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Coronary Adventitial and Perivascular Adipose Tissue Inflammation in Patients With Vasospastic Angina

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ABSTRACT

BACKGROUND Recent studies suggested that perivascular components, such as perivascular adipose tissue (PVAT) and adventitial vasa vasorum (VV), play an important role as a source of various inflammatory mediators in cardiovascular disease.

OBJECTIVES The authors tested their hypothesis that coronary artery spasm is associated with perivascular inflammation in patients with vasospastic angina (VSA) using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography/computed tomography (PET/CT).

METHODS This study prospectively examined 27 consecutive VSA patients with acetylcholine-induced diffuse spasm in the left anterior descending artery (LAD) and 13 subjects with suspected angina but without organic coronary lesions or coronary spasm. Using CT coronary angiography and electrocardiogram-gated ^{18}F-FDG PET/CT, coronary PVAT volume and coronary perivascular FDG uptake in the LAD were examined. In addition, adventitial VV formation in the LAD was examined with optical coherence tomography, and Rho-kinase activity was measured in circulating leukocytes.

RESULTS Patient characteristics were comparable between the 2 groups. CT coronary angiography and ECG-gated ^{18}F-FDG PET/CT showed that coronary PVAT volume and coronary perivascular FDG uptake significantly increased in the VSA group compared with the non-VSA group. Furthermore, optical coherence tomography showed that adventitial VV formation significantly increased in the VSA group compared with the non-VSA group, as did Rho-kinase activity. Importantly, during the follow-up period with medical treatment, both coronary perivascular FDG uptake and Rho-kinase activity significantly decreased in the VSA group.

CONCLUSIONS These results provide the first evidence that coronary spasm is associated with inflammation of coronary adventitia and PVAT, where ^{18}F-FDG PET/CT could be useful for disease activity assessment. (Morphological and Functional Change of Coronary Perivascular Adipose Tissue in Vasospastic Angina [ADIPO-VSA Trial]; UMIN000016675) (J Am Coll Cardiol 2018;71:414–25) © 2018 by the American College of Cardiology Foundation.

Coronary artery spasm plays an important role in the pathogenesis of a wide range of ischemic heart disease, not only in variant angina but also in other forms of angina pectoris and myocardial infarction (1,2). Recent studies have demonstrated that coronary spasm is also frequently noted in Caucasians and in Asians (3). We have previously demonstrated that activation of Rho-kinase,

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a molecular switch for vascular smooth muscle contraction, is a central mechanism of coronary spasm in animals and humans (1,4,5). We also have recently demonstrated that optical coherence tomography (OCT) enables the precise measurement of vasa vasorum (VV) area and that adventitial inflammatory changes, including VV formation, play important roles in the pathogenesis of coronary spasm in pigs and humans (6–8).

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The perivascular components, such as VV and perivascular adipose tissue (PVAT), have attracted much attention as sources of vascular inflammation (9). Indeed, PVAT is regarded as an active endocrine and paracrine organ that produces a variety of cytokines (e.g., interleukin [IL]-1β) (9). Epicardial adipose tissue volume measured by cardiac computed tomography (CT) is also significantly associated with cardiovascular events (10,11). However, it remains to be fully elucidated whether coronary artery spasm is associated with perivascular inflammation including coronary adventitia and PVAT in patients with vasospastic angina (VSA).

Functional alternations of the coronary artery are associated with PVAT inflammation (9–11). We have recently demonstrated that coronary PVAT volume is increased at the spastic coronary segment of VSA patients by CT coronary angiography (CTCA) (12), which suggests involvement of PVAT inflammation in the pathogenesis of the spasm. Currently, ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET)/CT is widely used to detect inflammation because it reflects the metabolic activity of glucose, which is known to be enhanced in inflamed tissue (13,14). Indeed, we have recently demonstrated that ^{18}F-FDG PET/CT is useful for the assessment of coronary perivascular inflammation in pigs in vivo (15). However, it remains to be examined whether ^{18}F-FDG PET/CT is also useful to assess disease activity and functional changes in the coronary adventitia and PVAT in VSA patients.

In the present study, we thus prospectively examined whether coronary artery spasm was associated with perivascular inflammation in VSA patients using ^{18}F-FDG PET/CT, and if so, whether imaging modalities (CTCA and OCT) were useful for detecting morphological alternations of coronary adventitia and PVAT and whether ^{18}F-FDG PET/CT was also useful to assess disease activity after medical treatment.

METHODS

The ethics committee of Tohoku University Graduate School of Medicine (No. 2014-1-720) approved the study protocol, which was performed in compliance with the Declaration of Helsinki (UMIN000016675). Written informed consent was obtained from all patients before study entry. The detailed methods are available in the Online Appendix.

