Quantitative analysis of coronary endothelial function with generator-produced rubidium-82 PET - Comparison with oxygen 15-labeled water PET-

Keiichiro Yoshinaga, MD, PhD[1], Osamu Manabe, MD[2]; Chietsugu Katoh, MD, PhD[3], Li Chen, MSc[4], Ran Klein, MASc, PhD[2,4], Masanao Naya, MD, PhD[5], Robert A. deKemp, PhD[4]; Kathryn Williams, MS[4], Rob S.B. Beanlands, MD, FRCPC, FACC[4], and Nagara Tamaki, MD, PhD[2].

[1]Department of Photobiology, [2]Department of Nuclear Medicine, [3]Department of Health Sciences, [5]Department of Cardiology Hokkaido University of Graduate School of Medicine, Sapporo, Japan,

[4]National Cardiac PET Centre, Division of Cardiology, University of Ottawa Heart Institute, Ottawa, Ontario, Canada.

Correspondence and reprint requests:

Keiichiro Yoshinaga, MD, PhD
Associate Professor
Department of Photobiology, Division of Molecular・Cellular Imaging, Hokkaido University Graduate School of Medicine
Kita15 Nishi7, Kita-Ku, Sapporo, Hokkaido, Japan 060-8638
Phone: 81-11-706-5152, Fax: 81-11-716-7155
Email address: *kyoshi@med.hokudai.ac.jp*

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Abstract

Purpose Endothelial dysfunction is the earliest abnormality in the development of coronary atherosclerosis. Rubidium-82 (^{82}Rb) is a generator-produced positron emission tomography (PET) myocardial perfusion tracer that is becoming more widely used. We aimed to develop a method for quantitative assessment of coronary endothelial function using the myocardial blood flow (MBF) response during a cold pressor test (CPT) in smokers, measured using ^{82}Rb PET and ii) compare the results with those measured using ^{15}O-water PET.

Methods MBF was assessed at rest and during CPT with ^{82}Rb and ^{15}O-water in 9 controls and 10 smokers. A one-compartment model with tracer extraction correction was used to estimate MBF with both tracers. CPT response was calculated as the ratio of MBF during CPT to MBF at rest.

Results At rest, measurements of MBF for smokers vs. controls were not different using ^{15}O-water (0.86±0.18 vs. 0.70±0.13, $p = 0.426$) than they were using ^{82}Rb (0.83± 0.23 vs. 0.62±0.20, $P = 0.051$). Both methods showed a reduced CPT response in smokers vs. controls (^{15}O-water, 1.03±0.21 vs. 1.42 ± 0.29, $p = 0.006$; ^{82}Rb, 1.02 ± 0.28 vs. 1.70 ± 0.52, $p < 0.001$). There was high reliability [intra-class correlation coefficients: 0.48 (0.07, 0.75)] of MBF measurement between ^{82}Rb and ^{15}O-water during CPT.

Conclusions Using CPT, ^{82}Rb MBF measurements detected coronary endothelial dysfunctions in smokers. ^{82}Rb MBF measurements were comparable to those made using the ^{15}O-water approach. Thus, ^{82}Rb PET may be applicable for risk assessments or evaluation of risk factor modification in subjects with coronary risk factors.

Keywords Blood flow· endothelium· positron emission tomography· smoking

Introduction

Coronary endothelial cells protect the coronary artery by providing a mechanical barrier and releasing bioactive factors [1]. Coronary endothelial dysfunction is the earliest abnormality in the development of coronary atherosclerosis and is also independently associated with future cardiac events [2]. Several coronary risk factors adversely affect endothelial function [3-6]. Among these risk factors, smoking is the leading cause of preventable cardiovascular death and is a related factor in more than 450,000 deaths annually in the United States[7]. Developing simple, objective and widely available coronary-specific endothelial function testing is particularly important for identifying high-risk subjects for future cardiovascular events and is also important for monitoring new or established therapeutic interventions [1].

Invasive Doppler flow measurements during intracoronary administration of the endothelium-dependent vasodilator, acetylcholine, is the standard approach for direct measurement of coronary endothelial function [8]. However, this technique is invasive and less practical for routine clinical use or clinical trials that require large patient populations. An alternative non-invasive approach, ultrasonographic evaluation of brachial artery vasodilatation capacity, is widely used for detecting peripheral vascular endothelial dysfunction [9]. However, this approach does not directly evaluate coronary endothelial function.

Positron emission tomography (PET) can non-invasively measure myocardial blood flow (MBF)[10] and estimate coronary endothelial function using the cold pressor test (CPT) [11-12]. We have demonstrated coronary endothelial dysfunction in young smokers otherwise no coronary risk factors testing using ^{15}O-water PET and CPT[13] . We also assessed the effects of therapeutic interventions in patients with hypertension using ^{15}O-water PET and CPT [4]. In most previous studies, PET MBF measurements have been performed using either ^{15}O-water or

^{13}N-ammonia, which require immediate access to an on-site cyclotron [14-15]. This requirement makes it difficult to evaluate risk assessment or therapeutic interventions in larger populations. Rubidium-82 (^{82}Rb) is a PET perfusion tracer produced from a strontium-82 (^{82}Sr)/ ^{82}Rb generator and is widely used in PET centers without immediate access to a cyclotron for the diagnosis of coronary artery disease [15-16]. Thus, it is possible to apply ^{82}Rb perfusion studies in larger populations [17-18]. However, there are limited data reported on MBF quantification using ^{82}Rb [19-23], and to our knowledge there has been no published data evaluating the MBF response during CPT to measure coronary endothelial function. The purpose of the current study was to develop coronary endothelial function measurements using ^{82}Rb, evaluate MBF response during CPT in smokers, and compare these results to those observed using ^{15}O-water PET.

Methods

Study subjects

Ten smokers and 9 healthy volunteers participated in the study. All participants were men. Smokers had been smoking for at least the past 10 consecutive years. The Brinkman index, number of cigarettes per day × the years of smoking, was 498.0±159.8. All participants had a normal resting electrocardiogram and did not have a history of cardiovascular disease and were not taking any cardiac medications. All healthy control subjects had a low pre-test likelihood of coronary artery disease (<5%) based on risk factors [24].

The study was approved by the Hokkaido University Graduate School of Medicine Human Research Ethics Board. Written informed consent was obtained from all subjects.

Study protocol

Each subject underwent rest and CPT imaging with both ^{15}O-water and ^{82}Rb PET. The ^{15}O-water and ^{82}Rb imaging were performed in randomized order (mean interval 17.6 ± 13.1 days).

