Atherosclerotic Coronary Plaque Is Associated With Adventitial Vasa Vasorum and Local Inflammation in Adjacent Epicardial Adipose Tissue in Fresh Cadavers

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Background: The coronary adventitia has recently attracted attention as a source of inflammation because it harbors nutrient blood vessels, termed the vasa vasorum (VV). This study assessed the link between local inflammation in adjacent epicardial adipose tissue (EAT) and coronary arterial atherosclerosis in fresh cadavers.

Methods and Results: Lesion characteristics in the left anterior descending coronary artery of 10 fresh cadaveric hearts were evaluated using integrated backscatter intravascular ultrasound (IB-IVUS), and the density of the VV and levels of inflammatory molecules from the adjacent EAT were measured for each of the assessed lesions. The lesions were divided into lipid-rich, lipid-moderate, and lipid-poor groups according to percentage lipid volume assessed by IB-IVUS. Higher expression of inflammatory molecules (i.e., vascular endothelial growth factor A [VEGFA] and VEGFB) was observed in adjacent EAT of lipid-rich (n=11) than in lipid-poor (n=11) lesions (7.99±3.37 vs. 0.45±0.85 arbitrary units [AU], respectively, for VEGFA; 0.27±0.15 vs. 0.11±0.07 AU, respectively, for VEGFB; P<0.05). The density of adventitial VV was greater in lipid-rich than lipid-poor lesions (1.50±0.58% vs. 0.88±0.23%; P<0.05).

Conclusions: Lipid-rich coronary plaques are associated with adventitial VV and local inflammation in adjacent EAT in fresh cadavers. This study suggests that local inflammation of EAT is associated with coronary plaque progression via the VV.

Key Words: Coronary plaque; Epicardial adipose tissue; Fresh cadaver; Inflammatory molecules; Vasa vasorum

The adventitia, the outermost layer of the vessel wall, has received considerable attention in recent years. Previous studies suggested that neovascularization of the adventitial vasa vasorum (VV) plays a key role in the development of human atherosclerotic plaques. Adipocytokines secreted from perivascular adipose tissue have direct access to the adjacent arterial wall via diffusion or the VV. Previously, we reported that human coronary atherosclerosis is associated with inflammation in epicardial adipose tissue (EAT). In that study we found that infiltration of macrophages, and expression of pro- and anti-inflammatory cytokines, was increased in the epicardial fat of patients with coronary artery disease (CAD) compared with non-CAD patients. However, little is known about the relationship between the VV and local inflammation in EAT around coronary plaques in humans. Recently, the characteristics of coronary plaques have been evaluated by intravascular imaging in the clinical field. Invasive imaging modalities, such as intravascular ultrasound (IVUS), are considered the gold-standard method to measure the progression of atherosclerotic plaques. Integrated backscatter (IB)-IVUS, which uses the average power of backscatter radiofrequency signals, enables analysis of tissue components of coronary plaques in vivo. However, little is known about the relationship between coronary plaque characteristics and inflammation in EAT in humans. Because Tokushima University Graduate School of Biomedical Sciences has a laboratory that uses fresh cadaveric human bodies, we decided to investigate this relationship in cadaveric human hearts.

The aim of the present study was to investigate the...
relationships among coronary plaque characteristics, adventitial VV, and inflammation in adjacent EAT using fresh cadaveric hearts.

Methods

Cadavers and Study Design

After receiving approval from the Institutional Review Board of Tokushima University, we undertook this study using 10 fresh, frozen cadavers that had been stored at \(-20^\circ\text{C}\). Hearts were removed from the cadavers and cleaned with normal saline. Then, a guidewire was introduced into the left anterior descending artery (LAD) so that the LAD could be evaluated by IB-IVUS. For each heart, at least 2 samples were obtained in which the coronary plaque burden was over 25%. For each lesion, the area density of the adventitial VV was measured by immunohistological examination and the expression of inflammatory molecules in the adjacent EAT was determine (Figure 1A). Lesions were divided into 3 groups (lipid-rich, lipid-moderate, and lipid-poor groups) according to percentage lipid volume assessed by IB-IVUS, and VV area density and the expression inflammatory molecules were compared between 2 groups (i.e., lipid-rich and lipid-poor groups; Figure 1B).

