1.5 ms, flip angle = 55°, field of view = 350 × 350 mm, reconstruction matrix = 256 × 256, reconstruction pixel size = 1.37 × 1.37 mm, slice thickness = 5 mm, and phases per cardiac cycle = 20) (Fig. 1). The imaging plane for PC-MRI was placed perpendicular to the CS, approximately 1 to 2 cm proximal to the entrance of the CS into the right atrium and distal to the merging portion of the middle cardiac vein. CS flow measurements were obtained during a suspended shallow breath-hold using an electrocardiography-triggered turbo phase contrast field echo sequence with k-space segmentation (repetition time = 5.6 ms, echo time = 3.6 ms, flip angle = 15°, slice thickness = 5 mm, field of view = 240 × 194 mm, acquisition matrix = 160 × 112, acquisition pixel size = 1.5 × 1.7 mm, velocity encoding = ± 50 cm/s, reconstruction

matrix = 256 × 256, reconstruction resolution = 0.9 × 0.9 mm, and phases per cardiac cycle = 20).

Cold pressor test

After acquisition of cine MRI of the LV and PC-MRI of the CS at rest, the subject's foot was immersed in a MRI-compatible ice-water bath (at a temperature of approximately 7°C) for 2 minutes. After 1 minute of foot immersion, flow measurement of the CS was performed during the CPT. Heart rate and blood pressure were measured using a noninvasive and MRI-compatible electrocardiogram and calf blood pressure monitor at baseline and at 30-s intervals throughout each study. The rate-pressure product (RPP) was calculated as systolic blood pressure × heart rate.

Image analysis

Cine MRI and PC-MRI were analyzed using a workstation (ViewForum, Philips Medical Systems, Best, The Netherlands). For measurement of LV mass and LV volume, two independent observers manually traced epi- and endo-cardial borders of the LV wall on short axis cine images at end-diastole and end-systole. LV mass was calculated as the sum of myocardial areas multiplied by the slice thickness and the density (1.05 g/ml) of myocardial tissue [22].

CS flow was measured by tracing the CS contour on each magnitude image throughout the cardiac cycle by two independent observers blinded to both subject group (control vs. smoker) and state (rest vs. CPT). The area of the CS was recorded, and the traced region was automatically transferred to the corresponding phase

images in order to measure average flow velocity in the CS. Phasic blood flow was calculated as the product of the area and spatial average flow velocity. Mean volume flow was derived by means of integration of phasic flow over time. To compensate for the through-plane motion and phase offset error, a second region of interest was traced for each phase image on the adjacent tissue of CS [18, 23]. Flow velocity in each pixel within the vessel lumen was corrected by subtracting the mean velocity in the second region of interest. According to previous studies [23-25], MBF (ml/min/g) was calculated as follows: MBF = (CS flow / (RPP $\times$ LV mass)) $\times$ 7500. The difference between MBF during the CPT and at rest and the percent change of MBF during the CPT were calculated as follows:

ΔMBF = MBF during the CPT − MBF at rest, and

Percent change of MBF (%) = ΔMBF / MBF at rest $\times$ 100.

Statistical analysis

Data were statistically analyzed using SPSS software, version 11.5 (SPSS, Inc., Chicago, IL, USA). Continuous values are presented as mean ± standard deviation or as median and interquartile range (IQR). MRI data are reported as an average of measurements recorded by two observers. Differences between control subjects and smokers in age, height, weight, BMI, LV end-diastolic volume, ejection fraction, LV mass, MBF, ΔMBF, and percent change of MBF were tested with the unpaired Student's *t*-test. Differences in LV end-systolic volume between control subjects and smokers were analyzed by the Mann-Whitney *U* test. The effect of the CPT on blood pressure, heart rate, and MBF was analyzed by the paired Student's *t*-test. For investigating the effect of the CPT on RPP, the Wilcoxon signed-rank test was used.

For correlation analysis, we calculated Spearman's correlation coefficients. Inter- and intra-observer variabilities in measuring MBF and ΔMBF were evaluated using an intra-class correlation coefficient (ICC), the Bland-Altman method [26] and a repeatability coefficient. The repeatability coefficient was calculated as 1.96 times the standard deviation of the differences, as proposed by Bland and Altman [26]. All *P* values < 0.05 were considered to represent statistical significance.

Results

Clinical characteristics of all 26 subjects are shown in Table 1. There were no significant differences in clinical characteristics between smokers and non-smokers. No significant differences were found between non-smokers and smokers for LV end-diastolic volume, end-systolic volume, ejection fraction, heart rate or LV mass (159.6 ± 14.4 ml vs. 163.8 ± 24.2 ml, *P* = 0.60; 62.2 ± 7.6 ml vs. 62.4 ± 13.9 ml, *P* = 0.96; 63.1 ± 7.6% vs. 62.1 ± 4.2%, *P* = 0.68; 61.9 ± 9.3 beats/min vs. 63.5 ± 9.9 beats/min, *P* = 0.67; and 88.1 ± 9.4 g vs. 90.4 ± 11.6 g, *P* = 0.58, respectively).

PC-MRI was acquired at rest and during the CPT at diagnostic quality in all subjects. Representative CS volume flow curves in the cardiac cycle are shown in Figure 2. Peak volume flow in the CS was observed during early diastole. Hemodynamic parameters and MBF values at rest and during the CPT are shown in Table 2.

In non-smokers, MBF was 0.88 ± 0.19 ml/min/g at rest, and significantly increased to 1.13 ± 0.26 ml/min/g during the CPT (*P* = 0.0001). In smokers, MBF was 0.94 ± 0.26 ml/min/g at rest and 0.96 ± 0.30 ml/min/g during the CPT (*P* = 0.73). Individual ΔMBF values and percent change of MBF are shown in Figure 3. Mean

ΔMBF during the CPT in smokers (0.02 ± 0.20 ml/min/g [95% CI: 0.16 to 0.35 ml/min/g]) was significantly lower than in non-smokers (0.26 ± 0.18 ml/min/g [95% CI: -0.10 to 0.14 ml/min/g], *P* = 0.005). The mean percent change of MBF in smokers was also significantly lower than in non-smokers (3.6 ± 19.7% vs. 30.7 ± 21.3%, *P* = 0.003). There was no significant correlation between pack-years and percent change of MBF in smokers (*r* = -0.39, *P* = 0.20) (Fig. 4).