PET Acquisition Protocol

Participants were instructed to fast for > 6h, and to abstain from caffeine-containing products for > 24 hours prior to PET studies [4, 25-26]. Smokers were also instructed to refrain from smoking for >12h before the PET studies. Participants were positioned with the heart roughly centered in the field of view in a whole body PET scanner (ECAT HR+, Siemens/CTI Knoxville, Tennessee). All emission and transmission scans were performed in the 2-dimensional acquisition mode.

^{15}O-Water

Immediately following a 6-minute transmission scan for attenuation correction, participants inhaled 2,000 MBq of ^{15}O-labeled CO (0.14% CO mixed with room air) for 1 minute and were then imaged over 5 min to obtain a blood volume image [26-27]. The physical half life of ^{15}O-labeled CO is 2 minutes. Therefore, an interval of 10 minutes between ^{15}O-labeledCO inhalation and ^{15}O-labeled water injection allowed for tracer decay [27]. 1,500 MBq of ^{15}O-labeled water was administered intravenously at a slow infusion rate (2 minutes) at rest, and a 20-frame dynamic PET acquisition was initiated with varying frame duration (6 × 5 seconds, 6 × 15 seconds, 8× 30 seconds, total = 6 min) [26-27].

A second transmission scan was performed and the participant's foot (order was randomly selected in order to avoid adaptation [25]) was immersed into ice water up to the ankle [4, 27]. PET data acquisition was started 60 seconds after the beginning of CPT using the same dynamic

sequence as at rest, and CPT continued for a total of 4 minutes [4, 27]. During the entire exam stress, symptoms, heart rate, blood pressure, and the electrocardiogram were monitored continuously.

^{82}Rb

The ^{82}Rb protocol was similar to that of ^{15}O-water except that immediately following the transmission scan, 1,480 MBq of ^{82}Rb (Bracco Diagnosis, Princeton, NJ) was administered intravenously over one minute [22]. A 10-minute, 24-frame dynamic scan was initiated with increasing frame duration (12 × 10 seconds, 2 × 30 seconds, 1 × 60 seconds, 1 × 120 seconds, 1 × 240 seconds) [18, 21-22]. To avoid adaptation to CPT, ^{82}Rb measurement was performed with immersion of the opposite foot used during ^{15}O-water use.

Quantification of MBF

Images were reconstructed using filtered back-projection reconstruction with a 10 mm and 12 mm Hanning filter for ^{15}O-water and ^{82}Rb respectively, and attenuation and decay were corrected. Each image consisted of 63 transaxial slices each having 128×128 voxels with dimensions 3.4×3.4×2.4 mm. MBF quantification followed using a software program developed in-house [28] that semi-automatically defines regions of interest (ROIs) for left ventricular (LV) cavity blood pool and the LV myocardium. The program then performs MBF quantification using a 1-tissue-compartment tracer kinetic model [21, 28]. The MBF quantification processes differed slightly for ^{15}O-water and ^{82}Rb:

^{15}O-Water

MBF was analyzed by a semiautomatic program as previously reported [28]. The frames of the water image were summed and the blood volume image (CO) was then subtracted to generate

a myocardial uptake image. The uptake image was used to reorient the original image to create short-axis slices semi-automatically and to automatically create a myocardial ROI. The blood volume image was similarly reoriented and then automatically segmented to define a blood pool ROI with integral intensity equal to 85% of maximum pixel intensity. The ROIs were applied to the entire dynamic image to generate LV blood and myocardium time activity curves (TACs), LV(t) and R(t) respectively.

The myocardium was modeled as a partial volume mixture of arterial blood, Ca(t), and tissue, Ct(t), activity concentrations as in Eq. 1 where the PTF-denoted perfusable tissue fraction, Va is the arterial blood volume, and ρ is the density of tissue (1.04 g/ml).

$$R(t) = PTF \cdot \rho \cdot Ct(t) + Va \cdot Ca(t) \quad \text{Eq. 1}$$

The change in tissue activity concentration was modeled using the 1-tissue compartment model in Eq. 2 where F denotes blood flow in mL/min/g. The parameter p is the partition coefficient of water in the myocardium and is equal to 0.91.

$$dCt(t)/dt = F \cdot Ca(t) - (F/p) \cdot Ct(t) \quad \text{Eq. 2}$$

In the LV blood cavity, activity concentration was modeled as a partial volume mix of β=85% arterial blood and (1-β=15%) tissue as shown in Eq. 3.

$$LV(t) = \beta \cdot Ca(t) + (1-\beta) \cdot \rho \cdot Ct(t) \quad \text{Eq. 3}$$

Equations 1-3 were solved with a non-linear least squares analysis, and F was used as the global estimate of MBF.

^{82}Rb

With ^{82}Rb, a myocardial uptake image was generated by summing the last 4-6 minutes of the image sequence, and a blood volume image was created by summing the first 2 minutes of the

image sequence. As with ^{15}O-water, the images were reoriented and segmented semi-automatically to generate LV blood and myocardial TACs.

The measured tissue time-activity-curve in the whole-myocardial ROI, R(t), was estimated using Eq. 1 and the change in tissue activity concentration was modeled using

$$dCt(t)/dt = K1 \cdot Ca(t) - k2 \cdot Ct(t) \quad \text{Eq. 4}$$

where K1 (mL/min/g) is the uptake ratio from blood into the tissue and k2 (/min) is the outflow ratio from myocardial tissue into the blood Ca (t) (Bq/ml). Radioactivity in the LV blood pool was calculated using equation Eq. 3 with β=85%.

The values of a, Va, K1 and k2 were estimated using Eq. 1, 3 and 4 with a non-linear least squares analysis. Conversion from K1 to MBF was estimated with the modified Renkin-Crone model [21-22].

Evaluation of CPT response and coronary vascular resistance

CPT response was calculated as the ratio of MBF during CPT to MBF at rest [6, 12, 27]. Coronary vascular resistance (CVR) was determined as the ratio of mean arterial blood pressure to MBF (mm Hg/[mL/g/min]). The ΔCVR, increase in absolute units, was defined as the difference between CVR during CPT and at rest [4-5, 27].

Statistical analysis

Continuous variables are presented as means and standard deviations. Categorical variables are presented as frequencies with percentages. For the analysis of patient baseline characteristic differences, Wilcoxon rank sum tests were used for continuous variables. Fisher's exact tests were used for categorical variables.