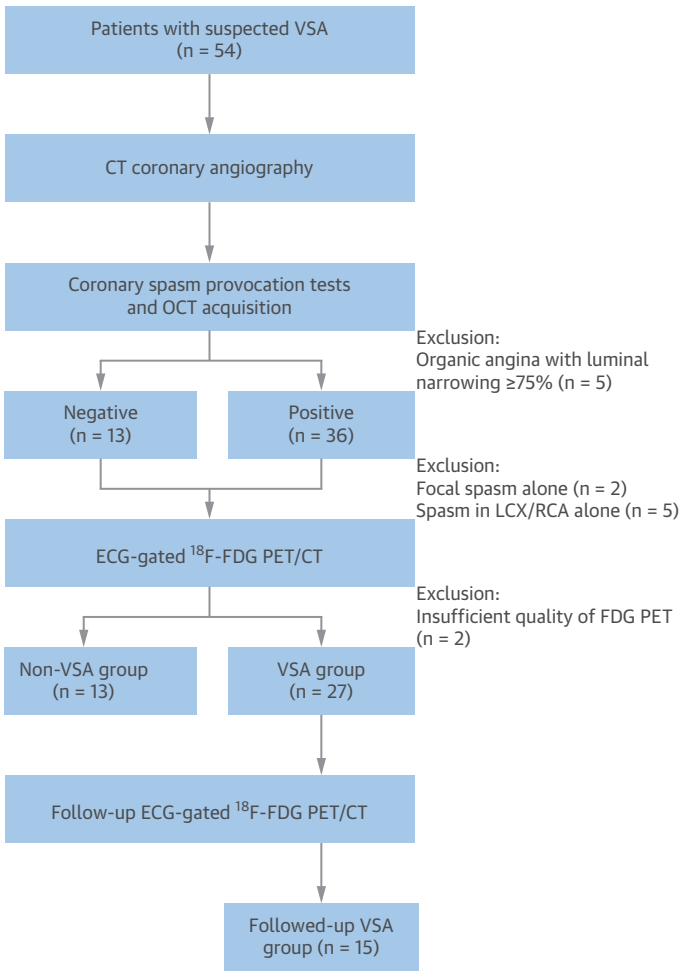
STUDY PATIENTS. The details of patient enrollment are provided in the Online Appendix. From March 2015 to September 2016, we prospectively enrolled a total of 54 consecutive eligible patients, from whom written informed consent was obtained at our Tohoku University Hospital (**Figure 1**). Inclusion criteria included age 20 years or older and rest angina due to suspected VSA. Exclusion criteria included acute coronary syndrome, left ventricular ejection fraction of 50% or less, serum creatinine level of 1.2 mg/dl or higher, history of an adverse reaction to contrast media, history of cardiac surgery, severe asthma, diabetes mellitus with insulin therapy, inflammatory or autoimmune diseases with steroid therapy, stent implantation in the left anterior descending coronary artery (LAD), organic coronary stenosis, focal spasm alone, spasm that occurred in the left circumflex artery (LCx)/right coronary artery (RCA) alone, and insufficient quality of ^{18}F-FDG PET/CT. We performed electrocardiogram (ECG)-gated CTCA and control coronary angiography and excluded 5 patients with luminal narrowing ≥75%. After control coronary angiography, we performed a coronary spasm provocation test with intracoronary acetylcholine (ACh). Diffuse spasm was diagnosed when luminal narrowing was noted from the proximal to the distal segment of the coronary artery, whereas focal spasm was defined as a discrete luminal narrowing (>90%) localized in the major coronary artery (16). In addition, we performed ECG-gated ^{18}F-FDG PET/CT. Among 36 patients with a positive provocation test, 9 were excluded, including 2 with focal spasm alone, 5 with spasm in the LCx or RCA alone, and 2 with insufficient quality of ^{18}F-FDG PET/CT. Finally, 27 VSA patients and 13 subjects with suspected angina but without organic coronary lesions or coronary spasm were enrolled in the VSA group and the non-VSA group, respectively. Of the VSA group, 15 patients were followed up and underwent ^{18}F-FDG PET/CT after a median follow-up of 23 months.

CORONARY SPASM PROVOCATION TEST WITH ACh AND QUANTITATIVE CORONARY ANGIOGRAPHY. We performed a provocation test of coronary spasm with ACh in accordance with the Japanese Circulation

ABBREVIATIONS AND ACRONYMS

ACh = acetylcholine
CT = computed tomography
CTCA = computed tomography coronary angiography
ECG = electrocardiogram
FDG = fluorodeoxyglucose
IL = interleukin
LAD = left anterior descending coronary artery
LCx = left circumflex artery
OCT = optical coherence tomography
PET = positron emission tomography
PVAT = perivascular adipose tissue
RCA = right coronary artery
ROI = region of interest
SUV = standardized uptake value
TBR = target-to-background ratio
VSA = vasospastic angina
VV = vasa vasorum

FIGURE 1 Study Flow Chart



^{18}F-FDG PET/CT = ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography; CT = computed tomography; ECG = electrocardiogram; FDG = fluorodeoxyglucose; LCX = left circumflex coronary artery; OCT = optical coherence tomography; PET = positron emission tomography; RCA = right coronary artery; VSA = vasospastic angina.

Society guidelines as previously reported (16-19). We performed quantitative coronary angiography to assess coronary vasomotor responses (Online Figure 1). The diagnosis of VSA was made when a total or subtotal (>90%) coronary artery narrowing accompanied by chest pain or ischemic ECG changes was noted (17). We also evaluated the extent of coronary vasoconstriction at segment 7 for correlations with coronary PVAT volume and perivascular FDG uptake.

MEASUREMENT OF CORONARY PVAT VOLUME WITH CTCA. We performed the measurement of adipose tissue volume with a dual-source 2×128 detector-row CT scanner (SOMATOM Definition, Siemens Medical, Forchheim, Germany) as previously reported (12). Adipose tissue volume was expressed as the volume index corrected by body surface area: (volume [cm^3]/body surface area [m^2]) (12). Measurement of coronary PVAT volume with CTCA was performed by 2 independent cardiologists (H.U. and S.T.) blinded to knowledge of the study groups. Concordance correlation coefficient values for intraobserver and interobserver agreement of coronary PVAT volume with CTCA measurements were confirmed in the present study (0.95 and 0.91 for intraobserver and interobserver agreement, respectively).

MEASUREMENT OF CORONARY PERIVASCULAR FDG UPTAKE WITH ECG-GATED ^{18}F-FDG PET/CT. To suppress physiological myocardial FDG uptake and avoid affecting measurement of coronary perivascular FDG uptake, we adopted a protocol of at least 18 h of fasting before PET imaging (20). In addition to at least 18 h of fasting, the evening meal on the day before PET imaging was a very low-carbohydrate and high-fat diet (21). Furthermore, to suppress motion artifact and avoid affecting measurement of coronary perivascular FDG uptake, we used ECG-gated ^{18}F-FDG PET/CT. The study patients received an intravenous administration of ^{18}F-FDG (6 MBq [0.162 mCi]/kg body weight) (21). Three hours after FDG injection, 3-dimensional PET imaging was performed with a list mode-capable PET/CT scanner (Biograph True Point 40, Siemens AG Medical Solutions, Forchheim, Germany) in the chest (21,22). In this study, the PET and CT images at 75% to 100% R-R interval were used for the analysis. The standardized uptake value (SUV) was calculated using the maximal pixel activity value within an approximately 28 mm^2 (a circle with a diameter of 6 mm) region of interest (ROI) placed on the coronary perivascular segment. The measurement of coronary perivascular FDG uptake was acquired as the average of 9 ROIs placed along the LAD (3 ROIs placed in the proximal, mid, and distal LAD, respectively) (23). Special attention was paid to avoid spillover activity from the myocardium. The SUV was corrected for blood activity by dividing the average blood SUV estimated from the ascending aorta to obtain a blood-corrected SUV, also known as target-to-background ratio (TBR) (22). Measurement of FDG uptake with ^{18}F-FDG PET/CT was performed by an independent radiologist and cardiologist (K. Takanami and H.U.) blinded to knowledge of the study groups. Concordance correlation coefficient values for intraobserver and interobserver agreement of coronary perivascular FDG uptake with ^{18}F-FDG PET/CT measurements were confirmed in the present

study (0.92 and 0.88 for intraobserver and interobserver agreement, respectively).