Inter- and intra-observer variation in MBF measurements are summarized in Table 3. The ICC between the measurements by the two observers was 0.91 for MBF at rest, 0.96 for MBF during the CPT and 0.90 for ΔMBF. Bland-Altman plots of inter- and intra-observer variabilities in measuring ΔMBF and percent change of MBF are presented in Figures 5 and 6, respectively. The mean difference between the two observers was 0.03 ± 0.10 ml/min/g for ΔMBF and 2.3 ± 12.7% for percent change of MBF.

Discussion

The present study demonstrated that noninvasive assessment of MBF response to CPT using PC-MRI can provide detection of altered endothelial function in coronary circulation. Impaired coronary endothelial function in healthy smokers was detected with PC-MRI at 1.5 tesla without administration of contrast medium or exposure to ionizing radiation.

For evaluating coronary endothelial function, acetylcholine infusions during X-ray coronary angiography or coronary flow measurement by an intracoronary Doppler flow wire have been used [27]. However, the clinical utility of these methods is limited to patients with a high probability of coronary artery disease due to their

invasiveness. A noninvasive approach that permits assessment of coronary endothelial function is desirable. Several investigators have used myocardial perfusion positron emission tomography (PET) to assess MBF before and during the CPT [24-25, 28-30]. One previous PET study demonstrated that cigarette smoking causes impairment of endothelium-dependent coronary vasodilatory capacity and abnormal coronary flow response to CPT [28]. However, myocardial perfusion PET is a more complex and time-consuming method than MRI. MRI flow measurement does not expose patients to ionizing radiation nor does it require venous injection of contrast medium; thus, an MR approach using a 1.5 tesla MR system can be used in many clinical hospitals.

In the present study, MRI measurement of MBF in non-smokers was 0.88 ± 0.19 ml/min/g at rest, in good agreement with resting MBF values reported in previous studies using myocardial perfusion PET [28-30]. A previous PET study using ^{15}O-water demonstrated that MBF at rest was 0.80 ± 0.14 ml/min/g in 12 young non-smokers without coronary risk factors [29]. Another PET study using ^{13}N-ammonium demonstrated that MBF at rest was 0.75 ± 0.17 ml/min/g in 10 healthy subjects without coronary risk factors [28]. In addition, MRI measurement of resting MBF in the present study was consistent with values calculated from CS flow divided by LV mass, a method used by Kawada et al. [18] (0.74 ± 0.23 ml/min/g) and by Schwitter et al. [31] (0.53 ± 0.14 ml/min/g).

Because MBF at rest and after sympathetic stimulation is strongly determined by cardiac workload, we corrected MBF values for corresponding RPP in this study. According to previous studies [8, 29-30, 32-34], the percent change of MBF during CPT in healthy young non-smokers was 4.5-37% with RPP correction and 40-55% without correction. Siegrist et al. [25] reported that the change of MBF during the CPT

was significantly lower after correcting by RPP. In our study, the percent change of MBF during the CPT in non-smokers was approximately 30%, in concordance with the results of previous studies using RPP-corrected MBF.

In the current study, impairment of coronary endothelial function in young healthy smokers was detected using a noninvasive PC-MRI approach. Mean ΔMBF during the CPT in smokers (0.02 ± 0.20 ml/min/g) was significantly lower than in non-smokers (0.26 ± 0.18 ml/min/g, $P = 0.005$). The impaired MBF response to the CPT in smokers observed in the present study is in agreement with the findings of previous PET studies [28-29]. Schindler et al. [28] investigated MBF response to the CPT in smokers using ^{13}N-ammonium PET. They found that ΔMBF during the CPT in smokers was -0.08 ± 0.16 ml/min/g. In the present study, the 95% CI of ΔMBF was 0.16 to 0.35 ml/min/g for non-smokers, and -0.10 to 0.14 ml/min/g for smokers, indicating that an MRI approach with the CPT can detect impaired coronary endothelial dysfunction in young smokers.

Accuracy of phase-contrast flow volume MRI measurements in small blood vessels depends on spatial resolution. A previous study demonstrated that accurate blood flow volume can be determined with PC-MRI if the number of pixels per vessel diameter is 3 or greater [35]. In our current study, PC-MRI was acquired with a field of view of 240 × 194 mm, acquisition matrix of 160 × 112, acquisition pixel size of 1.5 × 1.7 mm and reconstruction resolution of 0.9 × 0.9 mm. These parameters allowed for > 3 pixels per vessel diameter. Thus, the spatial resolution in the present study was acceptable for quantifying flow volume in the CS.

As reviewed by Czernin and Waldherr [36], cigarette smoking acutely increases coronary blood flow in healthy individuals by up to 40%. In this study, to investigate the long-term effect of cigarette smoking on MBF response to CPT, all

subjects abstained from smoking for at least 12 hours before MRI examination. The mechanism by which smoking causes endothelial dysfunction is thought to be primarily the effects of oxidant chemicals. Oxidant chemicals degrade nitric oxide and reduce nitric oxide release, therefore antagonizing the actions of nitric oxide to dilate blood vessels and inhibiting platelet aggregation [8]. A previous study demonstrated that coronary endothelial dysfunction was reversible by short-term smoking cessation in young smokers, but not in middle-aged smokers [37]. Long-term smoking exposure could lead to more advanced coronary endothelial dysfunction and atherosclerosis possibly via oxidative stress.

Several limitations need to be acknowledged in this study. First, the number of subjects was limited and a larger number of subjects might be necessary to confirm our findings. However, an impaired CS flow response to the CPT in young male smokers was clearly demonstrated with the phase contrast cine MRI with sufficient inter- and intra-observer variability in this study. In addition, MBF at rest and during the CPT as well as ΔMBF observed in this study are in good accordance with the results by previous PET studies [28-30]. The current study shows that MR CPT method has great potential for noninvasive assessment of coronary endothelial function. Second, only male smokers and non-smokers were enrolled in the present study. A recent study in young, healthy men and women demonstrated a higher increase in MBF during the CPT in women than in men, presumably because estrogen improves coronary endothelial function [32]. Because MBF response to CPT is smaller in men, the feasibility and reproducibility of assessing ΔMBF during the CPT is more difficult in men than in women. Thus, the major aims of this study, to test the feasibility and reproducibility of the MR CPT method, have been validated in the current study. However, further study is necessary to evaluate the effect of smoking

on coronary endothelial function in women. Third, the present technique was not compared with invasive measurement of CS flow, which is considered to be a gold standard measurement. However, since the purpose of the present study was to explore the feasibility of assessing coronary endothelial function in asymptomatic volunteers, it was not considered ethically appropriate to perform intracoronary catheterization or transesophageal echocardiography in the present study.