For within-group comparisons, Wilcoxon signed rank tests were used to compare differences of continuous variables measured by ^{15}O-water and ^{82}Rb PET. For between-group comparisons, the general linear model was used to compare differences between smokers and controls for ^{15}O-water and ^{82}Rb PET separately in order to adjust the confounding effect of baseline characteristic difference (age and total cholesterol). The inter-rater reliability of rest MBF, MBF during CPT, and CPT response measurements between ^{15}O-water and ^{82}Rb were assessed using intra-class correlation coefficients (ICC) [29] which is a best measure of reliability for continuous data and also evaluated graphically using a Bland and Altman plot which demonstrates not only the overall degree of agreement, but also whether the agreement is related to the underlying value of the item [30]. The strength of ICC was determined using the cut-offs of 0.5,0.3,0.1 for high, moderate and low levels of agreement according to Cohen's effect size convention [31]. A *P* value < 0.05 was considered statistically significant. Statistical calculations were carried out using SAS 9.2 software (SAS Institute, Inc. Cary, North Carolina).

Results

The CPT stress was well tolerated by all participants. None of the participants had ischemic electrocardiography changes or chest pain during CPT.

Subject characteristics

The baseline characteristics of the 19 subjects are shown in Table 1. Smokers were older than the healthy controls ($p = 0.014$) and had a higher total cholesterol ($p = 0.041$) and a higher Brinkman index ($p < 0.001$).

Hemodynamics

Heart rate and systolic blood pressure at rest were similar in smokers and controls, both in ^{15}O-water and ^{82}Rb PET studies. Both groups had similar rest rate pressure products (RPP) with ^{15}O-water and ^{82}Rb PET (Table 2). In both groups, CPT stimulation significantly increased RPP with either ^{15}O-water (smokers; 7799.0 ± 1223.9 to 10335.9 ± 1657.3, $p = 0.004$, controls; 6646.0 ± 1131.5 to 10462.4 ± 3172.0, $p = 0.005$) or ^{82}Rb (smokers; 7469.4 ± 1238.2 to 10117.6 ± 1304.9, $p < 0.001$, controls; 6152.7 ± 831.9 to 9757.8 ± 2258.7, $p < 0.001$). Both groups had similar percent change of RPP during CPT in ^{15}O-water and ^{82}Rb studies, indicating comparable hemodynamic study conditions during ^{15}O-water and ^{82}Rb PET studies (smokers; $p = 0.375$, controls; $P = 0.910$). There was no significant difference in percentage of change of RPP during CPT between the smokers and controls either with ^{15}O -water (34.5 ± 22.5% vs. 56.1 ± 29.3%, $p = 0.095$) or with ^{82}Rb PET (38.7 ± 29.4% vs. 58.3 ± 27.9%, $p = 0.095$).

Rest MBF and MBF during CPT

The rest MBF was similar between smokers and controls when measured using both ^{15}O-water and ^{82}Rb (Table 3, Fig. 1).

Control subjects had significantly increased MBF during CPT stress with ^{15}O-water (from 0.70 ± 0.13 mL/min/g to 0.99 ± 0.21 mL/g/min, $p = 0.004$) and ^{82}Rb (from 0.62 ± 0.20 mL/min/g to 1.02 ± 0.36 mL/min/g, $p = 0.008$). On the other hand, there was no significant change in MBF during CPT in smokers with ^{15}O-water (from 0.86 ± 0.18 mL/min/g to 0.88 ± 0.22 mL/min/g, $p = 0.922$) or ^{82}Rb (from 0.83 ± 0.23 to 0.81 ± 0.09, $p = 0.770$). Therefore, the CPT response (ratio of the MBF during CPT to the rest MBF) in smokers was significantly lower than in the controls with ^{15}O-water ($p = 0.006$) and ^{82}Rb ($p < 0.001$; Fig. 2).

The rest CVR measurement for the smokers and the controls was similar using both ^{15}O-water and ^{82}Rb (Table 4). Control subjects had reduced CVR during CPT stress with ^{15}O-water and ^{82}Rb but the difference was not significant (p = 0.426 and p = 0.098). CVR increased in the smokers during CPT stress both with ^{15}O-water and ^{82}Rb. The CVR increases were significant (p = 0.020 and p = 0.027). The decline of ΔCVR was significantly greater in controls than in smokers with ^{15}O-water (-7.95 ± 24.64 mm Hg/[mL/ g/min] vs. 20.6 ± 20.7 mm Hg/[mL/g/min], p = 0.035) and with ^{82}Rb (-25.8 ± 35.1 mm Hg/[mL/ g/min] vs. 21.7 ± 23.1 mm Hg/[mL/ g/min], p = 0.002). There was no significant difference inΔCVR between ^{15}O-water and ^{82}Rb in controls (p = 0.11).

Reliability of MBF measurements between ^{15}O-Water and ^{82}Rb

According Cohen's effect size covention, the comparability of MBF measurements between ^{15}O-water and ^{82}Rb was high during CPT [ICC: 0.48 (0.07, 0.75)] and moderate at rest [ICC: 0.29 (-0.16, 0.64)] and CPT response [ICC: 0.36 (-0.08, 0.68)]. However, there was no significant difference among these measurements. Bland Altman plots were also used to plot the difference between ^{15}O-water and ^{82}Rb (^{15}O-water -^{82}Rb) against the mean of the two values with 95% confidence limits of agreement for MBF measurements. Overall Bland Altman plots showed close agreement for MBF measurements between ^{15}O-water and ^{82}Rb at rest, during CPT, and in the CPT response, however the difference was found to decrease with the magnitude of MBF measurement in the CPT response although the relation was week (Fig. 3).

Discussion

^{82}Rb MBF measurements detected coronary endothelial dysfunction using CPT. There were close agreements between rest and CPT stress MBF measurements using ^{82}Rb PET compared to rest and CPT stress MBF measurements made using ^{15}O-water PET, which were considered to be the standard. ^{82}Rb MBF measurements identified decreased flow response during CPT, suggesting coronary endothelial dysfunction in smokers, in accordance with ^{15}O-water PET findings. To our knowledge, the current study is the first to demonstrate that MBF measurements determined using PET with the generator-produced ^{82}Rb tracer and combined with CPT have potential as a biomarker of coronary endothelial function.