MORPHOMETRIC ANALYSIS AND MEASUREMENT OF ADVENTITIAL VV AREA ON OCT. After intracoronary administration of isosorbide dinitrate (2 mg), acquisition of OCT (Fast View, Terumo, Tokyo, Japan) was performed in 25 of 27 VSA patients and 13 non-VSA subjects, as previously reported (7,8,24,25). Morphometric analysis on OCT was performed by 2 independent cardiologists (H.U. and J.S.) blinded to knowledge of the study groups (Online Figure 2). Concordance correlation coefficient values for intraobserver and interobserver agreement of VV area density on the OCT measurements were confirmed in the present study (0.92 and 0.89 for intraobserver and interobserver agreement, respectively).

MEASUREMENT OF RHO-KINASE ACTIVITY IN CIRCULATING LEUKOCYTES. Western blot analysis for Rho-kinase activity in circulating leukocytes was performed with venous blood samples obtained before coronary angiography used as a useful biomarker for diagnosis and disease activity assessment of VSA (26).

FOLLOW-UP STUDY. After a median follow-up of 23 months, 15 of the 27 patients in the VSA group underwent ECG-gated ^{18}F-FDG PET/CT and venous blood sampling and were then examined using a questionnaire about angina symptoms. Follow-up examinations were performed as evaluated similarly at the baseline examinations.

STATISTICAL ANALYSIS. Continuous variables are expressed as mean ± SEM and categorical variables as n and percentages. Unpaired Student's *t*-test for normal distribution and Mann-Whitney *U* test for asymmetrical distribution were used to analyze differences in continuous variables. Chi-square test was used for categorical variables. Correlations between continuous variables were analyzed with a linear regression model. Paired Student's *t*-tests were used to compare variables before and after medical treatment. Jonckheere-Terpstra trend test was used to assess the trend in multiple variables. The Lin concordance correlation coefficient values for intraobserver and interobserver agreement were calculated. Statistical analysis was performed with IBM SPSS Statistics 22 (IBM, New York, New York). A value of $p < 0.05$ was considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS. Clinical characteristics of the study subjects were all comparable between the VSA group and the non-VSA group in terms of age, sex, body weight, body mass index, percent body fat, coronary risk factors, left ventricular ejection fraction, serum cardiac markers, and medical treatments (**Table 1,** Online Table 1). The prevalence of organic stenosis ≤50% in the LAD was also comparable between the 2 groups (Online Table 2).

TABLE 1 Baseline Clinical Characteristics and Treatments of the Non-VSA Group and the VSA Group

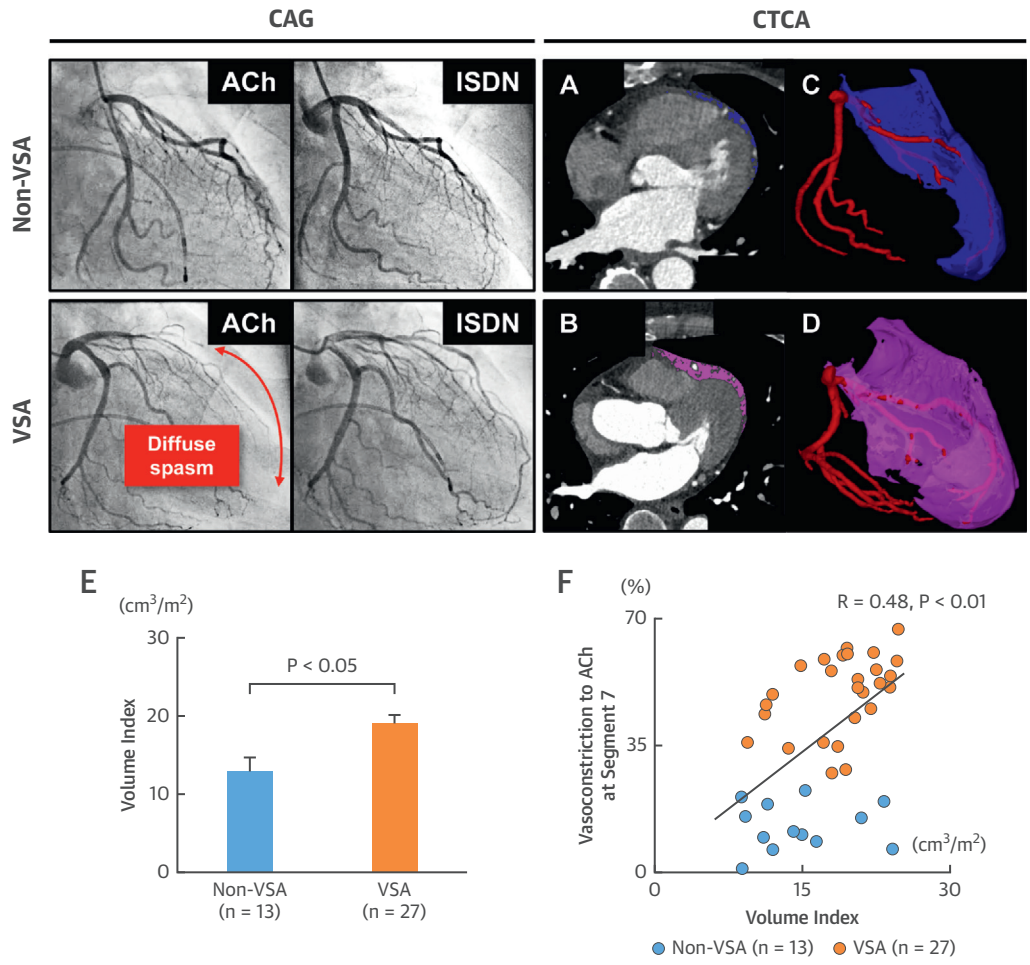
	Non-VSA (n = 13)	VSA (n = 27)	p Value
Age, yrs	65.4 ± 3.3	62.1 ± 2.1	0.40
Male	8 (61)	16 (59)	0.89
Body weight, kg	59.1 ± 2.4	59.5 ± 3.5	0.93
Body mass index, kg/m^2	22.1 ± 0.7	22.7 ± 1.0	0.55
Percent body fat, %	24.4 ± 2.0	26.1 ± 1.4	0.49
Hypertension	5 (38)	11 (41)	0.89
Diabetes mellitus	1 (8)	2 (7)	0.97
LDL cholesterol, mg/dl	109.2 ± 8.8	106.9 ± 4.9	0.81
HDL cholesterol, mg/dl	55.9 ± 5.4	58.9 ± 3.7	0.67
Current smoker	3 (23)	6 (22)	0.95
Former smoker	2 (15)	5 (19)	0.81
Positive family history of CVD	3 (23)	2 (8)	0.18
LVEF, %	68.6 ± 1.8	66.9 ± 1.1	0.44
Cardiac markers			
hs-CRP, mg/dl	0.06 ± 0.31	0.05 ± 0.01	0.55
BNP, pg/ml	20.4 ± 4.1	21.2 ± 3.8	0.89
Troponin I, μg/l	0.010 ± 0.001	0.010 ± 0.002	0.72
Medical treatments			
CCB	8 (62)	17 (63)	0.93
Long-acting nitrate	2 (15)	6 (22)	0.61
Potassium channel opener	2 (8)	9 (14)	0.39
ACE inhibitor or ARB	5 (38)	6 (22)	0.41
Beta-blocker	0 (0)	6 (22)	0.07
Statin	4 (31)	9 (33)	0.87
Antiplatelet	3 (23)	11 (41)	0.27