In conclusion, an impaired CS flow response to the CPT in young male smokers was clearly demonstrated with PC-MRI by using a 1.5 tesla MR system that is widely available. This technique has sufficient inter- and intra-observer variability. MRI measurement of the response to the CPT could be of great value for assessing coronary endothelial dysfunction and coronary microvascular disease.

Acknowledgments The authors would like to thank the Advanced School for Core Investigators program organized by the Asian Society of Cardiovascular Imaging for providing valuable suggestions and comments on our research.

Figure Legends

Fig. 1 Coronary sinus (CS) flow measurement by phase-contrast cine MRI. **a** A representative axial cine image of the CS with slice orientation for flow imaging (*solid line*). Representative phase-contrast cine images are also depicted: **b** magnitude image and **c** velocity map of the CS (*arrows*).

Fig. 2 Representative coronary sinus (CS) volume flow curves in the cardiac cycle at baseline and during the cold pressor test (CPT). Peak volume flow in CS was observed during early diastole.

Fig. 3 Individual ΔMBF values and percent change of MBF in non-smokers and smokers. The mean ΔMBF during the CPT in non-smokers was 0.26 ± 0.18 ml/min/g. The mean ΔMBF during the CPT in smokers was 0.02 ± 0.20 ml/min/g, and was significantly lower than in non-smokers (P = 0.005). The mean percent change of MBF in smokers was significantly lower than in non-smokers (3.6 ± 19.7% vs. 30.7 ± 21.3%, P = 0.003).

Fig. 4 Relationship between percent change of MBF and pack-years. Number of pack-years is calculated as packs smoked per day multiplied by years as a smoker. There was no significant correlation between pack-years and percent change of MBF in smokers (r = -0.39, P = 0.20, y = -0.79x + 13.3).

Fig. 5 Bland-Altman plots of (**a**) inter-observer and (**b**) intra-observer variability of ΔMBF (ml/min/g). The mean difference in ΔMBF inter-observer variability was 0.03 ±

0.10 ml/min/g and intra-observer variability was 0.02 ± 0.07 ml/min/g.

Fig. 6 Bland-Altman plots of (**a**) inter-observer and (**b**) intra-observer variability of percent change of myocardial blood flow (MBF). The mean difference in MBF inter-observer variability was 2.3 ± 12.7% and intra-observer variability was 2.6 ± 10.5%.

References

1. Vallance P, Chan N (2001) Endothelial function and nitric oxide: clinical relevance. Heart 85(3):342-350
2. Vanhoutte PM (2003) Endothelial control of vasomotor function: from health to coronary disease. Circ J 67(7):572-575
3. Halcox JP, Schenke WH, Zalos G et al (2002) Prognostic value of coronary vascular endothelial dysfunction. Circulation 106(6):653-658
4. Suwaidi JA, Hamasaki S, Higano ST et al (2000) Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 101(9):948-954
5. Higashi Y, Noma K, Yoshizumi M et al (2009) Endothelial function and oxidative stress in cardiovascular diseases. Circ J 73(3):411-418
6. Kirma C, Akcakoyun M, Esen AM et al (2007) Relationship between endothelial function and coronary risk factors in patients with stable coronary artery disease. Circ J 71(5):698-702
7. Ambrose JA, Barua RS (2004) The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 43(10):1731-1737
8. Benowitz NL (2003) Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. Prog Cardiovasc Dis 46(1):91-111
9. Hill JM, Zalos G, Halcox JP et al (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 348(7):593-600
10. Nitenberg A, Paycha F, Ledoux S et al (1998) Coronary artery responses to physiological stimuli are improved by deferoxamine but not by L-arginine in non-insulin-dependent diabetic patients with angiographically normal coronary arteries and no other risk factors. Circulation 97(8):736-743
11. Nabel EG, Ganz P, Gordon JB et al (1988) Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. Circulation 77(1):43-52
12. Monahan KD, Feehan RP, Sinoway LI et al (2013) Contribution of sympathetic activation to coronary vasodilatation during the cold pressor test in healthy men: effect of ageing. J Physiol 591(Pt 11):2937-2947
13. Hwang HJ, Youn HJ, Lee MY et al (2012) Change of coronary flow velocity during the cold pressor test is related to endothelial markers in subjects with chest pain and a normal coronary angiogram. Clin Cardiol 35(2):119-124
14. Antony I, Aptecar E, Lerebours G et al (1994) Coronary artery constriction caused by the cold pressor test in human hypertension. Hypertension 24(2):212-219
15. Nitenberg A, Valensi P, Sachs R et al (2004) Prognostic value of epicardial coronary artery constriction to the cold pressor test in type 2 diabetic patients with angiographically normal coronary arteries and no other major coronary risk factors. Diabetes Care 27(1):208-215
16. van Rossum AC, Visser FC, Hofman MB et al (1992) Global left ventricular perfusion: noninvasive measurement with cine MR imaging and phase velocity mapping of coronary