IVUS Data Acquisition

Following the passage of a 0.014-inch guidewire into the LAD, the IVUS catheter (40 MHz; ViewIT; Terumo, Tokyo, Japan) was introduced over the wire and positioned as distal to the LAD as possible. Saline was infused into the LAD via constant manual perfusion pressure to obtain clear images. The guidewire was removed from the vessel before IVUS in order to reduce associated artifacts. Data were collected at an auto pull-back rate of 0.5 mm/s and analyzed using an IVUS imaging system (VISIWAVE; Terumo). For each lesion, 5 IB-IVUS images (5 mm in length) were captured at 1-mm intervals using a motorized pull-back system.

Conventional IVUS and IB-IVUS Parameters

IB-IVUS parameters were measured in the LAD of 10 relationships among coronary plaque characteristics, adventitial VV, and inflammation in adjacent EAT using fresh cadaveric hearts.

![Figure 1.](image1.png)

(A) Study design for using integrated backscatter intravascular ultrasound (IB-IVUS) and measuring the adventitial vasa vasorum (VV) area density and inflammatory molecules in adjacent epicardial adipose tissue (EAT) of cadaveric hearts. (B) Lesion classification. In all, 37 lesions were divided into 3 groups (lipid-rich, lipid-moderate, and lipid-poor groups) to measure the VV area density and inflammatory molecules.

![Figure 2.](image2.png)

Classification according to percentage lipid volume of coronary plaques. The lipid-rich group was defined as having a value greater than the mean+0.5 SD, whereas the lipid-poor group was defined as having a value less than mean value–0.5 SD.
fresh cadaveric hearts. The cross-sectional lumen area, cross-sectional vessel area within the external elastic membrane, and plaque area (external elastic membrane area minus lumen area) were calculated using software attached to the IVUS system. Plaque volume was calculated using integration. The distance from the ostium of the LAD to the proximal endpoint of the lesions was also measured.

The IB values for each histological category were defined by comparing histological images reported in a previous study. Plaque properties were classified into 4 types by combining spectral parameters of posterior scattering signals of IVUS: lipid pool, fibrosis, dense fibrosis, or calcification. The percentage area of each component was automatically measured in each plaque. The percentage of calcification. The percentage area of each component was automatically measured in each plaque. The percentage area of each component was automatically measured in each plaque.

Measurement of Inflammatory Molecules

After performing IB-IVUS, the vessels in which the coronary plaque burden was >25% and adjacent EAT were trimmed from at least 2 lesions from 1 LAD. The expression of inflammatory molecules (vascular endothelial growth factor A [VEGFA], VEGFB, VEGFC, C-C motif chemokine ligand 2 [CCL2], adiponectin [ADIPOQ], and glycerol-3-phosphate dehydrogenase [G3PDH]) was measured in each EAT. Total RNA was extracted using the illustra RNAspin RNA Isolation Kit (GE Healthcare). cDNA was synthesized from 100 ng total RNA extracted from tissues and cells using a QuantiTect Reverse Transcription kit (Qiagen). Real-time quantitative polymerase chain reaction (qPCR) was performed using an Mx3000P (Agilent Technologies) and Power SYBR Green PCR Master Mix (Applied Biosystems). The primer sequences used were as follows: VEGFA, 5′-AAGGAGGAGGGCA GAATCAT-3′ (sense) and 5′-ATCTGCATTGGTAGTTGAG TTGGA-3′ (antisense); VEGFB, 5′-GGCTTAGAGCTC AACCAGA-3′ (sense) and 5′-GACAAGGGATGGGA GAAGAG-3′ (antisense); VEGFC, 5′-AAAGAACCCTG CCCAGAAAT-3′ (sense) and 5′-GAAAATCCTGGGCT CAACAAGC-3′ (antisense); CCL2, 5′-CCCAAGTCACCT GCGTTAT-3′ (sense) and 5′-AGATCTCCTTGCGCAC AATG-3′ (antisense); ADIPOQ, 5′-GTGATGGCACAG ATGGCAC-3′ (sense) and 5′-ACATGTAATGGCAC CGTGA-3′ (antisense); G3PDH, 5′-TGGGTGAGGCAAC CAGAAG-3′ (sense) and 5′-GCTAAGCGATTGGTG GTGC-3′ (antisense). Expression was normalized against that of G3PDH and is given in arbitrary units (AU).

