Echocardiographic Epicardial Adipose Tissue Thickness Is Associated with Symptomatic Coronary Vasospasm during Provocative Testing

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**Background:** Epicardial adipose tissue (EAT) is the ectopic visceral fat surrounding the heart, which plays an important role in atherosclerosis of the coronary arteries via endothelial damage. Several studies have also suggested that vasospasm with angina (VSA) causes endothelial dysfunction in the coronary arteries. The aim of this study was to evaluate the thickness of EAT in the anterior interventricular groove (EAT-AIG) using echocardiography in patients who had no obstructive coronary artery disease and were suspected of having VSA.

**Methods:** Sixty-five patients who underwent intracoronary acetylcholine provocation testing for clinical indications were prospectively enrolled. VSA was diagnosed by coronary artery stenosis increase of >90% and the presentation of chest pain with ischemic changes on electrocardiography.

**Results:** Subjects were divided into two groups, with and without significant coronary spasm (VSA group, 30 patients; non-VSA group, 35 patients), consistent with acetylcholine provocation testing. EAT-AIG thickness was significantly greater in the VSA group than in the non-VSA group (8.2 ± 2.7 vs 6.1 ± 2.5 mm, P = .002). By receiver operating characteristic analysis, EAT-AIG thickness had a high C statistic (area under the curve = 0.81, P < .001) after adjustment for conventional risk factors (smoking, diabetes mellitus, and dyslipidemia). EAT-AIG thickness had incremental diagnostic value over other conventional risk factors (area under the curve = 0.81 vs 0.64, P for comparison = .020).

**Conclusions:** EAT-AIG thickness, which is noninvasively and easily measured using transthoracic echocardiography, can be one of multiple clinical variables associated with VSA. (J Am Soc Echocardiogr 2017;30:1021-7.)

**Keywords:** Echocardiography, Epicardial adipose tissue, Vasospasm in patients with angina, Anterior interventricular groove

Previous studies have shown that increased epicardial adipose tissue (EAT) thickness is associated with known cardiovascular risk factors. Echocardiography can determine the regional thickness of EAT and has some advantages over other methods (i.e., it is noninvasive, relatively economical, and easy to perform for screening). Echocardiographic EAT thickness has been defined as the thickness of the low-echoic area on the free wall of the right ventricle (RV) in the parasternal long- and short-axis views. EAT is not uniformly distributed, as adipose tissue concentrates primarily in the interventricular and atrioventricular grooves rather than in nongroove segments, such as the free wall of the RV. We recently reported that the echocardiographic thickness of EAT in the anterior interventricular groove (EAT-AIG), obtained using a higher frequency linear probe, was well correlated with EAT volume measured by computed tomography and associated with coronary artery disease (CAD). Vasospasm with angina (VSA) is caused by focal or diffuse spasm of a major coronary artery, resulting in a high-grade obstruction. Although it is sometimes thought that patients with VSA have relatively favorable outcomes, their clinical outcomes strongly depend on early diagnosis. Because the treatment strategies for VSA and other chest pain or discomfort diseases are substantially different, it is important to...
METHODS

Study Population

We consecutively enrolled 792 patients who underwent their first coronary angiographic examinations from May 1, 2012, to November 1, 2014, at Tokushima University Hospital. In the present study, the analyzed population was patients who had no significant coronary artery stenosis but were suspected to have VSA (chest pain or chest discomfort at rest). Therefore, patients who demonstrated ≥50% stenosis on coronary angiography (n = 482) or lacked typical VSA symptoms (n = 212) were excluded. Subjects were also excluded if they refused to undergo the intracoronary acetylcholine (ACh) stress test (n = 33). After these exclusions, 65 patients remained for the final analysis (Figure 1). They were scheduled to undergo intracoronary ACh stress testing to diagnosis VSA. We also enrolled 30 age- and gender-matched healthy control subjects who were selected from our healthy volunteer database on the basis of a comprehensive history and physical examination, to determine the normal value of EAT. The study was approved by the institutional review board of Tokushima University Hospital (no. 2183-1), and written informed consent was obtained from all subjects.

All subjects were classified into either the VSA group or the non-VSA group. The VSA group was defined as patients who had coronary spasm in any coronary artery during the intracoronary ACh stress test. Hypertension was defined as systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg or current treatment with antihypertensive medication. Diabetes mellitus was defined as fasting blood glucose 126 mg/dL, glycated hemoglobin ≥ 6.5%, and/or the need for oral hypoglycemic agents. Dyslipidemia was defined as plasma total cholesterol > 220 mg/dL or the use of lipid-lowering therapy. Smoking was defined as current or previous use of cigarettes.