Values are mean ± SEM or n (%).

ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blocker; BNP = brain natriuretic peptide; CCB = calcium channel blocker; CVD = cardiovascular disease; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein; LVEF = left ventricular ejection fraction; VSA = vasospastic angina.

EVALUATION OF CORONARY PVAT VOLUME WITH CTCA. Cross-sectional CT images and 3-dimensional reconstructed CT images of coronary PVAT showed that coronary PVAT volume was increased at the spastic LAD in the VSA group compared with the non-VSA group (**Figures 2A to 2D**). Quantitative volumetric analysis showed that coronary PVAT volume index at the spastic LAD was significantly greater in the VSA group than in the non-VSA group (18.8 ± 0.8 cm^3/m^2 vs. 14.7 ± 1.4 cm^3/m^2; $p < 0.05$) (**Figure 2E**). Importantly, there was a significant positive correlation between the extent of coronary PVAT volume

FIGURE 2 Coronary Angiograms and CT Images of Coronary PVAT Volume

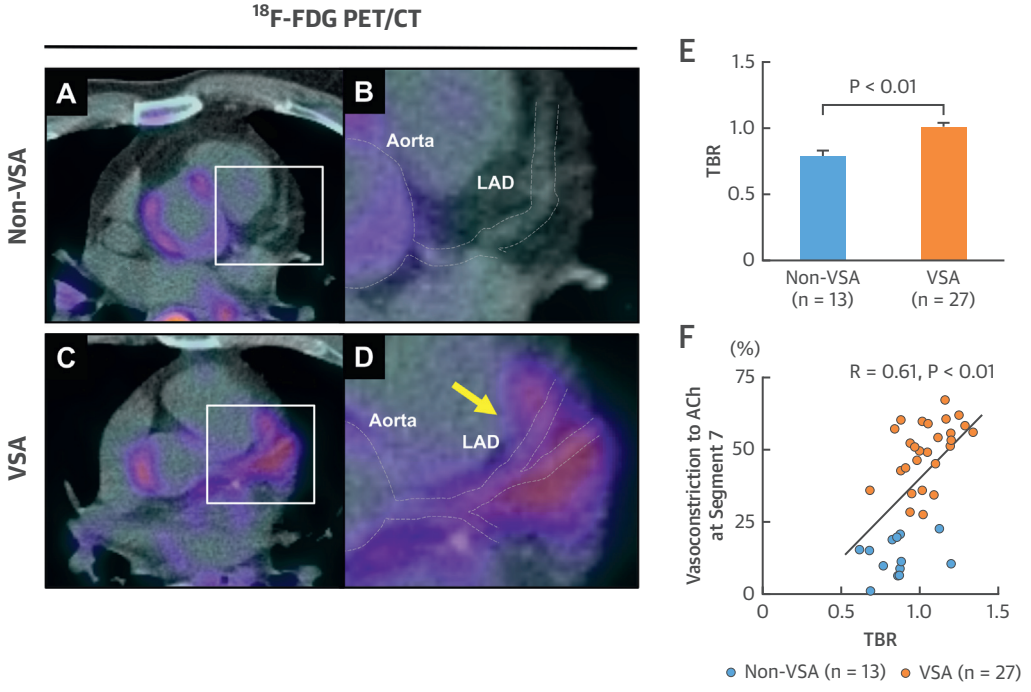


Representative coronary angiograms after intracoronary ACh and ISDN, cross-sectional CTCA images, and 3-dimensional reconstructed CT images of coronary perivascular adipose tissue at the spastic LAD in a non-VSA subject **(A, C)** and a VSA patient **(B, D)**. Coronary PVAT volume of LAD was larger in a VSA patient than in a non-VSA subject. Quantitative volumetric analysis showed that coronary PVAT volume index at the spastic LAD was significantly greater in the VSA group than in the non-VSA group **(E)**. There was a significant positive correlation between the extent of coronary PVAT volume index and that of coronary vasoconstricting responses to ACh **(F)**. ACh = acetylcholine; CAG = coronary angiography; CTCA = computed tomography coronary angiography; ISDN = isosorbide dinitrate; LAD = left anterior descending coronary artery; PVAT = perivascular adipose tissue; other abbreviations as in Figure 1.

index and that of coronary vasoconstricting responses to ACh in the VSA group ($R = 0.48$, $p < 0.01$) (Figure 2F).

EVALUATION OF CORONARY PERIVASCULAR FDG UPTAKE WITH ^{18}F-FDG PET/CT. ^{18}F-FDG PET/CT images showed that coronary perivascular FDG uptake was markedly enhanced at the spastic LAD in the VSA group compared with the non-VSA group (Figures 3A to 3D). Quantitative analysis showed that coronary perivascular TBR at the spastic LAD was significantly greater in the VSA group than in the non-VSA group (1.04 ± 0.03 vs. 0.85 ± 0.04, both $p < 0.01$) (Figure 3E). Importantly, there was a significant positive correlation between the extent of coronary perivascular TBR and that of coronary vasoconstricting responses to ACh in the VSA group ($R = 0.61$, $p < 0.01$) (Figure 3F). Intriguingly, there was a significant positive correlation between the extent of coronary PVAT volume index and that of coronary perivascular TBR in the VSA group but not in the non-VSA group (Online Figure 3).