Measurement of the Density of the Adventitial VV

Vessels trimmed from the LAD vessels were cut into 5- to 7-mm slices and fixed in 10% neutralized formalin. Paraffin-embedded sections were cut into 5-μm slices. All sections were stained with hematoxylin-eosin. Immunohistochemical staining was performed using mouse anti-human CD31 antibody. Adventitial VV area density was calculated using the following formula (Figure 3):

Adventitial VV area density=VV area/(area of intima + media + adventitia)

Statistical Analysis

All data are expressed as the mean±SD. The significance of differences in each parameter between the lipid-rich and lipid-poor groups was determined using unpaired Student’s t-test. Significance was set at 2-tailed P<0.05. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria). More precisely,
### Results

#### Baseline Characteristics

Ten fresh, frozen cadavers (5 male, 5 female) were used in this study. The mean age was 81.1 years (range 69–96 years). None of the cadavers had died of cardiovascular disease. The clinical characteristics and causes of death for all 10 cadavers are given in Table 1.

#### IVUS Measurements and Lesion Classification

Conventional and IB-IVUS parameters are given in Table 2. The 37 lesions were divided into 3 groups according to the percentage lipid volume assessed by IB-IVUS. The mean percentage lipid volume was 32.7±6.0%. There were 11 lesions in each of the lipid-rich (percentage lipid volume >35.7%) and lipid-poor (percentage lipid volume <29.7%) groups (Figure 2). There were significant differences between these 2 groups in conventional and IB-IVUS parameters. Mean vessel area, mean lumen area, and plaque volume were larger in the lipid-rich than lipid-poor group, whereas percentage fibrous volume, percentage dense fibrous volume, and percentage calcified volume were higher in the lipid-poor than lipid-rich group.

#### Expression of Inflammatory Molecules

The expression of inflammatory molecules in adjacent EAT is shown in Figure 4. Expression of VEGFA and VEGFB in adjacent EAT was higher for lipid-rich than in lipid-poor lesions (7.99±3.37 vs. 0.45±0.85 AU, respectively, for VEGFA; 0.27±0.15 vs. 0.11±0.07 AU, respectively, for VEGFB; P<0.05). CCL2 expression was slightly higher in lipid-rich lesions (0.50±0.15 vs. 0.30±0.26 AU; P=0.06), whereas ADIPOQ expression was slightly higher in lipid-poor lesions (0.016±0.012 vs. 0.009±0.005 AU; P=0.08).

### Discussion

Recent reports suggested that adipokines and inflammatory molecules secreted from the EAT significantly affect the myocardium and coronary arteries. The quality of the EAT, such as inflammatory status, determines cardiac and coronary vascular function. Several adipokines and inflammatory molecules secreted from the EAT can diffuse through interstitial fluid across the adventitia, media, and intima, and may interact with VV, endothelial cells, and vascular smooth muscle cells of the coronary vasculature, resulting in inflammation, endothelial and smooth muscle cell proliferation, atherosclerosis, and destabilization of atherosclerotic plaques.

However, little is known about the association between local inflammation in the EAT and the growth of coronary adventitial VV in vivo. In the present study we found that inflammatory molecules in adjacent EAT and the progression of VV are associated with the characteristics of coronary plaques.

#### Fresh Frozen Cadaveric Study

In this study we used fresh cadaveric hearts without formaldehyde fixation. The advantages of using fresh cadaveric hearts include the ability to measure the expression of inflammatory molecules under the same conditions as in living tissues and the absence of any adverse effects of formaldehyde on observers: formaldehyde is toxic, causes mucosal irritation, and respiratory damage, and is carcinogenic. In this study, the cadavers were safely and accurately observed in the laboratory.