Echocardiographic Examination

Echocardiography was performed using commercially available ultrasound machines (Vivid E9 IGE Healthcare, Milwaukee, WI, iE33 [Philips Healthcare, Best, The Netherlands]; Aplio 500 [Toshiba Medical Systems, Tochigi, Japan]; or α10, Preirus [Hitachi, Tokyo, Japan]) 1 day before coronary angiography. The details of EAT thickness measurements have already been described in our previous report. Using a high-frequency linear probe (7.5–11 MHz), EAT thickness was measured at end-systole in two locations: in the AIG, where the left anterior descending coronary artery (LAD) runs, and on the free wall of the RV, away from any major epicardial artery. While assessing EAT-AIG thickness, we searched for the distal portion of the LAD and carefully rotated the probe until a longitudinal section was identified. EAT-AIG thickness was measured as the distance from the outer wall of the myocardium to the visceral layer of the epicardium. EAT-RV thickness was measured using the method previously reported by Iacobellis and Willens. The thickness of pericardial adipose tissue over the free wall of the RV outside of the parietal pericardium was also measured at end-systole from the same image used to measure EAT-RV thickness. Measurements were performed during three cardiac cycles for each parameter, and the mean for each parameter was used for analysis. The reproducibility of EAT-AIG thickness, expressed as the intraclass correlation coefficient, has been described in detail by our group as 0.98 and 0.91 for intraobserver and interobserver variation.

Provocation Testing of VSA

The intracoronary infusion of ACh was performed according to the guidelines for diagnosis and treatment of patients with vasospastic angina. The administration of vasoactive drugs, including calcium channel blockers, nitrates, β-adrenergic blockers, and other vasodilators, was stopped for ≥2 days before angiography. Before performing vasospasm provocation testing with ACh, controlled coronary angiography was performed. ACh was injected from the same angle over a period of 20 sec into the right coronary artery (RCA; at a dose of 20 or 50 μg) and the left coronary artery (LCA; at a dose of 20, 50, or 100 μg) according to the clinical condition. Angiography was subsequently performed 1 min after the start of each injection. Angiography was also performed after presentation of ischemic changes on electrocardiography or chest pain. Coronary spasm was defined as total or subtotal occlusion (≥90% stenosis) accompanied by episodes of chest pain or ischemic ST-segment changes on electrocardiography (Figure 2).

Statistical Analysis

Data are presented as mean ± SD. Student’s t test was used to compare continuous variables between the two groups (VSA and non-VSA). When comparing between three groups (VSA, non-VSA, and normal), we added the control group to use the analysis of variance with the Bonferroni method. Logistic regression was used to calculate odds ratios and 95% CIs after adjustment for potential confounders. Potential confounders, such as smoking, diabetes mellitus, and dyslipidemia, were entered in multivariate models. The performance of clinical risk factors (smoking, diabetes mellitus, and dyslipidemia) plus various combinations of EAT-AIG thickness was assessed using the area under the curve in receiver operating characteristic analysis. To evaluate the correlation of EAT-AIG thickness and presence of VSA, two models were constructed and compared using receiver operating characteristic analysis. Model 1, the basic model, consisted of clinical risk factors alone. Model 2 included the variables in model 1 plus EAT-AIG thickness. The DeLong method was used to compare the C statistic. We conducted bootstrapping with 2,000 resamples to assess the internal validation. Statistical analysis was performed using standard statistical software packages (SPSS version 21.0, IBM SPSS, Chicago, IL; MedCalc version 15.8, MedCalc Software, Mariakerke, Belgium, and R version 3.3.3 [IR Foundation for Statistical Computing, Vienna, Austria]). P values <.05 were considered to indicate statistical significance.

Abbreviations

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<tr>
<td>ACh</td>
<td>Acetylcholine</td>
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<td>AIG</td>
<td>Anterior interventricular groove</td>
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<td>CAD</td>
<td>Coronary artery disease</td>
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<td>EAT</td>
<td>Epicardial adipose tissue</td>
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<td>LAD</td>
<td>Left anterior descending coronary artery</td>
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<td>RCA</td>
<td>Right coronary artery</td>
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<td>RV</td>
<td>Right ventricle</td>
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<td>VSA</td>
<td>Vasospasm with angina</td>
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Figure 1 Flowchart of the recruitment of patients.