FIGURE 3 Coronary Perivascular FDG Uptake Evaluated With ^{18}F-FDG PET/CT



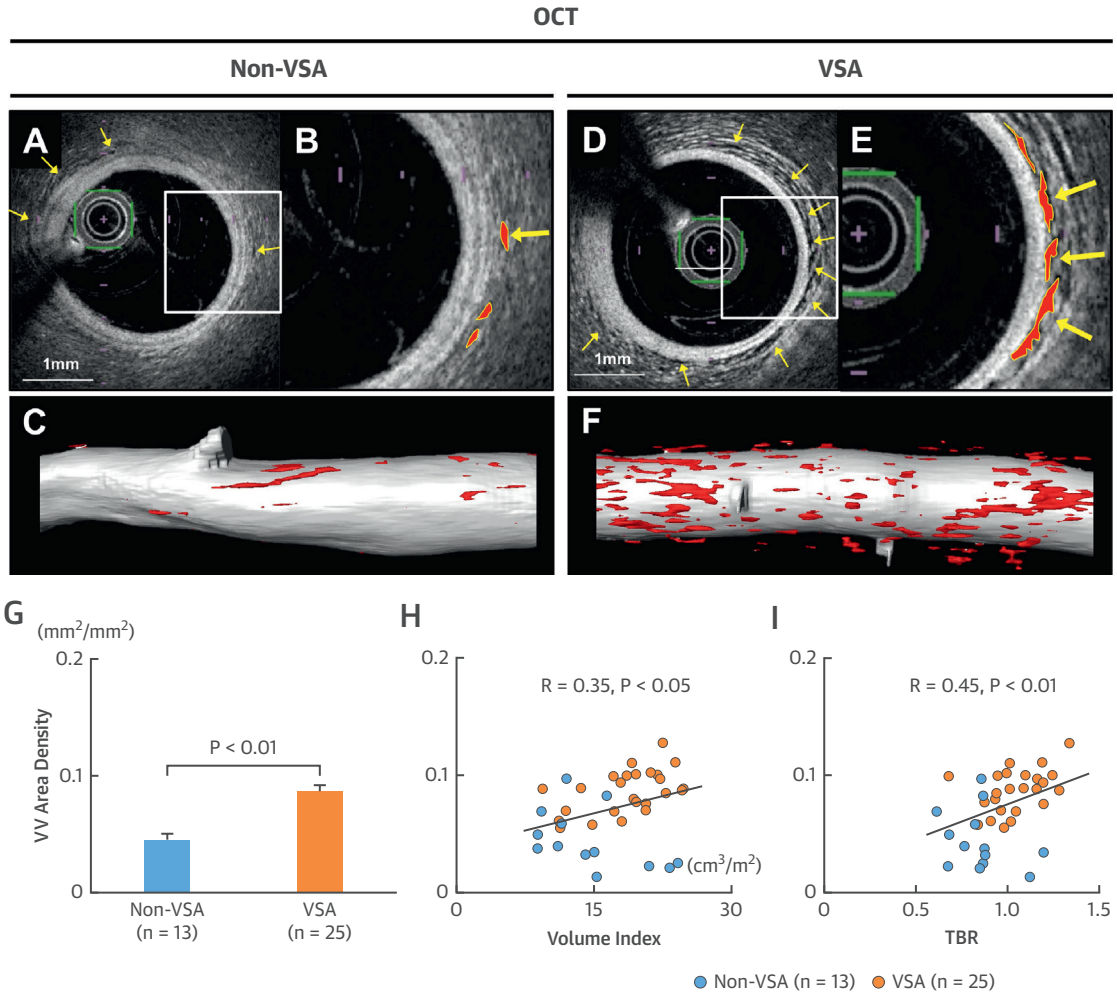
Representative ^{18}F-FDG PET/CT images of a non-VSA subject **(A, B)** and a VSA patient **(C, D)**. Coronary perivascular FDG uptake was enhanced at the spastic LAD in the VSA group compared with the non-VSA group. Quantitative analysis showed that coronary perivascular TBR at the spastic LAD was significantly greater in the VSA group than in the non-VSA group **(E)**. There was a significant positive correlation between the extent of coronary perivascular TBR and that of coronary vasoconstricting responses to ACh **(F)**. **Yellow arrow** shows coronary perivascular FDG uptake in LAD. TBR = target-to-background ratio; other abbreviations as in Figures 1 and 2.

ADVENTITIAL VV FORMATION ON OCT AND RELATIONSHIPS WITH CORONARY PVAT VOLUME AND CORONARY PERIVASCULAR FDG UPTAKE. All morphometric parameters with OCT were statistically comparable between the 2 groups (Online Table 3). OCT examination showed that adventitial VV area density per a cross-sectional OCT image at the spastic LAD was significantly greater in the VSA group than in the non-VSA group (0.086 ± 0.006 mm^2 vs. 0.045 ± 0.006 mm^2; $p < 0.01$) (Figures 4A to 4G). Notably, there was a significant positive correlation between the extent of coronary PVAT volume index and that of VV formation in the VSA group ($R = 0.35$, $p < 0.05$) (Figure 4H). In addition, there was a significant positive correlation between the extent of coronary perivascular TBR and that of VV formation in the VSA group ($R = 0.45$, $p < 0.01$) (Figure 4I).

RHO-KINASE ACTIVITY IN CIRCULATING LEUKOCYTES AND RELATIONSHIPS WITH CORONARY PVAT VOLUME AND CORONARY PERIVASCULAR FDG UPTAKE. Rho-kinase activity in circulating leukocytes was significantly higher in the VSA group than in the non-VSA group (1.21 ± 0.05 mm^2 vs. 0.91 ± 0.07 mm^2; $p < 0.05$) (Figure 5A). Importantly, there was a significant positive correlation between the extent of coronary PVAT volume index and that of Rho-kinase activity in the VSA group ($R = 0.36$, $p < 0.05$) (Figure 5B). In addition, there was a significant positive correlation between the extent of coronary perivascular TBR and that of Rho-kinase activity in the VSA group ($R = 0.53$, $p < 0.01$) (Figure 5C).