Endothelial function measurements using CPT

The CPT activates the sympathetic nervous system. Normally, β -adrenergic activation induces direct coronary vasodilatation which increases coronary blood flow [6, 12]. This increasing coronary blood flow enhances shear stress on endothelial cells, which release nitric oxide (NO). NO induces vascular dilatation in normal endothelia [1]. In contrast, various risk factors, such as hyperlipidemia, hypertension, diabetes, and smoking, attenuate this vasomotor response to NO. In the present study, smokers had reduced MBF during CPT using ^{15}O-water (and ^{82}Rb) PET, indicating coronary endothelial dysfunction. This data agrees with previous studies using ^{15}O-water or ^{13}N-ammonia PET and CPT [3, 13]. Smokers also increased CVR during CPT using ^{15}O-water (and ^{82}Rb) PET. This data agrees with previous study using ^{15}O-water PET and CPT [27].

Smoking-associated endothelial dysfunction

Subjects who smoke represent the largest population among those subjects with coronary risk factors. Smoking is the leading cause of preventable death and is one of the leading preventable causes of future cardiovascular events [32]. Cigarette smoking alters the vascular endothelium via oxidative stress[3]. In some smokers, endothelial dysfunction has been observed to be the only early sign of vascular dysfunction [3]. Thus, the endothelial function measurements are especially important for detecting early vascular dysfunction and risk stratification [1, 9]. In the present study, CPT response was significantly reduced in smokers, indicating coronary endothelial dysfunction regardless of whether this was measured using ^{15}O-water or ^{82}Rb PET. Bland Altman plot revealed agreement between the two approaches. Thus, ^{82}Rb PET may detect coronary endothelial dysfunction in smokers as can the ^{15}O-water PET approach.

^{82}Rb PET endothelial function measurements

^{15}O-labeled water is freely diffusible across the capillary membrane over a wide range of flows and therefore the extraction fraction remains constant and high. Thus, ^{15}O-labeled water is considered the gold standard for non-invasive MBF quantification [33]. We have also applied this approach in the risk assessment in smokers [13] and also applied the assessment of therapeutic interventions [4]. In the present study, CPT response and ΔCVR with ^{15}O-labeled water were impaired in smokers when compared to controls, in agreement with previous data [5, 27, 34]. In previous studies, a simple net retention model was applied for myocardial blood flow quantification using ^{82}Rb. This model is easy to implement and requires less processing time. However, the data are highly dependent on the time at which the model equation is evaluated and a predetermined perfusable tissue fraction (*PTF*). Compartment models can provide acceptable

myocardial blood flow estimation [20] by modeling the full physiology with regards to the tracer. Using the one-tissue compartment, we previously reported good repeatability of MBF measurements using ^{82}Rb [22]. Thus, we applied one-tissue compartment models to MBF quantification in the present study. On the other hand, there are limited data reported on MBF quantification using mathematical models with ^{82}Rb [19-23], and there are no data evaluating MBF response during CPT for the measurement of coronary endothelial function. In the current study, ^{82}Rb identified altered coronary endothelial function in smokers as did ^{15}O-labeled water. Similar results have been reported previously [3, 35].

The present study further expands on previous studies regarding ^{82}Rb MBF measurement in normal subjects [21-22]. In the present study, the variability of CPT response in controls with ^{82}Rb was slightly higher (not significant) than with ^{15}O-labeled water. ^{82}Rb has a relatively high positron range which may reduce image spatial resolution [36], increase myocardium spillover into the blood TAC, thereby reducing MBF accuracy. However, ^{82}Rb as well as ^{15}O-water detected an altered CPT response in smokers, indicating that the spillover correction can effectively correct spillover effects. Lortie et al. estimated ^{82}Rb MBF using the single-compartment model and compared it to ^{13}N-ammonia PET. Differences between the ^{82}Rb and ^{13}N-ammonia uptake rate (K1) were smaller at rest compared to those for dipyridamole stress [21]. Lautamaki et al. also reported significant correlations between MBF measured using microspheres and MBF measured using ^{82}Rb PET in the physiological flow range of less than 3 mL/g/min [37]. The (unidirectional) extraction of ^{82}Rb is approximately 70% at rest and decreases with increasing MBF. Therefore, theoretically ^{82}Rb MBF measurements are less variable in the lower flow range [21, 37]. CPT stress increases MBF by only 30% to 50% compared to rest MBF, and usually MBF during CPT is less than 2.0 mL/min/g [3, 14-15]. In the

present study, MBF measured using ^{15}O-water and ^{82}Rb at rest and during CPT was less than 1.7 mL/min/g in smokers and controls. Thus ^{82}Rb MBF measurements at rest and during CPT both in smokers and in controls are not greatly affected by reduced extraction fraction that occurs at higher flow rates..

Clinical implications for endothelial function testing

Defining biomarkers for endothelial function may play an important role in the prevention of future cardiovascular events. New tests for endothelial function should be simple, non-invasive, and widely available [9]. Flow-mediated dilatation (FMD) has been used for risk stratification or for monitoring therapeutic interventions in relatively large populations [9]. In some studies, brachial artery endothelial function correlated with coronary endothelial function [9]. However, FMD measurements are not direct measurements of coronary endothelial function. In an alternative approach, PET MBF measurements with CPT have been applied in patients with coronary risk factors [3, 12, 14, 27]. PET MBF measurements represent a physiological approach using a bioactive tracer that is non-invasive and relatively simple to apply. In the past, these PET measurements have been performed by using either ^{13}N-ammonia or ^{15}O-labeled water. However, ^{13}N-ammonia and ^{15}O-labeled water require an on-site cyclotron. Thus, these approaches have not been widely available. In addition, most previous studies that assessed therapeutic interventions evaluated only limited study populations [4, 11, 14, 35]. ^{82}Rb is produced from a ^{82}Sr/^{82}Rb generator and is widely used in centers without immediate access to a cyclotron for diagnosis of coronary artery disease [15-16]. PET scanners are being installed on an increasing basis. More than 1,000 PET or PET/CT scanners have been installed in North America to date [38]. Most centers use PET scanners for oncology imaging. It would also be

possible to perform MBF measurements using ^{82}Rb. ^{82}Rb has a very short physical half-life (76 seconds) and the generator can provide radiotracer doses at 10-minute intervals, allowing for repeat MBF measurements to be taken within a short time span [15]. In the current protocol, rest and CPT response measurements were completed in approximately 30 minutes which is a similar timeframe for FMD measurements[9]. Thus, ^{82}Rb PET endothelial function tests can be applied to many patients in a clinical setting [38]. Recent ^{82}Rb perfusion studies evaluated larger populations of patients with coronary artery disease and in fact, Lertsburapa et al. evaluated 1,441 patients [17]. Present data may support the role of ^{82}Rb measurements with CPT as a new endothelial functional biomarker that is relatively simple to implement and could become widely available.