#### Inflammation in Adjacent EAT

VEGF induces the migration and proliferation of endothelial cells, increases vascular permeability, and plays a role in tumor growth, adipose tissue expansion, age-related

### Table 1. Baseline Characteristics of the Cadavers Used in the Present Study

| Cadaver No. | Age, sex  | Cause of death* |
|-------------|-----------|-----------------|
| 1           | 70, Female| Muscular dystrophy |
| 2           | 80, Female| Caducity         |
| 3           | 96, Female| Pneumonia        |
| 4           | 70, Male  | Amyloidosis      |
| 5           | 77, Male  | Respiratory failure |
| 6           | 95, Female| Caducity         |
| 7           | 69, Female| Multiple organ failure |
| 8           | 74, Female| Brain cancer     |
| 9           | 93, Male  | Chronic obstructive pulmonary disease |
| 10          | 87, Female| Senile decay     |

*Causes of death are taken from death certificates.

### Table 2. Conventional IVUS and Integrated Backscatter IVUS Parameters

|                      | All (n=37) | Lipid-rich group (n=11) | Lipid-poor group (n=11) | P-value  |
|----------------------|------------|-------------------------|-------------------------|----------|
| Distance from the LAD ostium (mm) | 34.0±15.2  | 29.1±13.9               | 39.1±16.8               | 0.14     |
| Mean vessel area (mm²)   | 9.1±1.3    | 11.0±3.0                | 6.9±3.0                 | 0.005    |
| Mean lumen area (mm²)    | 4.4±0.6    | 5.2±1.2                 | 3.8±1.5                 | 0.009    |
| Plaque volume (mm³)      | 18.8±3.3   | 24.1±8.8                | 13.5±6.3                | 0.004    |
| Fibrous volume (%)       | 55.1±3.7   | 46.0±8.9                | 62.0±4.5                | 0.0003   |
| Lipid volume (%)         | 32.7±6.0   | 47.5±11.8               | 19.2±8.1                | 0.0002   |
| Dense fibrous volume (%) | 10.4±2.8   | 5.4±3.2                 | 16.1±8.3                | 0.0007   |
| Calcified volume (%)     | 1.8±0.7    | 1.0±1.1                 | 2.7±2.1                 | 0.03     |

LAD, left anterior descending artery; IVUS, intravascular ultrasound. Data are given as the mean±SD.
Coronary Plaque and Epicardial Adipose Inflammation

Progression and morphology.\textsuperscript{1,6,28} Adventitial focal VV formation coincides with early coronary atherosclerotic changes, such as focal coronary spasm.\textsuperscript{29} In clinical settings, imaging devices, such as optical coherence tomography (OCT), are available to assess the neovessels of coronary

macular degeneration, and diabetic retinopathy.\textsuperscript{15,16} CCL2 is expressed primarily by inflammatory and endothelial cells. Expression of CCL2 is upregulated by proinflammatory stimuli and tissue injury, which are associated with atherosclerotic lesions.\textsuperscript{17,18} A recent study reported that blood CCL2 concentrations were higher in patients with vulnerable coronary plaques.\textsuperscript{19} Previously, we reported on the relationship between CAD and EAT.\textsuperscript{6,7,20–22} In the present study, the expression of VEGFs and CCL2 in focal adjacent EAT increased in the lipid-rich group, suggesting that inflammation in focal adjacent EAT is associated with coronary plaque vulnerability. Conversely, ADIPOQ expression was slightly lower in lipid-rich coronary plaque lesions. Previous studies demonstrated a cardioprotective action of AdipoQ in vascular endothelial cells, smooth muscle cells, and cardiac myocytes.\textsuperscript{23–25} The findings of the present study suggest that AdipoQ inhibits the progression of vulnerable plaques.