venous outflow. Radiology 182(3):685-691

17. Hood WB Jr (1968) Regional venous drainage of the human heart. Br Heart J 30(1):105-109
18. Kawada N, Sakuma H, Yamakado T et al (1999) Hypertrophic cardiomyopathy: MR measurement of coronary blood flow and vasodilator flow reserve in patients and healthy subjects. Radiology 211(1):129-135
19. Lund GK, Watzinger N, Saeed M et al (2003) Chronic heart failure: global left ventricular perfusion and coronary flow reserve with velocity-encoded cine MR imaging: initial results. Radiology 227(1):209-215
20. Watzinger N, Lund GK, Saeed M et al (2005) Myocardial blood flow in patients with dilated cardiomyopathy: quantitative assessment with velocity-encoded cine magnetic resonance imaging of the coronary sinus. J Magn Reson Imaging 21(4):347-353
21. Maroules CD, Chang AY, Kontak A et al (2010) Measurement of coronary flow response to cold pressor stress in asymptomatic women with cardiovascular risk factors using spiral velocity-encoded cine MRI at 3 Tesla. Acta Radiol 51(4):420-426
22. Semelka RC, Tomei E, Wagner S et al (1990) Normal left ventricular dimensions and function: interstudy reproducibility of measurements with cine MR imaging. Radiology 174(3 Pt 1):763-768
23. Koskenvuo JW, Hartiala JJ, Knuuti J et al (2001) Assessing coronary sinus blood flow in patients with coronary artery disease: a comparison of phase-contrast MR imaging with positron emission tomography. AJR Am J Roentgenol 177(5):1161-1166
24. Naya M, Tsukamoto T, Morita K et al (2007) Olmesartan, but not amlodipine, improves endothelium-dependent coronary dilation in hypertensive patients. J Am Coll Cardiol 50(12):1144-1149
25. Siegrist PT, Gaemperli O, Koepfli P et al (2006) Repeatability of cold pressor test-induced flow increase assessed with H(2)(15)O and PET. J Nucl Med 47(9):1420-1426
26. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1(8476):307-310
27. Deanfield JE, Halcox JP, Rabelink TJ (2007) Endothelial function and dysfunction: testing and clinical relevance. Circulation 115(10):1285-1295
28. Schindler TH, Nitzsche EU, Munzel T et al (2003) Coronary vasoregulation in patients with various risk factors in response to cold pressor testing: contrasting myocardial blood flow responses to short- and long-term vitamin C administration. J Am Coll Cardiol 42(5):814-822
29. Morita K, Tsukamoto T, Naya M et al (2006) Smoking cessation normalizes coronary endothelial vasomotor response assessed with 15O-water and PET in healthy young smokers. J Nucl Med 47(12):1914-1920
30. Alexanderson E, Rodriguez-Valero M, Martinez A et al (2009) Endothelial dysfunction in recently diagnosed type 2 diabetic patients evaluated by PET. Mol Imaging Biol 11(1):1-5
31. Schwitter J, DeMarco T, Kneifel S et al (2000) Magnetic resonance-based assessment of

global coronary flow and flow reserve and its relation to left ventricular functional parameters: a comparison with positron emission tomography. Circulation 101(23):2696-2702

32. Moro PJ, Flavian A, Jacquier A et al (2011) Gender differences in response to cold pressor test assessed with velocity-encoded cardiovascular magnetic resonance of the coronary sinus. J Cardiovasc Magn Reson 13:54

33. Hwang KH, Lee BI, Kim SJ et al (2009) Evaluation of coronary endothelial dysfunction in healthy young smokers: Cold pressor test using [(15)O]H(2)O PET. Appl Radiat Isot 67(7-8):1199-1203

34. Schindler TH, Facta AD, Prior JO et al (2007) Improvement in coronary vascular dysfunction produced with euglycaemic control in patients with type 2 diabetes. Heart 93(3):345-349

35. Hofman MB, Visser FC, van Rossum AC et al (1995) In vivo validation of magnetic resonance blood volume flow measurements with limited spatial resolution in small vessels. Magn Reson Med 33(6):778-784

36. Czernin J, Waldherr C (2003) Cigarette smoking and coronary blood flow. Prog Cardiovasc Dis 45(5):395-404

37. Naya M, Morita K, Yoshinaga K et al (2011) Long-term smoking causes more advanced coronary endothelial dysfunction in middle-aged smokers compared to young smokers. Eur J Nucl Med Mol Imaging 38(3):491-498

Figure
Click here to download Figure: Fig 1.ppt

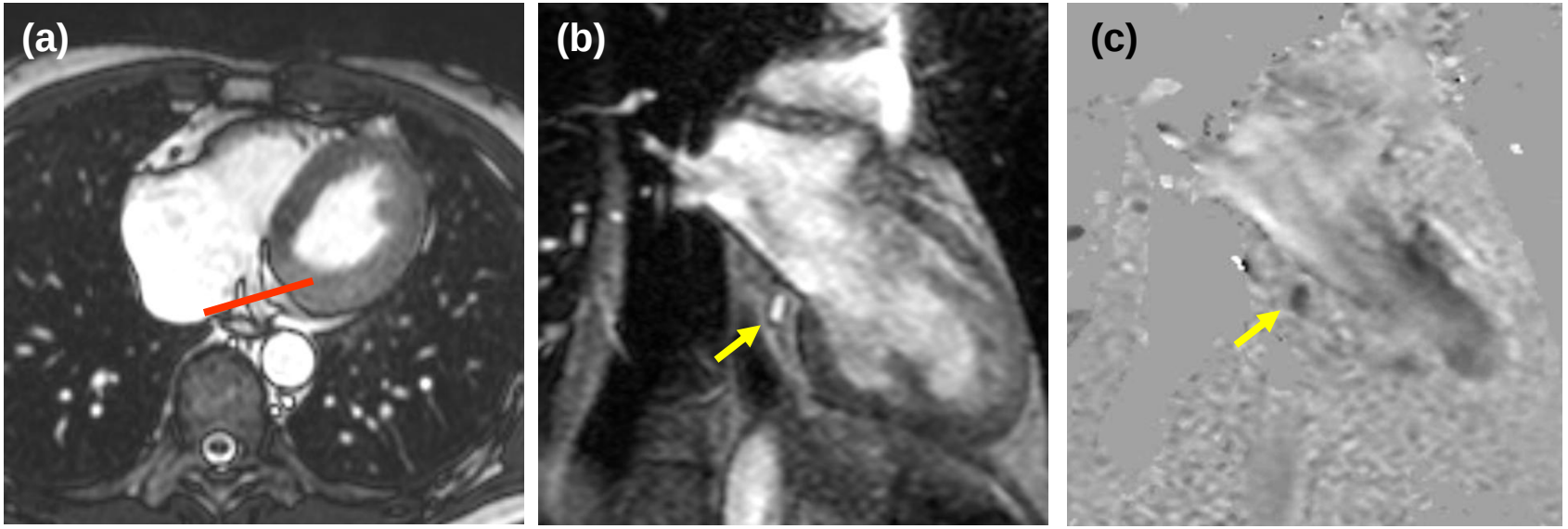

Fig. 1 Coronary sinus (CS) flow measurement by phase-contrast cine MRI. **a** A representative axial cine image of the CS with slice orientation for flow imaging (*solid line*). Representative phase-contrast cine images are also depicted: **b** magnitude image and **c** velocity map of the CS (*arrows*).