792 patients underwent their first coronary angiographic examinations from May 1, 2012 to Nov 1, 2014

- ≥50% luminal narrowing (n=482)
- <50% luminal narrowing (n=310)
  - Without typical VSA symptoms (n=212)
  - Presumptive VSA (n=98)
    - No consent (n=33)

65 patients diagnosed as presumptive VSA and underwent intracoronary acetylcholine provocation testing

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Figure 2 Representative recordings. VSA group: a 73-year-old woman presented with chest discomfort. EAT was thickened, and we suspected coronary artery disease or VSA. Coronary angiography revealed that there was no significant organic stenosis. We added ACh provocation testing. Significant coronary spasm was observed, and we diagnosed VSA. Non-VSA group: an 87-year-old man presented with chest discomfort. EAT was not thickened. The coronary artery had no significant stenosis, and ACh provocation testing was negative. PAT, Pericardial adipose tissue.
RESULTS

Patient Characteristics

On the basis of the results of ACh provocation testing, subjects were divided into two groups with (VSA group: 30 patients; mean age, 66 ± 6 years; 19 men) and without (non-VSA group: 35 patients; mean age, 65 ± 6 years; 18 men) significant coronary spasm. We added age-matched healthy control subjects (control group: 30 patients; mean age, 65 ± 6 years; 17 men) in the analysis. Clinical characteristics of the patients in the VSA group, non-VSA group, and control group are presented in Table 1. There were no significant differences in age, gender, body mass index, and blood pressure among all three groups. Neither were there any differences in cardiovascular risk factors between the VSA and non-VSA groups. In the non-VSA group, all patients underwent provocative testing with ACh 50 mg in the RCA and ACh 100 mg in the LCA. In the VSA group, for the provocative test in the RCA, two subjects received ACh 20 mg and 22 subjects ACh 50 mg. For the provocative test in the LCA, no subjects received ACh 20 mg, three received ACh 50 mg, and 17 received ACh 100 mg. Thus, many patients underwent provocative testing with the maximum dose of ACh.

Echocardiographic Parameters and Measurements of EAT Thickness

We were able to measure EAT thicknesses using echocardiography in all subjects. Echocardiographic parameters and measurements of EAT thickness are presented in Table 2. There were no significant differences in left ventricular and left atrial size and Doppler findings between the VSA and non-VSA groups. EAT-AIG thickness was significantly greater in the VSA group than in the non-VSA group (8.2 ± 2.7 vs 6.1 ± 2.5 mm, P = .002). Interestingly, there was no difference of EAT-AIG thickness between the control and non-VSA groups. No differences in EAT-AIG thickness were found between patients with no visible stenosis (0% stenosis, n = 50) and those with mild stenosis (1%–49% stenosis, n = 15; 7.2 ± 2.9 vs 6.9 ± 2.2 mm, P = .733). The prevalence of VSA was similar in both of these groups (42% vs 60%, P = .119). Furthermore, in the non-VSA group, there

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<th>Table 1 Clinical background</th>
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<td>Healthy control (n = 30)</td>
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<td>Systolic blood pressure (mm Hg)</td>
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<td>Diastolic blood pressure (mm Hg)</td>
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Risk factors

| Diabetes mellitus | — | 8 (27%) | 5 (14%) | .229 |
| Hypertension | — | 16 (53%) | 18 (51%) | .881 |
| Dyslipidemia | — | 14 (47%) | 12 (34%) | .319 |
| Smoking | — | 17 (57%) | 12 (34%) | .073 |

Medication

| Diabetes mellitus drug | — | 5 (17%) | 2 (5%) | .178 |
| Calcium channel blocker | — | 9 (30%) | 17 (49%) | .129 |
| Statins | — | 12 (32%) | 9 (26%) | .231 |

Laboratory data

| HDL cholesterol (mg/dL) | — | 60 ± 18 | 61 ± 15 | .856 |
| LDL cholesterol (mg/dL) | — | 111 ± 33 | 113 ± 32 | .826 |
| LDL/HDL | — | 2.0 ± 0.7 | 2.0 ± 0.8 | .985 |
| AA (μg/mL) | — | 203 ± 54 | 182 ± 46 | .122 |
| DHA (μg/mL) | — | 163 ± 69 | 161 ± 69 | .924 |
| DHLA (μg/mL) | — | 41 ± 12 | 37 ± 13 | .230 |
| EPA (μg/mL) | — | 65 ± 35 | 82 ± 57 | .168 |
| EPA/AA | — | 0.34 ± 0.19 | 0.49 ± 0.38 | .073 |
| CRP (mg/dL) | — | 0.07 ± 0.05 | 0.08 ± 0.16 | .737 |
| Creatinine (mg/dL) | — | 0.78 ± 0.16 | 0.73 ± 0.24 | .284 |
| Glycated hemoglobin (%) | — | 5.9 ± 0.7 | 5.9 ± 0.9 | .661 |
| BNP (pg/mL) | — | 42 ± 51 | 35 ± 38 | .544 |