Study Limitations

The sample size of the present study was small. However the similar sample size was used for previous physiological studies that have enough power to validate such new methods [25] or evaluated therapeutic interventions [3-4, 12, 35]. Although the findings from the present study were sufficient to detect significant differences in coronary endothelial dysfunction between smokers and controls using ^{82}Rb PET as well as ^{15}O-water, further studies involving a larger cohort or multi-center studies are required to confirm our results and support wider use of this approach. The reliability of rest MBF, MBF during CPT, and CPT response between the ^{15}O-water and ^{82}Rb was evaluated using the intraclass correlation coefficient which is considered a best measure of reliability for continuous data and also confirmed using the Bland Altman plots. Overall the agreement of MBF measurements was good between the ^{15}O-water and ^{82}Rb. However, the difference was found to decrease with the magnitude of MBF in the CPT response

although the relation was weak. Increasing the sample size might improve the agreement, and therefore further studies with a larger cohort are required.

In the present study, the smokers were older than the control subjects and had higher total cholesterol. Therefore, between-group comparisons were performed using the general linear model adjustment for age and total cholesterol. So the confounding effect of differences in age and total cholesterol between two groups has been removed from the analysis

Kaufmann et al. reported that smokers had significantly reduced CFR with ^{15}O-labeled water [35] . Hyperemic MBF reflects the combined effect of coronary endothelial function and smooth muscle function [35]. Since we focused specifically on coronary endothelial function, pharmacological flow or CFR was not included in the study. Future studies are required to determine which specific measures of endothelial function, CPT PET flow or vasodilator CFR, are more valuable for early identification of patients at risk and early identification of effective therapies.

This study assessed the MBF in the whole LV myocardium but not in regional myocardial segments. Further studies are needed to evaluate regional MBF during CPT stress in CAD patients, where additional regional heterogeneity may be expected.

The present study included only male subjects. It is known that women have different vascular function according to their hormonal status, and therefore we did not include women in the present study. The present study aimed to clarify the usefulness of ^{82}Rb in comparison with ^{15}O-water as the tool of coronary endothelial function measurement. A further study of this topic, which would include women subjects, is required. .

Conclusion

On the basis of the results of this pilot study, ^{82}Rb MBF measurements appears to detect coronary endothelial dysfunction using CPT. There were close agreement in rest and stress flow measurements using both ^{82}Rb myocardial perfusion imaging and a ^{15}O-water PET approach. Thus, ^{82}Rb PET myocardial perfusion imaging may be applicable for risk assessments or evaluation of risk factor modification in subjects with coronary risk factors. Coronary endothelial function measurements using ^{82}Rb PET myocardial perfusion imaging may improve the management of patients with coronary risk factors. Larger studies will be needed to support the wider use of this approach.

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References

1. Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. Circulation. 2002;105:546-9.
2. Lerman A, Zeiher AM. Endothelial function: cardiac events. Circulation. 2005;111:363-8.
3. Campisi R, Czernin J, Schoder H, Sayre JW, Marengo FD, Phelps ME, et al. Effects of long-term smoking on myocardial blood flow, coronary vasomotion, and vasodilator capacity. Circulation. 1998;98:119-25.
4. Naya M, Tsukamoto T, Morita K, Katoh C, Furumoto T, Fujii S, et al. Olmesartan, but not amlodipine, improves endothelium-dependent coronary dilation in hypertensive patients. J Am Coll Cardiol. 2007;50:1144-9.
5. Prior JO, Schindler TH, Facta AD, Hernandez-Pampaloni M, Campisi R, Dahlbom M, et al. Determinants of myocardial blood flow response to cold pressor testing and pharmacologic vasodilation in healthy humans. Eur J Nucl Med Mol Imaging. 2007;34:20-7.
6. Schindler TH, Nitzsche EU, Schelbert HR, Olschewski M, Sayre J, Mix M, et al. Positron emission tomography-measured abnormal responses of myocardial blood flow to sympathetic stimulation are associated with the risk of developing cardiovascular events. J Am Coll Cardiol. 2005;45:1505-12.
7. Ezzati M, Henley SJ, Thun MJ, Lopez AD. Role of smoking in global and regional cardiovascular mortality. Circulation. 2005;112:489-97.
8. Zeiher AM, Drexler H, Wollschlager H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis. Circulation. 1991;84:1984-92.
9. Celermajer DS. Reliable endothelial function testing: at our fingertips? Circulation. 2008;117:2428-30.
10. Knuuti J, Bengel FM. Positron emission tomography and molecular imaging. Heart. 2008;94:360-7.
11. Bengel FM, Abletshauser C, Neverve J, Schnell O, Nekolla SG, Standl E, et al. Effects of nateglinide on myocardial microvascular reactivity in Type 2 diabetes mellitus--a randomized study using positron emission tomography. Diabet Med. 2005;22:158-63.
12. Di Carli MF, Tobes MC, Mangner T, Levine AB, Muzik O, Chakroborty P, et al. Effects of cardiac sympathetic innervation on coronary blood flow. N Engl J Med. 1997;336:1208-15.
13. Iwado Y, Yoshinaga K, Furuyama H, Ito Y, Noriyasu K, Katoh C, et al. Decreased endothelium-dependent coronary vasomotion in healthy young smokers. Eur J Nucl Med Mol Imaging. 2002;29:984-90.
14. Camici PG, Crea F. Coronary microvascular dysfunction. N Engl J Med. 2007;356:830-40.
15. Yoshinaga K, Chow BJ, dekemp RA, Thorn S, Ruddy TD, Davies RA, et al. Application of cardiac molecular imaging using positron emission tomography in evaluation of drug and therapeutics for cardiovascular disorders. Curr Pharm Des. 2005;11:903-32.
16. Bateman TM, Heller GV, McGhie AI, Friedman JD, Case JA, Bryngelson JR, et al. Diagnostic accuracy of rest/stress ECG-gated Rb-82 myocardial perfusion PET: comparison with ECG-gated Tc-99m sestamibi SPECT. J Nucl Cardiol. 2006;13:24-33.
17. Lertsburapa K, Ahlberg AW, Bateman TM, Katten D, Volker L, Cullom SJ, et al. Independent and incremental prognostic value of left ventricular ejection fraction determined by