Figure
Click here to download Figure: Fig 2-6.ppt

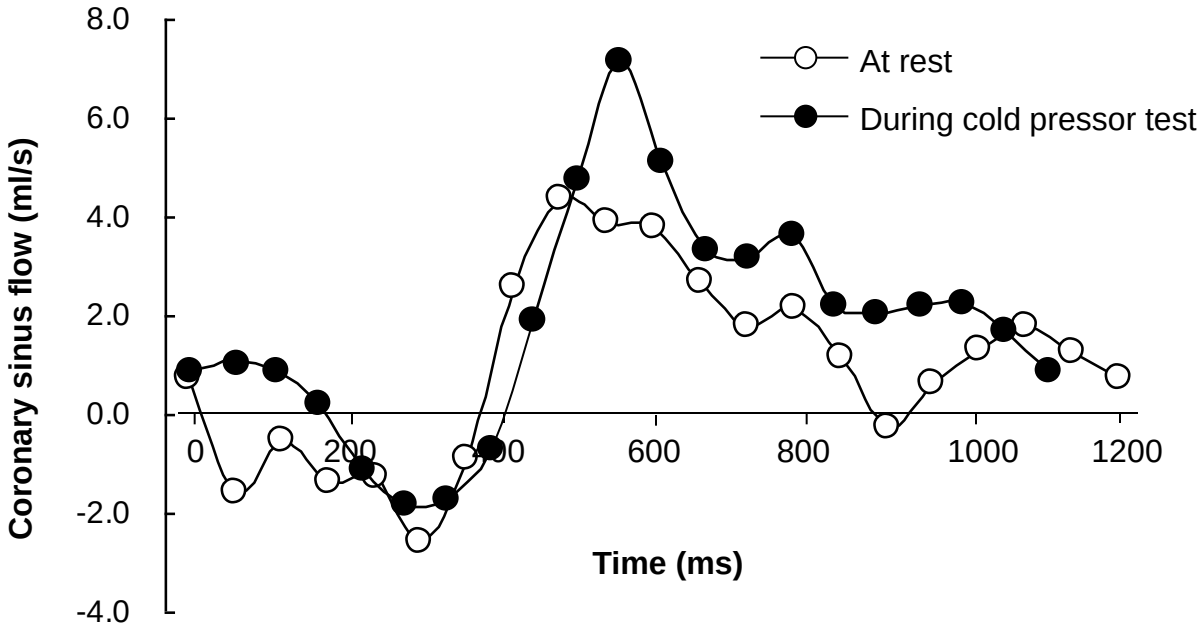

Fig. 2 Representative coronary sinus (CS) volume flow curves in the cardiac cycle at baseline and during the cold pressor test (CPT). Peak volume flow in CS was observed during early diastole.