CORONARY PVAT VOLUME, CORONARY PERIVASCULAR FDG UPTAKE, AND RHO-KINASE ACTIVITY AFTER MEDICAL TREATMENT. In the VSA group ($n = 15$), we compared coronary PVAT volume index, coronary perivascular TBR, and Rho-kinase activity before and after medical treatment. Clinical characteristics were comparable between the VSA patients who were followed up and those who were not (Online Table 4). Medications at baseline and follow-up in the VSA group are shown in Online Table 5. After the diagnosis of VSA was made, all patients were treated with calcium-channel blockers (CCBs). ^{18}F-FDG PET/CT images in a VSA patient showed that coronary

FIGURE 4 Adventitial VV Formation Evaluated With OCT and Relationships Between Coronary PVAT Volume/Perivascular FDG Uptake and Adventitial VV Formation



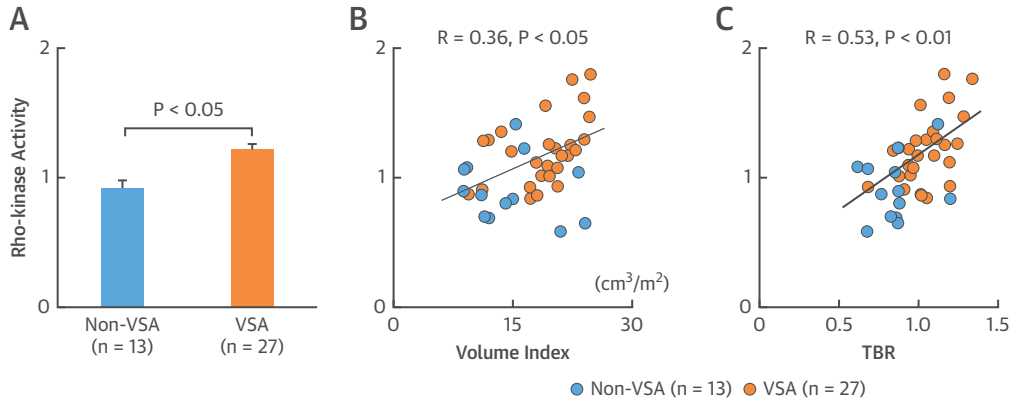
Representative cross-sectional OCT images and 3-dimensional reconstruction of OCT images of a non-VSA subject **(A to C)** and a VSA patient **(D to F)**. It is evident that coronary adventitial VV formation is enhanced in a VSA patient compared with a non-VSA subject. OCT examination showed that adventitial VV area density at the spastic LAD was significantly greater in the VSA group than in the non-VSA group **(G)**. There were significant positive correlations between the extent of VV formation and that of coronary perivascular adipose tissue volume index **(H)** and coronary perivascular TBR **(I)**. **Yellow arrows** show adventitial VV. VV = vasa vasorum; other abbreviations as in Figures 1 to 3.

perivascular FDG uptake at the spastic LAD was markedly reduced after medical treatment (Figures 6A to 6D). Quantitative analysis showed that although coronary PVAT volume index was unaltered, coronary perivascular TBR and Rho-kinase activity were significantly decreased after medical treatment (both $p < 0.01$) (Figures 6E to 6G). Moreover, there were significant trends between the extent of symptom improvement and percent change in coronary perivascular TBR (Figure 7A) and that of Rho-kinase activity (Figure 7B).

DISCUSSION

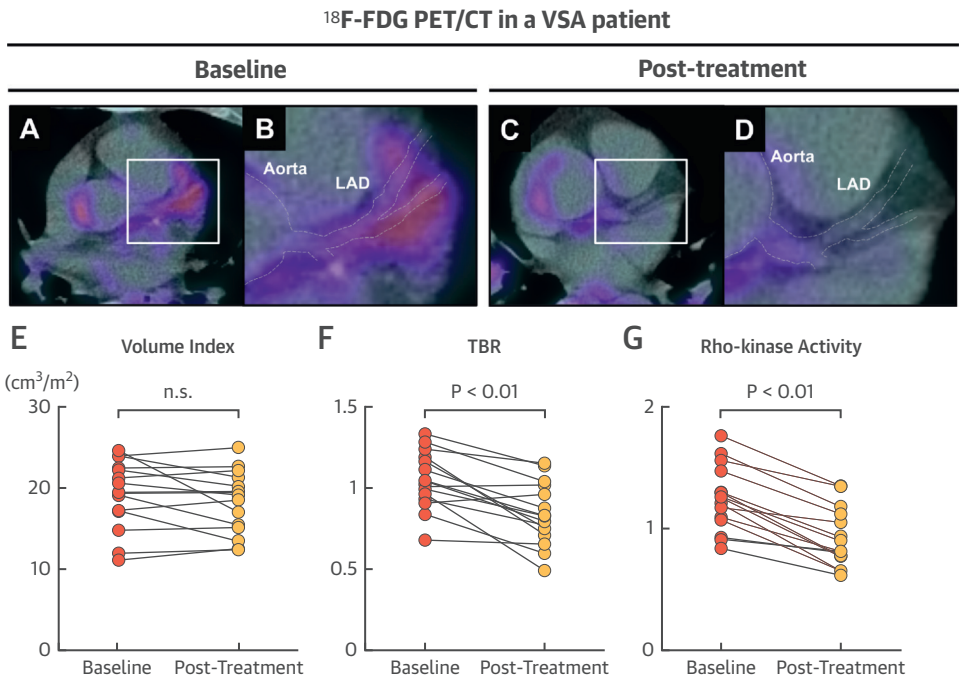
The major findings of the present study were that: 1) coronary perivascular FDG uptake with ^{18}F-FDG PET/CT was significantly increased in the VSA group compared with the non-VSA group; 2) the extents of coronary PVAT volume and coronary perivascular FDG uptake were positively correlated with those of adventitial VV formation on OCT and Rho-kinase activity of circulating leukocytes; and 3) the level of coronary perivascular FDG uptake significantly was

FIGURE 5 **Rho-Kinase Activity in Circulating Leukocytes and Relationships Between Coronary PVAT Volume/Perivascular FDG Uptake and Adventitial VV Formation**



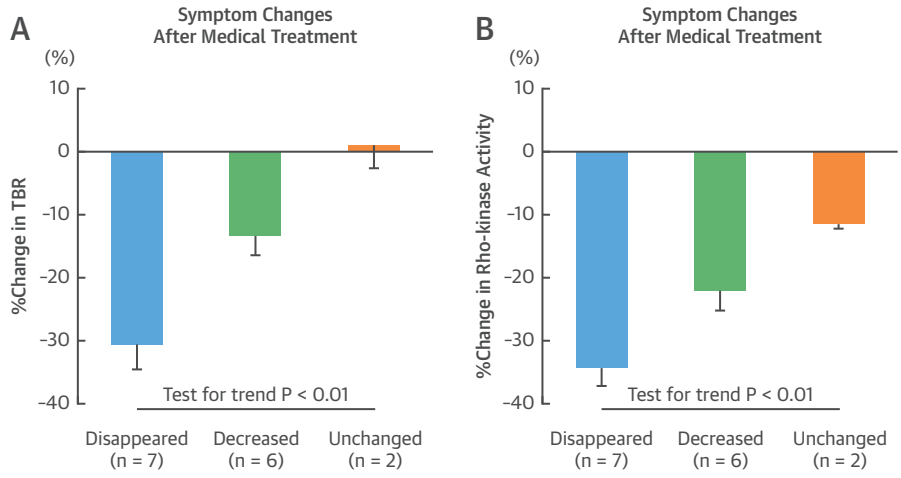
Rho-kinase activity in circulating leukocytes was significantly enhanced in the VSA group compared with the non-VSA group **(A)**. There were significant positive correlations between the extent of Rho-kinase activity and that of coronary perivascular adipose tissue volume index **(B)** and coronary perivascular TBR **(C)**. Abbreviations as in Figures 1 to 4.