stress gated rubidium 82 PET imaging in patients with known or suspected coronary artery disease. J Nucl Cardiol. 2008;15:745-53.
18. Yoshinaga K, Chow BJ, Williams K, Chen L, deKemp RA, Garrard L, et al. What is the prognostic value of myocardial perfusion imaging using rubidium-82 positron emission tomography? J Am Coll Cardiol. 2006;48:1029-39.
19. El Fakhri G, Kardan A, Sitek A, Dorbala S, Abi-Hatem N, Lahoud Y, et al. Reproducibility and accuracy of quantitative myocardial blood flow assessment with (82)Rb PET: comparison with (13)N-ammonia PET. J Nucl Med. 2009;50:1062-71.
20. Herrero P, Markham J, Shelton ME, Bergmann SR. Implementation and evaluation of a two-compartment model for quantification of myocardial perfusion with rubidium-82 and positron emission tomography. Circ Res. 1992;70:496-507.
21. Lortie M, Beanlands RS, Yoshinaga K, Klein R, Dasilva JN, DeKemp RA. Quantification of myocardial blood flow with 82Rb dynamic PET imaging. Eur J Nucl Med Mol Imaging. 2007;34:1765-74.
22. Manabe O, Yoshinaga K, Katoh C, Naya M, DeKemp RA, Tamaki N. Repeatability of rest and hyperemic myocardial blood flow measurements with Rb-82 dynamic PET. J Nucl Med. 2009;50:68-71.
23. Yoshida K, Mullani N, Gould KL. Coronary flow and flow reserve by PET simplified for clinical applications using rubidium-82 or nitrogen-13-ammonia. J Nucl Med. 1996;37:1701-12.
24. Diamond GA, Forrester JS. Analysis of probability as an aid in the clinical diagnosis of coronary-artery disease. N Engl J Med. 1979;300:1350-8.
25. Siegrist PT, Gaemperli O, Koepfli P, Schepis T, Namdar M, Valenta I, et al. Repeatability of cold pressor test-induced flow increase assessed with H(2)(15)O and PET. J Nucl Med. 2006;47:1420-6.
26. Yoshinaga K, Katoh C, Noriyasu K, Iwado Y, Furuyama H, Ito Y, et al. Reduction of coronary flow reserve in areas with and without ischemia on stress perfusion imaging in patients with coronary artery disease: a study using oxygen 15-labeled water PET. J Nucl Cardiol. 2003;10:275-83.
27. Furuyama H, Odagawa Y, Katoh C, Iwado Y, Yoshinaga K, Ito Y, et al. Assessment of coronary function in children with a history of Kawasaki disease using (15)O-water positron emission tomography. Circulation. 2002;105:2878-84.
28. Katoh C, Morita K, Shiga T, Kubo N, Nakada K, Tamaki N. Improvement of algorithm for quantification of regional myocardial blood flow using 15O-water with PET. J Nucl Med. 2004;45:1908-16.
29. Shrout P, Fleiss JL. Intraclass correlation: uses in assessing rater reliability. Psychological Bulletin. 1979;86:420-8.
30. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1:307-10.
31. Cohen J. A power primer. Psychological Bulletin. 1992;112:155-9.
32. Schroeder SA. Tobacco control in the wake of the 1998 master settlement agreement. N Engl J Med. 2004;350:293-301.
33. Bergmann SR, Fox KA, Rand AL, McElvany KD, Welch MJ, Markham J, et al. Quantification of regional myocardial blood flow in vivo with H215O. Circulation. 1984;70:724-33.

34. Prior JO, Quinones MJ, Hernandez-Pampaloni M, Facta AD, Schindler TH, Sayre JW, et al. Coronary circulatory dysfunction in insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. Circulation. 2005;111:2291-8.
35. Kaufmann PA, Gnecchi-Ruscone T, di Terlizzi M, Schafers KP, Luscher TF, Camici PG. Coronary heart disease in smokers: vitamin C restores coronary microcirculatory function. Circulation. 2000;102:1233-8.
36. Positron emission tomography. In: Cherry S, Sorenson, J, Phepes, M, editor. Physics in nuclear medicine. Philadelphia: Saunders; 2003. p. 325-59.
37. Lautamaki R, George RT, Kitagawa K, Higuchi T, Merrill J, Voicu C, et al. Rubidium-82 PET-CT for quantitative assessment of myocardial blood flow: validation in a canine model of coronary artery stenosis. Eur J Nucl Med Mol Imaging. 2009;36:576-86.
38. Machac J, Bacharach SL, Bateman TM, Bax JJ, Beanlands R, Bengel F, et al. Positron emission tomography myocardial perfusion and glucose metabolism imaging. J Nucl Cardiol. 2006;13:e121-51.

Figure legends

Fig. 1

MBF at rest and during CPT.

CPT = cold pressor test; MBF = myocardial blood flow.

Fig. 2

CPT response (MBF during CPT/MBF at rest).

CPT = cold pressor test; MBF = myocardial blood flow.

Fig. 3

Bland Altman plots showing the overall degree of agreement between ^{82}Rb MBF and ^{15}O -water for MBF measurements. The difference between ^{15}O-water and ^{82}Rb (^{15}O-water -^{82}Rb) are plotted against the mean of the two values with 95% confidence limits of agreement for MAF measurements at rest, during CPT, in the CPT response.

CPT = cold pressor test; MBF = myocardial blood flow.