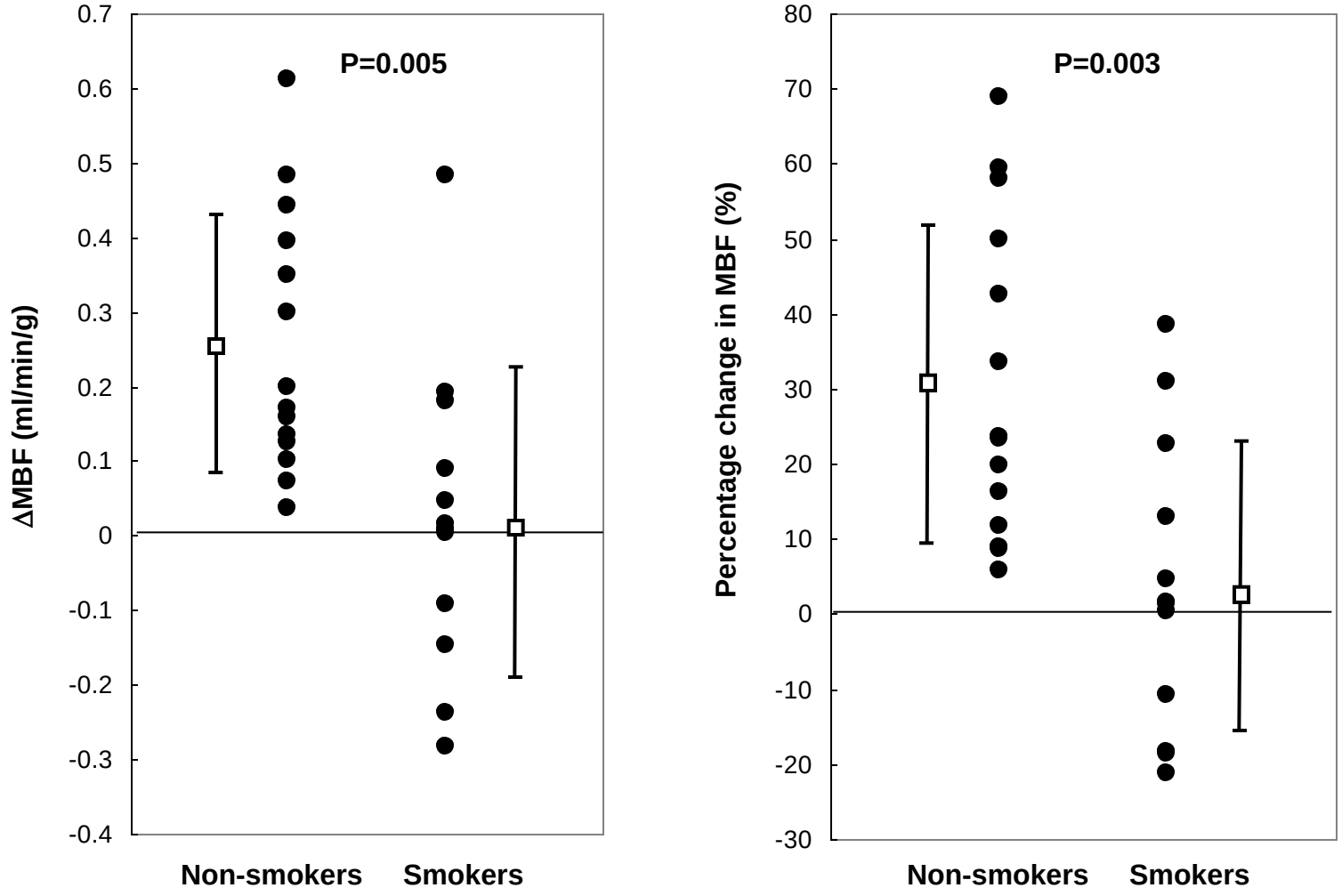

Fig. 3 Individual ΔMBF values and percent change of MBF in non-smokers and smokers. The mean ΔMBF during the CPT in non-smokers was 0.26 ± 0.18 ml/min/g. The mean ΔMBF during the CPT in smokers was 0.02 ± 0.20 ml/min/g, and was significantly lower than in non-smokers (*P* = 0.005). The mean percent change of MBF in smokers was significantly lower than in non-smokers (3.6 ± 19.7% vs. 30.7 ± 21.3%, *P* = 0.003).

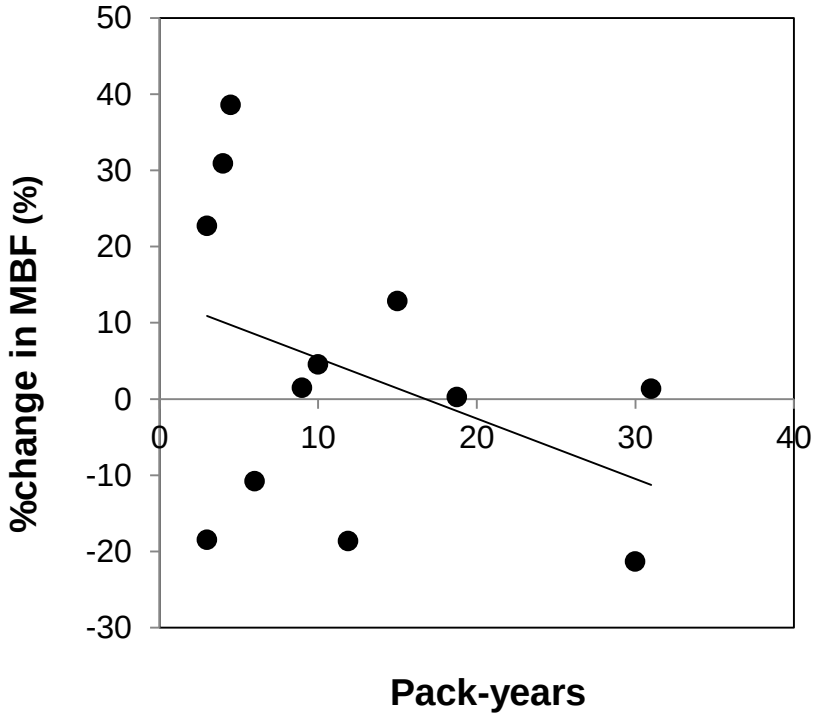

Fig. 4 Relationship between percent change of MBF and pack-years. Number of pack-years is calculated as packs smoked per day multiplied by years as a smoker. There was no significant correlation between pack-years and percent change of MBF in smokers (r = -0.39, P = 0.20, y = -0.79x + 13.3).