AA, Arachidonic acid; BMI, body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein; DHA, docosahexaenoic acid; DHLA, dihomo-gamma-linolenic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Data are expressed as mean ± SD or as number (percentage).
were 15 patients with 25% to 75% LAD stenosis after ACh infusion and 20 patients with no visible stenosis after ACh infusion. In the 15 patients with mild LAD stenosis after ACh infusion, there was a weak trend between the percentage of stenosis at LAD and EAT-AIG thickness (Supplemental Figure 1, available at www.onlinejase.com). Given these results, abnormal response with coronary stenosis may be associated with EAT-AIG thickness.

To determine the associated variables of VSA, we performed multivariate analysis of the association between baseline clinical and echocardiographic variables and VSA presence. The multivariate analysis for classification of VSA is presented in Table 3. After adjustment for smoking, diabetes mellitus, and dyslipidemia, EA T-AIG thickness was found to be significantly associated with VSA (χ² = 19.66; odds ratio, 1.55; 95% CI, 1.19–2.01; P = .001). EA T-AIG thickness had the highest C-statistic (0.73; P = .007) among adipose tissue thicknesses. By receiver operating characteristic curve analysis, EAT-AIG thickness > 6.9 mm was linked to the presence of significant VSA with 70% sensitivity and 71% specificity. EA T-AIG thickness showed a high area under the curve after adjustment for clinical variables (0.81; 95% CI, 0.70–0.91; P < .001). EA T-AIG thickness had incremental diagnostic value over other conventional risk factors (C statistic = 0.81 vs 0.64, P = .020; Figure 3). We assessed internal validation using the bootstrap method. We found that the differences in C statistics were similar between the original model and the modified model developed using the bootstrap method with 2,000 simulations (P = .020 for the original model, P = .019 for the bootstrap model).

### DISCUSSION

We sought to assess the correlation between EAT thickness, obtained via noninvasive echocardiography using a high-frequency linear probe, and VSA diagnosed using coronary angiography. Our study brings new insights to the understanding of VSA: (1) there were no significant differences in conventional echocardiographic findings between patients in the VSA and non-VSA groups, (2) there was a significant difference in EAT-AIG thickness between the VSA and non-VSA groups, and (3) EAT-AIG thickness had incremental diagnostic value over other conventional risk factors (area under the curve = 0.81 vs 0.64, P for comparison = .020). Therefore, we suggest that increased EAT-AIG thickness was well correlated with the presence of VSA.

### Correlates of VSA

More than 55 years ago, the first report of VSA as a clinical disease described episodes of rest angina that promptly respond to short-acting nitrates. There is a regional difference and a racial difference in the incidence rate of CAD. Generally, the incidence of CAD is higher in Europeans and Americans than Asians, including...
In previous studies, EAT is described as an organ involved in echocardiographic EAT-AIG thickness was greater in patients with CAD. Many investigators have shown that excessive EAT can be a marker of dyslipidemia, are well known. However, correlates of VSA have not been well established. From our data, EAT-AIG is found to be thicker in VSA. It might be a correlate of VSA with chest pain and without organic coronary artery stenosis.

Mechanism of Relationship between VSA and EAT

Many investigators have shown that excessive EAT can be a marker of the presence and severity of CAD. Furthermore, we reported that echocardiographic EAT-AIG thickness was greater in patients with CAD. In previous studies, EAT is described as an organ involved in the production of several inflammatory cytokines (e.g., interleukin-1β, interleukin-6, monocyte chemotactic protein–1, tumor necrosis factor–α). Inflammatory cytokines produced by infiltrating macrophages or by adipocytes are thought to locally contribute to the pathogenesis of coronary atherosclerosis. A recent study showed that inflammatory mediators released from EAT augmented coronary vasoconstriction. Another report suggested that EAT and vasa vasorum had been thought to be a source of inflammation. Indeed, enhanced EAT inflammation has been shown to promote progression of atherosclerosis. These results can support our result that EAT-AIG thickness is a marker of inflammation surrounding coronary arteries. Although the primary role of EAT is the secretion of cardioprotective adipokines such as adiponectin, excessive accumulation of EAT induces inflammation, leading to endothelium dysfunction in a coronary artery. In laboratory markers, there is a borderline difference in eicosapentaenoic acid/arachidonic acid ratio between the presence and absence of VSA. This result also supported our hypothesis, because the level of the eicosapentaenoic acid/arachidonic acid ratio has been well known as a marker of atherosclerosis.