FIGURE 6 **Changes in Coronary PVAT Volume, Coronary Perivascular FDG Uptake, and Rho-Kinase Activity Before and After Medical Treatment in VSA Patients**



Representative ^{18}F-FDG PET/CT images with a VSA patient at baseline and follow-up **(A to D)**. Coronary perivascular FDG uptake was markedly decreased in the spastic LAD after medical treatment. Quantitative analysis showed that although coronary perivascular adipose tissue volume index was not significantly decreased **(E)**, coronary perivascular TBR and Rho-kinase activity were significantly decreased after medical treatment **(F, G)**. Abbreviations as in Figures 1 to 3.

FIGURE 7 Symptom Improvement After Medical Treatment and Changes in Coronary Perivascular FDG Uptake and Those in Rho-Kinase Activity



There were significant trends between the extent of symptom improvement and percent change in coronary perivascular TBR **(A)** and that of Rho-kinase activity **(B)** during a median follow-up of 23 months in the group with VSA. Abbreviations as in Figures 1 and 3.

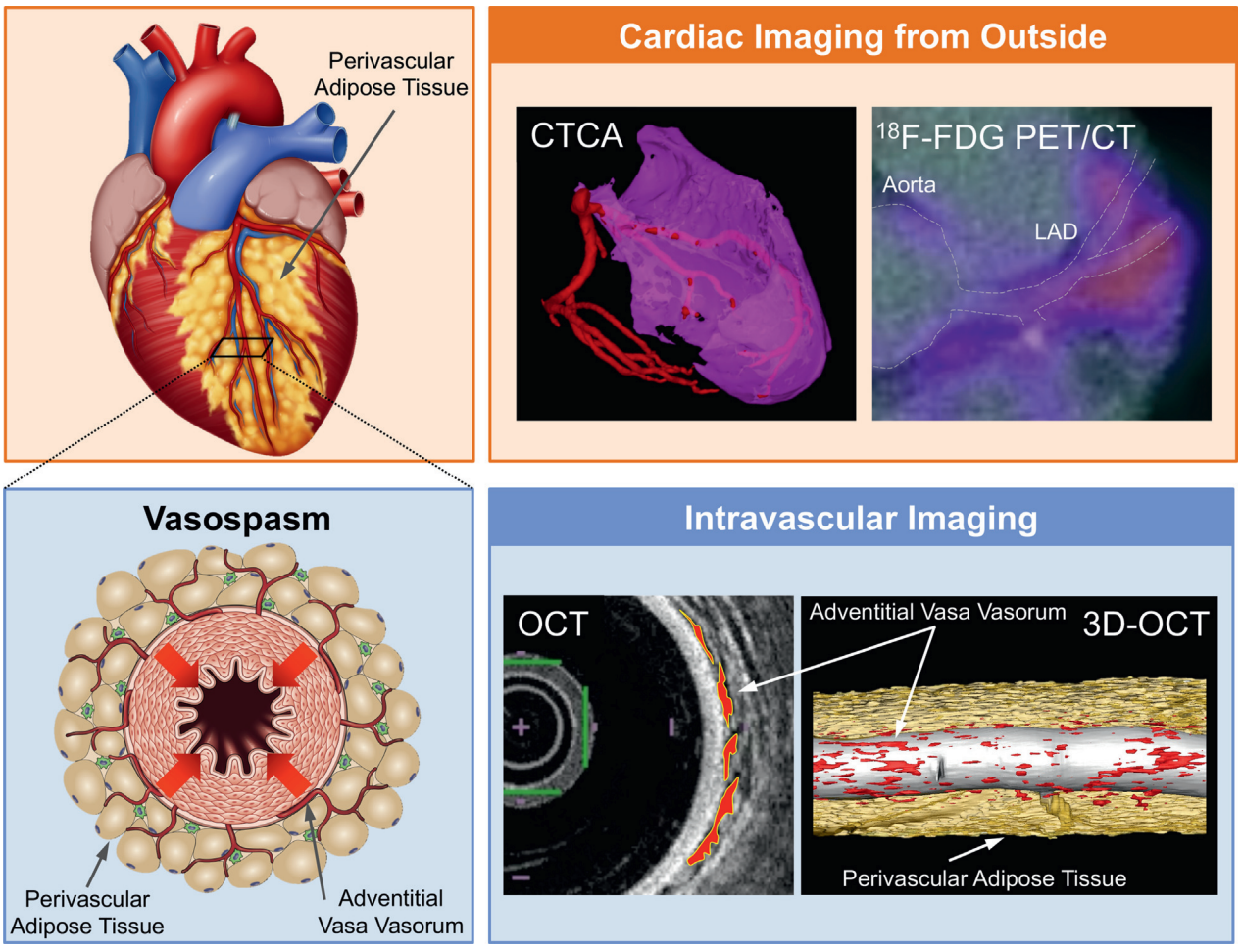
decreased at the follow-up after medical treatment. To the best of our knowledge, this is the first study that demonstrates that coronary artery spasm is associated with inflammation of coronary adventitia and PVAT through Rho-kinase activation, where ^{18}F-FDG PET/CT could be useful for disease activity assessment.

ROLES OF CORONARY ADVENTITIA AND PVAT IN VASOSPASTIC DISORDER. The adventitia completely surrounds the media and thus mediates communication with medial vascular smooth muscle cells (VSMCs), where coronary spasm is primarily caused by VSMC hypercontraction (1,27). The adventitia also interacts with its adjacent PVAT, which is linked to microvessels (VV) and nerves, to regulate vascular physiology, homeostasis, and structural remodeling, exerting major influences on the progression or regression of vascular disease (27). Indeed, we have previously demonstrated that adventitial VV formation is enhanced in association with coronary vasomotion abnormalities through Rho-kinase activation in a porcine model and in VSA patients (6,8). In the present prospective study, although body weight, body mass index, and percent body fat measured by bioelectrical impedance analysis were all comparable between the 2 groups, coronary PVAT volume at the spastic segment significantly increased in the VSA group compared with the non-VSA group. In addition, coronary PVAT volume at the spastic segment was significantly associated with that of VV. These findings suggest the important roles of coronary adventitia and PVAT in the pathogenesis of VSA.