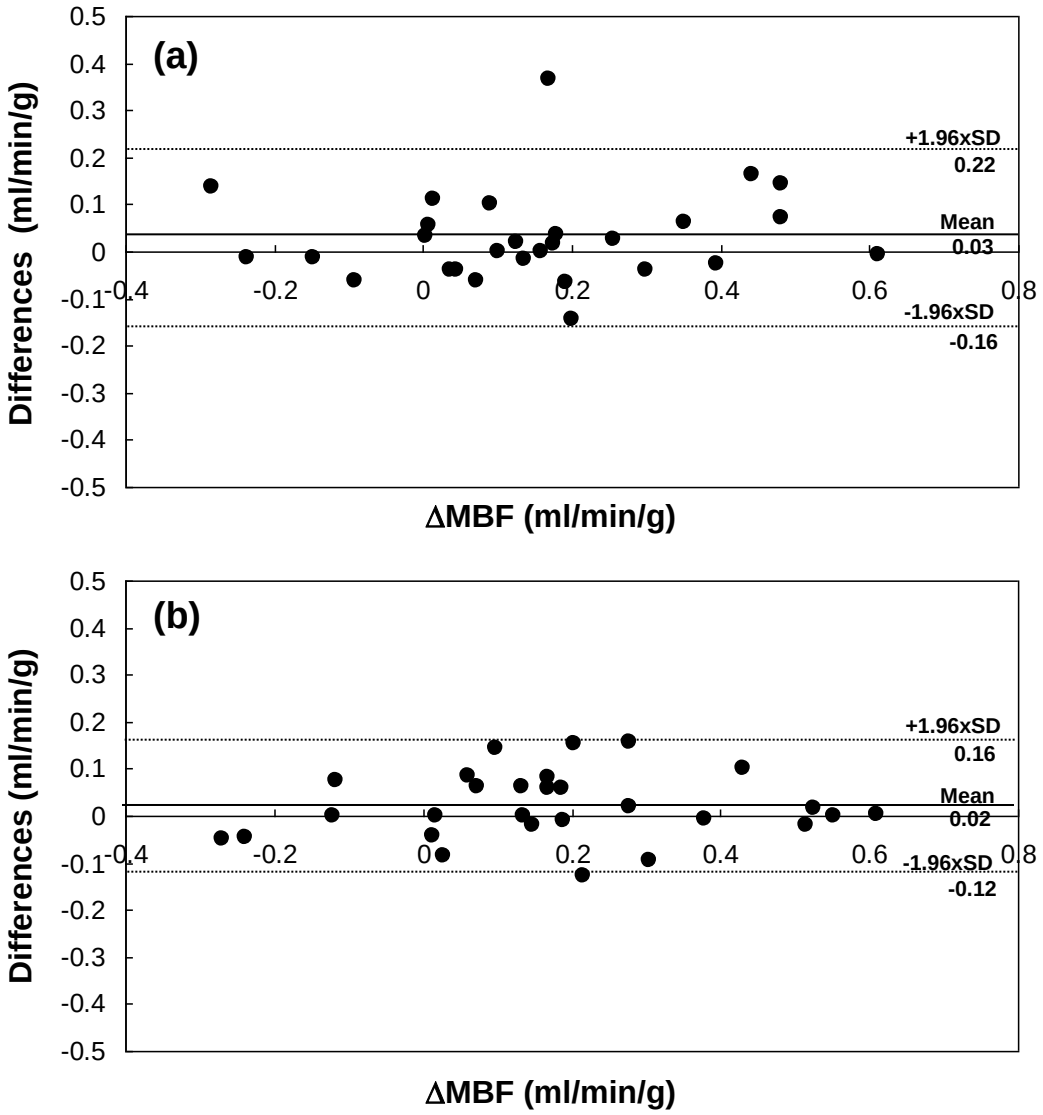

Fig. 5 Bland-Altman plots of (**a**) inter-observer and (**b**) intra-observer variability of ΔMBF (ml/min/g). The mean difference in ΔMBF inter-observer variability was 0.03 ± 0.10 ml/min/g and intra-observer variability was 0.02 ± 0.07 ml/min/g.

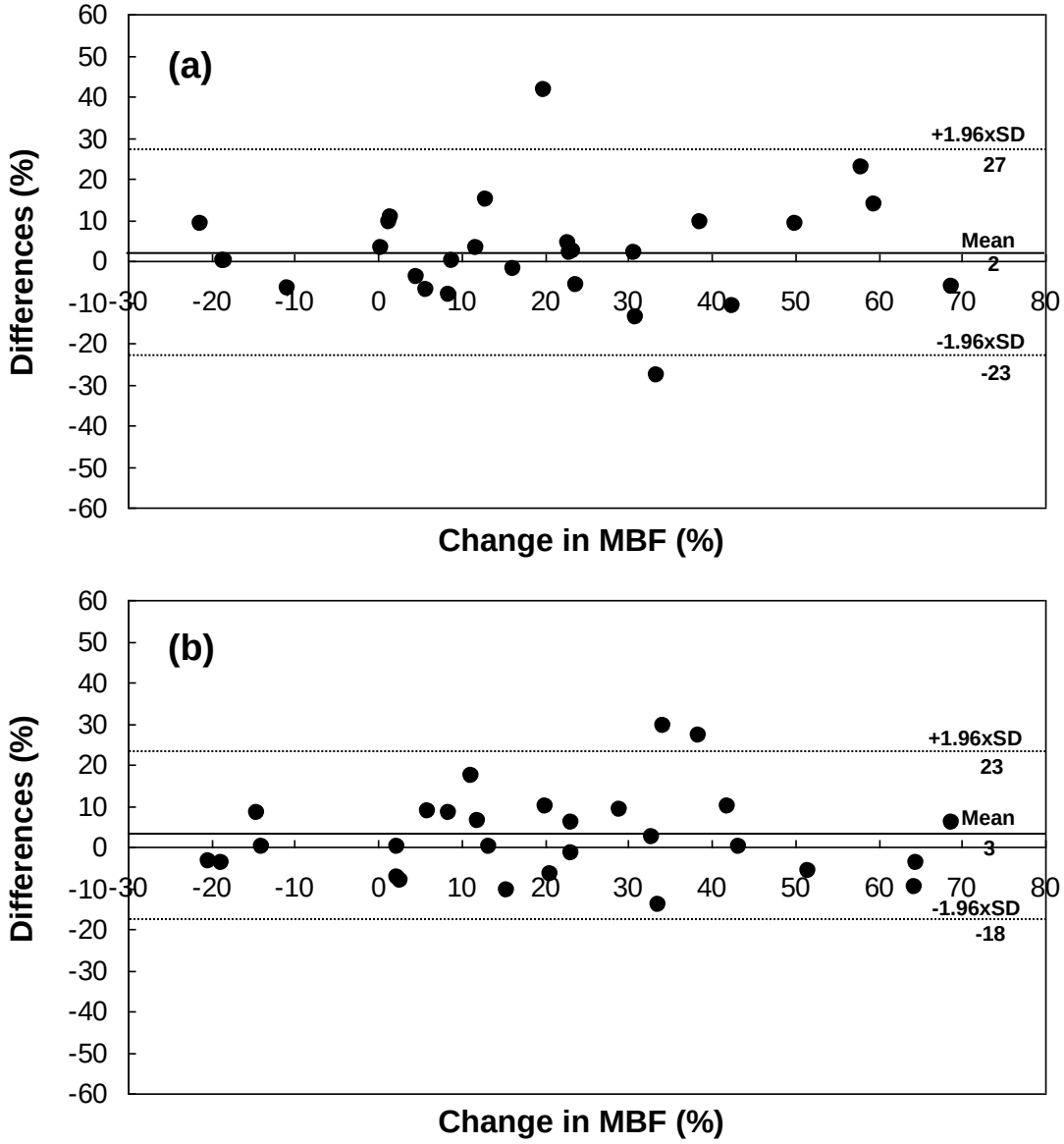

Fig 6. Bland-Altman plots of (**a**) inter-observer and (**b**) intra-observer variability of percent change of myocardial blood flow (MBF). The mean difference in MBF inter-observer variability was 2.3 ± 12.7% and intra-observer variability was 2.6 ± 10.5%.