Echocardiographic Measurement of EAT Thickness

Echocardiographic EAT measurement is a noninvasive and simple way to gauge location-specific EAT thickness. As first proposed by Iacobellis et al., EAT is measured at the right ventricular free wall in the parasternal long- and short-axis views. However, the distribution of EAT-RV is not uniform, and there are no coronary vessels in the right ventricular free wall. Recent evidence has suggested that location-specific EAT thickness at the groove was closely associated with CAD. In our previous study, we reported that measurement of EAT-AIG thickness by echocardiography, using a high-frequency linear probe, could be measured in 311 of 318 patients (98%). EAT-AIG thickness was highly correlated with EAT volume compared with EAT-RV thickness. In addition, EAT-AIG thickness has an additional and stronger prognostic value over EAT-RV thickness in the prediction of coronary artery lesions. Thus, we believed that measurement of EAT-AIG thickness can be useful in the clinical setting.

Location of EAT Thickness

The mechanism of vasospasm with the location of EAT is a matter of debate. A separate analysis examining LAD vasospasm alone related to EAT-AIG would be of great interest to this matter. In our cohort, there some patients lacked infusion of ACh into both the left and right coronary systems. When one coronary artery was positive, provoking vasospasm in the other artery was thought to be unnecessary in the clinical setting. Thus, for the analysis of LAD vasospasm alone, we excluded patients lacking of infusion into both coronary arteries. In patients with infusion into both coronaries, there were nine patients with LCA vasospasm alone, six patients with both LCA and RCA vasospasm, and two patients with RCA vasospasm alone. In these groups, EAT-AIG thickness was greater in three groups than in the non-VSA group (EAT-AIG: 6.1 vs 7.9 vs 8.2 vs 8.3 mm). Unfortunately, because of the limited number of patients, we were unable to determine the relationship between EAT location and coronary territory (Supplemental Figure 2, available at www.onlinejase.com). On the other hand, in previous studies, perivascular adipose tissue volume around the LAD was reported to be related only to LAD vasospasm. Thus, EAT may be mechanically linked to coronary vasospasm by production of inflammatory mediators released in proximity to the coronary arteries. To prove this hypothesis, further echocardiographic studies involving patients with LAD vasospasm alone are needed.

Clinical Implications

To the best of our knowledge, this is the first report of echocardiographic EAT-AIG thickness to help differentiate VSA with chest pain or discomfort. However, the accuracy of 70% is relatively low. EAT-AIG thickness is not sufficient enough to exclude VSA. This knowledge may be useful as a risk factor and for follow-up of patients with presumptive VSA. In addition, the results should be tested on a large cohort of patients with presumptive VSA using EAT-AIG thickness or EAT volume obtained from other cardiovascular imaging modalities (e.g., cardiac magnetic resonance imaging).

Limitations

The design of this clinical study was cross-sectional, and it was conducted at a single center with a relatively small number of subjects. The study
population consisted entirely of Japanese subjects, so the relevance of this study to patients of other ethnic backgrounds awaits further research. Moreover, we enrolled only high-risk patients with vasospasm, so the results of the present study may not be extrapolated to the general population. We did not conduct studies of inflammatory markers to assess the relationship between EAT and VSA. Finally, the cutoff value of EAT-AIG (6.9 mm) needs further validation in future studies.

CONCLUSIONS

EAT-AIG thickness is noninvasively and easily measured using transthoracic echocardiography and can be one of multiple clinical variables associated with VSA.

ACKNOWLEDGMENT

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.echo.2017.06.024.

REFERENCES

**Supplemental Figure 1** Relationship between LAD stenosis at ACh infusion and EAT-AIG thickness. There was a weak trend between stenosis of the LAD and EAT-AIG thickness.

**Supplemental Figure 2** Differences among groups without VSA, with LCA vasospasm alone, with both LCA and RCA vasospasm, and with RCA vasospasm alone. Among 17 patients with infusion into both coronary arteries, there were nine patients with LCA vasospasm alone, six patients with both LCA and RCA vasospasm, and two patients with RCA vasospasm alone. In these groups, EAT-AIG thickness was greater in three groups than in the non-VSA group (6.1 vs 7.9 vs 8.2 vs 8.3 mm).