Fig. 1

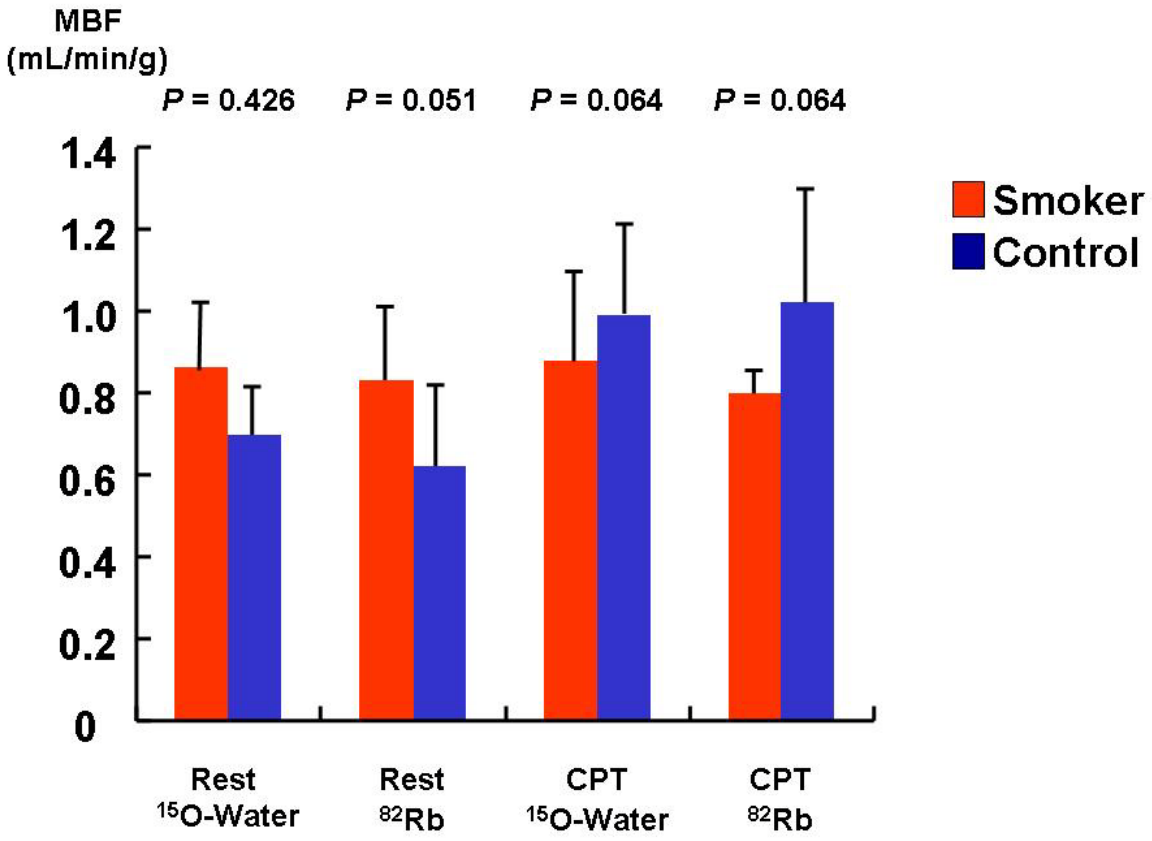
MBF
(mL/min/g)
P = 0.426
P = 0.051
P = 0.064
P = 0.064
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Rest
15O-Water
Rest
82Rb
CPT
15O-Water
CPT
82Rb
Smoker
Control

Fig. 2

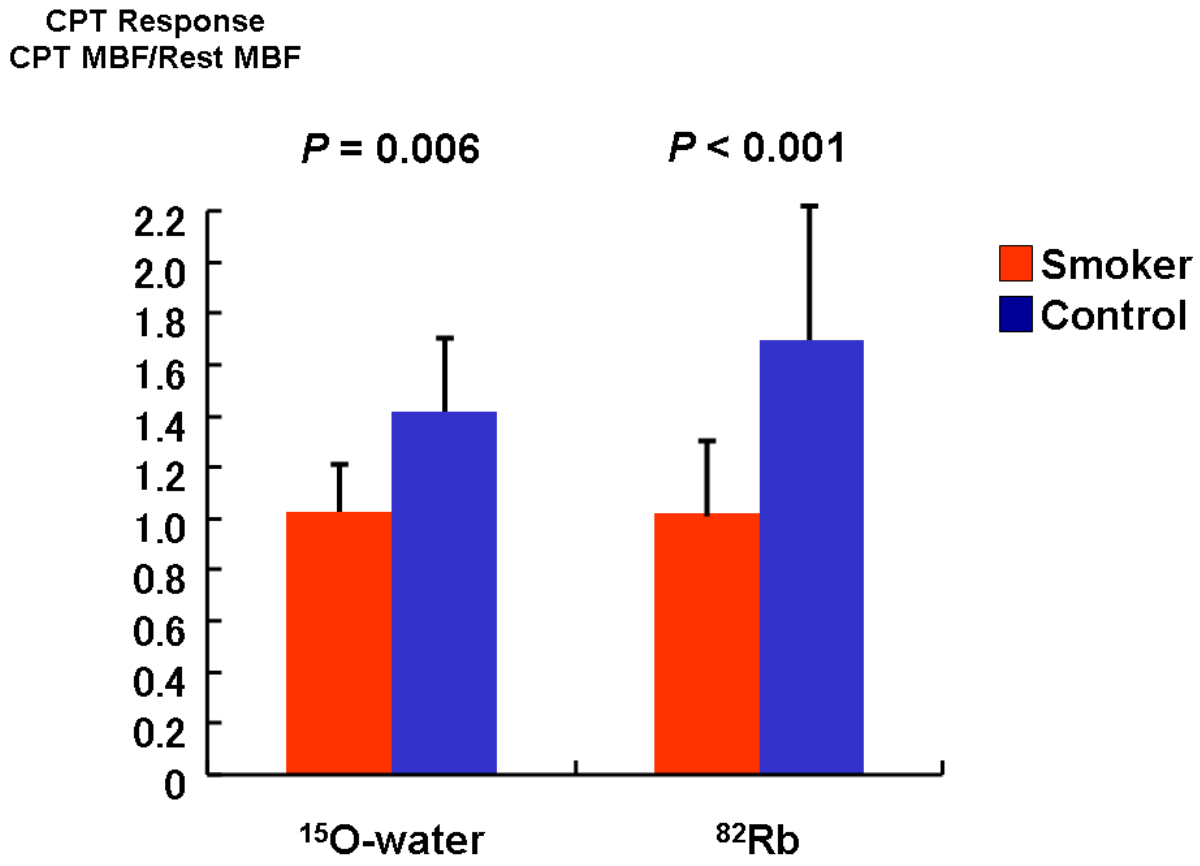
CPT Response
CPT MBF/Rest MBF
P = 0.006
P < 0.001
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Smoker
Control
15O-water
82Rb