Table
Click here to download Table: Eng_20130023_2_22_1028-13-084_Tables-ME-clean.doc

Table 1 Clinical characteristics of subjects

Variables	Non-smokers *n* = 14	Smokers *n* = 12	*P* value
Age (years)	31 ± 6	33 ± 8	0.30
Body mass index (kg/m^2)	22 ± 2	22 ± 3	0.75
Systolic blood pressure (mmHg)	118 ± 7	123 ± 14	0.38
Diastolic blood pressure (mmHg)	63 ± 6	66 ± 7	0.27
Heart rate (beats/min)	62 ± 9	64 ± 10	0.67
Total cholesterol (mg/dl)	200 ± 34	176 ± 28	0.09
Triglycerides (mg/dl)	105 ± 51	94 ± 57	0.67
Low-density lipoprotein cholesterol (mg/dl)	121 ± 27	111 ± 19	0.4
High-density lipoprotein cholesterol (mg/dl)	59 ± 13	53 ± 16	0.35
Serum creatinine (mg/dl)	0.90 ± 0.08	0.83 ± 0.14	0.80

All results are expressed as mean ± standard deviation

Table 2 Change in hemodynamics and myocardial blood flow (MBF) values during the cold pressor test (CPT)

	Measurement	Baseline (rest)	During CPT	% Change	*P* value
Non-smokers	Systolic blood pressure (mmHg)	118 ± 7	138 ± 12	18 ± 10	<0.001
n = 14	Diastolic blood pressure (mmHg)	63 ± 6	79 ± 9	25 ± 11	<0.001
	Heart rate (beats/min)	62 ± 9	74 ± 15	19 ± 17	0.001
	RPP (mmHg/min)	Median, 7110 IQR, 1081	Median, 9697 IQR, 3637	Median, 37 IQR, 21	0.001
	MBF (ml/min/g)	0.88 ± 0.19	1.13 ± 0.26	31 ± 21	<0.001
Smokers	Systolic blood pressure (mmHg)	123 ± 14	147 ± 18	20 ± 12	<0.001
n = 12	Diastolic blood pressure (mmHg)	66 ± 7	83 ± 11	26 ± 13	<0.001
	Heart rate (beats/min)	64 ± 10	75 ± 21	18 ± 27	0.045
	RPP (mmHg/min)	Median, 7543 IQR, 1360	Median, 9658 IQR, 6062	Median, 33 IQR, 32	0.004
	MBF (ml/min/g)	0.94 ± 0.26	0.96 ± 0.30	4 ± 20	0.73

All results except for rate-pressure product (RPP) are expressed as mean ± standard deviation. RPP values are expressed as median and interquartile range (IQR)

Table 3 Inter- and Intra-observer variation

Inter-observer variation	Intra-class correlation coefficient	Repeatability coefficient	Mean absolute difference
MBF at rest	0.91	0.19 ml/min/g	0.07 ± 0.07 ml/min/g
MBF during the CPT	0.96	0.14 ml/min/g	0.07 ± 0.05 ml/min/g
ΔMBF	0.90	0.19 ml/min/g	0.07 ± 0.07 ml/min/g
Percentage change in MBF	0.87	25.0%	8.9 ± 9.2%
Intra-observer variation	**Intra-class correlation coefficient**	**Repeatability coefficient**	**Mean absolute difference**
MBF at rest	0.95	0.15 ml/min/g	0.06 ± 0.05 ml/min/g
MBF during the CPT	0.98	0.10 ml/min/g	0.04 ± 0.04 ml/min/g
ΔMBF	0.95	0.14 ml/min/g	0.06 ± 0.05 ml/min/g
Percentage change in MBF	0.91	20.5%	8.0 ± 7.1%

MBF myocardial blood flow, *CPT* cold pressor test

The International Journal of CARDIOVASCULAR IMAGING Authorship and Disclosure Form

JASCI-13-084

MANUSCRIPT ID NUMBER

Altered coronary endothelial function in young smokers....

Article title (first few words)

First Author: Yasutaka Ichikawa

E-mail: yasutaka@clin.medic.mie-u.ac.jp

After submission of this agreement signed by all authors, changes of authorship or in the order of the authors listed will not be accepted by Springer.

AUTHORSHIP

I, the undersigned author(s), certify that:

- I have seen and approved the final version of the manuscript, and all subsequent versions.
- I have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data;
- I have drafted the article or revised it critically for important intellectual content.

I accept public responsibility for it, and believe it represents valid work. As an author of this article, I also certify that none of the material in the manuscript has been previously published, nor is it included in any other manuscript. I certify that this manuscript is not under consideration for publication elsewhere, nor has it been submitted or accepted in another publication in any form. The rights or interest in the manuscript have not been assigned to any third party.

Moreover, should the editor of *The International Journal of Cardiovascular Imaging* request the data upon which the manuscript is based, I shall produce it. I also certify that I have read and complied with the copyright information, as found on the journal's home page website.

FINANCIAL DISCLOSURE/CONFLICT OF INTEREST

I certify that any financial interests such as employment, stock ownership, honoraria, paid expert testimony, as well as any personal relationships, academic competition, and intellectual passion which may inappropriately influence my actions, have been disclosed on a separate attachment.

All funding sources supporting the work and all institutional or corporate affiliations of mine are acknowledged in a footnote.

I have had full access to all the data in the study (if applicable) and thereby accept full responsibility for the integrity of the data and the accuracy of the data analysis.

By checking the box next to my signature I assert that there are no conflicts of interest (both personal and institutional) regarding specific financial interests that are relevant to the work conducted or reported in this manuscript.

The International Journal of CARDIOVASCULAR IMAGING
Authorship and Disclosure Form

Springer

JASCI-13-084

MANUSCRIPT ID NUMBER

☐ Yasutaka Ichikawa
Author's signature

Yasutaka Ichikawa, Dec 16, 2013
Printed name and date

☐ Kakuya Kitagawa
Author's signature

Kakuya Kitagawa. Dec 16.2013
Printed name and date

☐ Shingo Kato
Author's signature

Shingo Kato Dec 16, 2013
Printed name and date

☐ Tadamori Hirano
Author's signature

Tadanori Hirano Dec. 16, 2013
Printed name and date

☐ Kaoru Dohi
Author's signature

Kaoru Dohi Dec 16, 2013
Printed name and date

☐ Masaaki Ito
Author's signature

Masaaki Ito Dec 17, 2013
Printed name and date

☐ Hajime Sakuma
Author's signature

Hajime Sakuma Dec 16, 2013
Printed name and date

☐
Author's signature

Printed name and date

Completed forms can be scanned and included as a pdf file during the online submission process as a supplemental file not for review. Please contact the appropriate editorial office should you experience problems with scanning the document.