There is growing evidence that signals that originate from the adventitia and PVAT play important roles in the regulation of vascular development, physiology, and vascular disease (27). Inflammatory cytokines secreted from inflamed coronary adventitia and PVAT are also involved in the pathogenesis of coronary spasm. Indeed, we demonstrated that the adventitial application of IL-1β with intact endothelium was able to cause coronary artery spasm in pigs in vivo (4). In addition, we have recently demonstrated that inflammatory changes in the coronary PVAT with enlarged adipocytes and elevated IL-1β levels are associated with coronary vasomotion abnormalities in a porcine model (15). In line with experimental findings, adventitial accumulation of mast cells was found in a patient with variant angina at autopsy (28), which suggests a potential role of inflamed coronary adventitia and PVAT in VSA patients. However, the detailed mechanism by which perivascular inflammation causes coronary artery spasm in humans remains to be fully elucidated.

INFLAMMATION OF CORONARY ADVENTITIA AND PVAT IN VSA PATIENTS. In the present study, we were able to demonstrate for the first time that not only coronary PVAT volume but also coronary perivascular FDG uptake is markedly enhanced in the spastic LAD in VSA patients compared with non-VSA

CENTRAL ILLUSTRATION **Multimodality Imaging Approach of Coronary Adventitia and PVAT in Patients With Vasospastic Angina**



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Cardiac images from outside the coronary artery with CTCA and ^{18}F-FDG PET/CT show coronary PVAT volume and coronary perivascular FDG uptake, respectively, as inflammatory changes of the coronary artery. In addition, intravascular images with OCT/3D-OCT show adventitial vasa vasorum formation as inflammatory changes of the coronary adventitia. In the present study, we used a multimodality approach with these 3 imaging tools to examine the inflammatory changes of coronary adventitia and PVAT in patients with vasospastic angina. 3D = 3-dimensional; ^{18}F-FDG PET/CT = ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography; CTCA = computed tomography coronary angiography; FDG = fluorodeoxyglucose; LAD = left anterior descending coronary artery; OCT = optical coherence technique; PVAT = perivascular adipose tissue.

subjects by using multimodality imaging with CTCA and ^{18}F-FDG PET/CT. In the preceding study, we demonstrated that perivascular FDG uptake detected by ^{18}F-FDG PET/CT actually reflects perivascular inflammation rather than vascular inflammation, in line with the histological findings in pigs in vivo (15). In addition, ECG-gated ^{18}F-FDG PET/CT enabled us to precisely assess the small structures, such as coronary artery and perivascular tissue, even in the beating heart. The extents of coronary PVAT volume and coronary perivascular FDG uptake were also positively associated with those of adventitial VV formation evaluated by OCT. These findings indicate an important role of perivascular inflammation in the pathogenesis of coronary artery spasm, as evaluated by cardiac imaging from outside and intravascular imaging from inside the coronary artery (**Central Illustration**).

Importantly, in the present study, we also were able to demonstrate that coronary perivascular FDG

uptake was significantly reduced after long-term medical treatment. Indeed, we have demonstrated that long-term treatment with CCBs suppresses coronary vasomotor abnormalities through decreased inflammation (29,30), which suggests an anti-inflammatory effect of CCBs on functional alternation of the coronary artery and its perivascular tissue, as demonstrated in the present study.

MECHANISMS OF CORONARY ARTERY SPASM. As mentioned above, coronary artery spasm is primarily caused by VSMC hypercontraction (1,4,5). We have previously demonstrated that enhanced Rho-kinase activity plays a central role in the molecular mechanisms of coronary artery spasm in animals and humans (1,4–6,30–33). Rho-kinase suppresses myosin phosphatase activity by phosphorylating MYPT1 and thus augments VSMC contraction (5). We also have recently demonstrated that Rho-kinase activity in circulating leukocytes is a useful biomarker for coronary artery spasm, not only for the diagnosis but also for the assessment of disease activity and efficacy of medical treatment (26). Importantly, in the present study, along with angina symptoms and Rho-kinase activity of circulating leukocytes, coronary perivascular FDG uptake was enhanced in active VSA patients and was remarkably decreased after medical treatment. These findings indicate that perivascular inflammation is the substrate for VSMC hypercontraction and that ^{18}F-FDG PET/CT can be a useful tool to assess disease activity in VSA patients.

STUDY LIMITATIONS. First, although we examined the association between inflammatory changes of adventitial VV/PVAT and coronary spasm, we did not clearly establish their causal relationship. Second, because we only enrolled patients with VSA in the LAD in the present study, it remains to be examined whether this is also the case for spasm in other coronary arteries (e.g., LCx and RCA). Third, we excluded patients with focal spasm because of its potentially different pathophysiology from diffuse spasm (24). Finally, because we did not examine VSA patients without CCB treatment, we were unable to directly demonstrate that CCBs had an anti-inflammatory effect on perivascular inflammation at the follow-up period in the VSA group.

CONCLUSIONS

These results provide the first evidence that the coronary perivascular inflammation associated with Rho-kinase activation can be the substrate for coronary spasm, which can be altered with medical treatment, and that ^{18}F-FDG PET/CT is useful for disease activity assessment of the vasospastic disorder in VSA patients.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Perivascular adipose tissue and adventitial vasa vasorum release inflammatory mediators that contribute to coronary vasospasm. Various imaging modalities, specifically CT coronary angiography, ^{18}F-FDG PET/CT, and OCT, can be useful for assessment of coronary perivascular inflammation and disease activity in patients with vasospastic angina.

TRANSLATIONAL OUTLOOK: Although invasive methods, such as coronary angiography and provocation with acetylcholine, are currently indispensable for diagnosis of vasospastic angina, a multimodality approach, especially ^{18}F-FDG PET/CT imaging, may become useful as a noninvasive tool for assessment of this disorder.

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KEY WORDS cardiac CT, coronary adventitia, coronary spasm, FDG PET, perivascular adipose tissue, Rho-kinase

APPENDIX For an expanded Methods section, please see the online version of this article.