Fig. 3

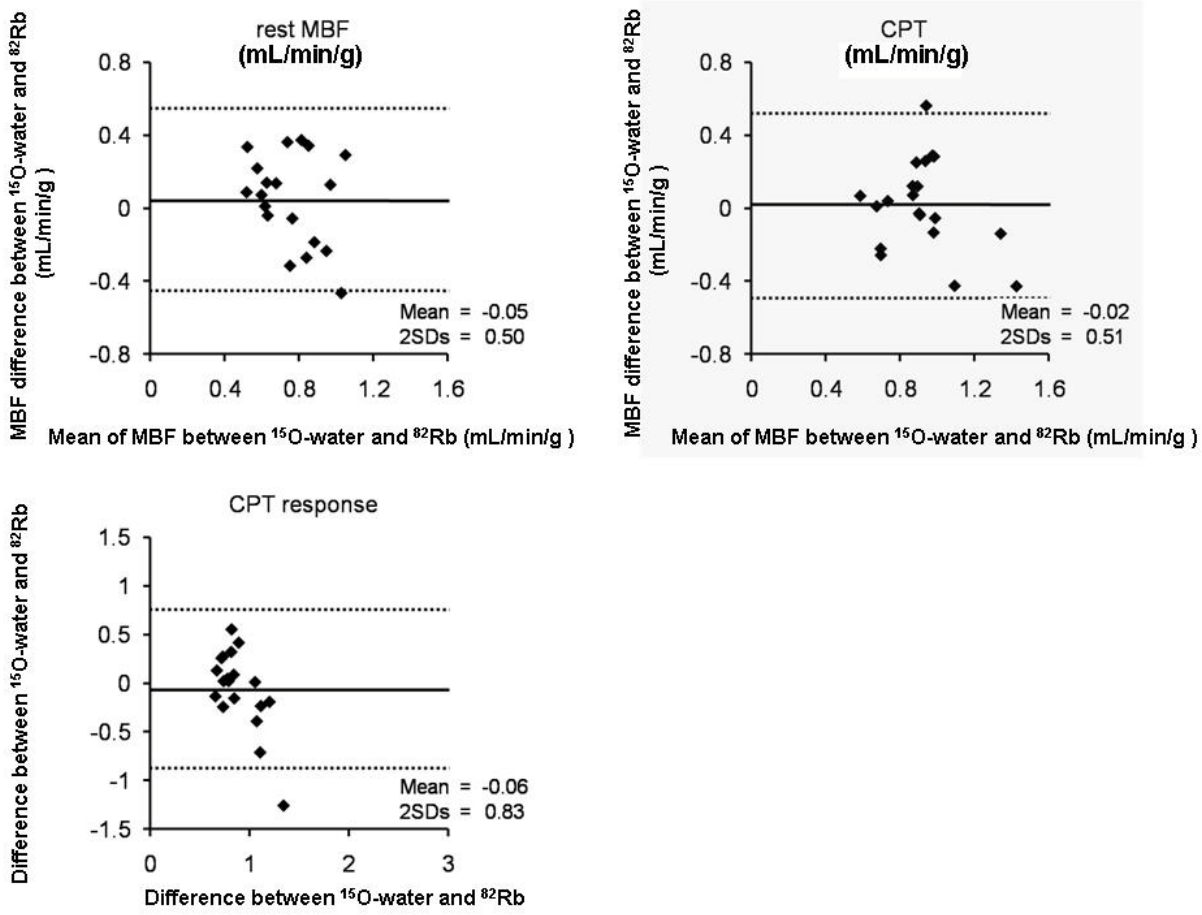
rest MBF
(mL/min/g)
MBF difference between 15O-water and 82Rb (mL/min/g)
Mean of MBF between 15O-water and 82Rb (mL/min/g)
Mean = -0.05
2SDs = 0.50
CPT
(mL/min/g)
MBF difference between 15O-water and 82Rb (mL/min/g)
Mean of MBF between 15O-water and 82Rb (mL/min/g)
Mean = -0.02
2SDs = 0.51
CPT response
Difference between 15O-water and 82Rb
Difference between 15O-water and 82Rb
Mean = -0.06
2SDs = 0.83

Table 1 Baseline subject characteristics

	Smokers (*n*=10)	**Controls (*n*=9)**
Age (yrs)	52.8 ± 9.4*	36.9 ± 11.9
Gender (M/F)	10/0	9/0
BMI	24.4 ± 2.2	22.7 ± 2.9
Systolic blood pressure (mmHg)	127.2 ± 14.6	116.6 ± 11.6
Total cholesterol (mg/dL)	205.1 ± 26.1**	170.4± 29.4
Brinkman index	498.0 ± 158.9†	0 ± 0

Data expressed as mean ± SD

BMI = body mass index; * $p = 0.014$; ** $p = 0.041$; †$p = 0.001$

Table 2 Rate pressure product (RPP) measurements at rest and during cold pressor test (CPT)

	Smokers (*n*=10)			**Controls (*n*=9)**		
	^{15}O-water	^{82}Rb	*p* value	^{15}O-water	^{82}Rb	*p* value
Rest	7779.0±1223.9	7469.4±1238.2	0.193	6646.0±1131.5	6152.7±831.9	0.098
CPT	10335.9±1657.3	10117.6±1304.9	0.625	10462.4±3172.0	9757.8±2258.7	0.359
% change (%)	34.5±22.5	38.7±29.4	0.375	56.1±29.3	58.3±27.9	0.910

Data expressed as mean ± SD. %change = percent change of RPP from rest during CPT; CPT = cold pressor test; RPP = rate pressure product.

Table 3 Myocardial blood flow (MBF) at rest and during Cold pressor test (CPT)

	^{15}**O-water**			^{82}**Rb**		
	Smokers (*n*=10)	Controls (*n*=9)	*p* value	Smokers (*n*=10)	Controls (*n*=9)	*p* value
Rest (mL/g/min)	0.86±0.18	0.70±0.13	0.426	0.83±0.23	0.62±0.20	0.051
CPT (mL/g/min)	0.88±0.22	0.99±0.21	0.064	0.81±0.09	1.02±0.36	0.064
CPT response	1.03±0.21	1.42±0.29	0.006	1.02±0.28	1.70±0.52	<0.001

Data expressed as mean ± SD. *P* value adjusted for age and total cholesterol.
CPT = cold pressor test.

Table 4 Coronary vascular resistance (CVR) at rest and during cold pressor test (CPT)

	^{15}**O-water**			^{82}**Rb**		
	Smokers (*n*=10)	Controls (*n*=9)	*p* value	Smokers (*n*=10)	Controls (*n*=9)	*p* value
Rest (mmHg/[mL/min/g])	111.0±24.6	117.0±20.0	0.931	110.5±27.6	135.5±38.9.	0.074
CPT (mmHg/[mL/min/g])	131.6±37.1	109.0±20.5	0.099	132.2±11.8	109.5±40.7	0.218
Δ CVR (mmHg/[mL/min/g])	20.6±20.7	-7.95±24.64	0.035	21.7±23.1	-25.8±35.1	0.002

Data expressed as mean ± SD. *p* value adjusted for age and total cholesterol.
CPT = cold pressor test; CVR= coronary vascular resistance.