Plaque Characterization Assessed by IVUS and Adventitial VV

In our conventional and IB-IVUS analyses, the large vessels had a higher lipid volume than small vessels. Previous studies reported that plaque in the proximal segment of the LAD has a significantly higher lipid content and lower fibrosis content than that in the distal segment.\textsuperscript{26} One possible reason for this is destabilization of atherosclerotic plaques resulting from a change in composition caused by low shear stress.\textsuperscript{26,27} The present study suggests that another reason is related to the growth of adventitial VV.

Adventitial VV is associated with coronary plaque

Figure 4. Expression of inflammatory molecules in adjacent epicardial adipose tissue (EAT). (A) Vascular endothelial growth factor A (VEGFA), (B) VEGFB, (C) VEGFC, (D) C-C motif chemokine ligand 2 (CCL2), and (E) adipocyte-specific protein (ADIPOQ) expression was compared among the 3 groups (lipid-rich, lipid-moderate, and lipid-poor groups). P-values are for comparisons between lipid-poor and lipid-rich lesions. There were no significant differences in the expression of inflammatory molecules in adjacent EAT between lipid-moderate lesions and other types of lesions.

Figure 5. Association between the percentage lipid volume and pathological vasa vasorum (VV) area density in lesions in the lipid-rich, lipid-moderate, and lipid-poor groups. P-values are for comparisons between lipid-poor and lipid-rich lesions. There were no significant differences in VV area density between lipid-moderate lesions and other types of lesions.
plagues. In this study, adventitial VV was observed during pathological examination. We found that the growth of adventitial VV was greater around lipid-rich coronary plaque lesions, and that the expression of inflammatory molecules in adjacent EAT was also higher in these lesions. These findings suggest that local inflammation in adjacent EAT affects plaque characteristics via the neovascularization of the VV.

Inward Progression Theory of Vascular Inflammation
Previous reports revealed an inward progression of vascular inflammation from the adventitia towards the media and intima.

It was also suggested that prominent VV neovascularization in the adventitia occurs prior to the initiation of coronary lesion formation in apolipoprotein E-deficient mice. In the present study, we demonstrated a significant relationship among lipid-rich coronary plaques, growth of adventitial VV, and higher expression of inflammatory molecules in adjacent EAT in fresh cadavers, supporting the important role of adventitial VV formation in the pathogenesis of atherosclerosis. We plan to assess the precise distribution of intraplaque neovessels and adventitial VV by OCT and pathological examination in order to clarify the mechanism of inward neovessel progression.

Clinical Implication
The findings of this study suggest that lipid-rich coronary lesions identified by IB-IVUS have inflammation in adjacent EAT and growth of adventitial VV. In clinical settings, it is important to follow the clinical course of such lipid-rich coronary lesions, even if they are non-stenotic. A previous study reported that the characteristics of plaques observed by IB-IVUS were consistent with the pathological findings of the coronary artery. Recent IB-IVUS studies also report that lipid plaques of non-culprit lesions are associated with the atherosclerotic progression of CAD. Our findings may explain one of the mechanisms underlying the clinical course and provide beneficial information about the future progression of CAD. Pathological examinations cannot be performed during an intervention in clinical settings. However, the findings of the present study suggest that IB-IVUS, which is readily available in clinical settings, can be used to examine inflammation in adjacent EAT and the characteristics of the VV. Thus, this method may provide useful information during interventions and in treatment planning.

Study Limitations
The present study has several limitations. First, the sample size was small because of the limited supply of fresh cadavers. In addition, the effects of repeated freeze and thaw cycles of cadavers on the qPCR results should not be discounted. We required freshly thawed cadavers to assay inflammatory molecules, restricting the sample size. We did not perform coronary angiography before using IVUS; therefore, it was difficult to introduce the guidewire into the LAD, and we sometimes removed the EAT to identify the route. Consequently, sampling sites were further restricted. Second, this study demonstrated an association among inflammatory molecules in EAT, the growth of VV, and coronary plaque vulnerability. However, it remains unclear whether the growth of VV directly affects the processing or healing of vulnerable plaques. It is necessary to accumulate clinical data, such as identification of VV using OCT, and observe plaque progression in order to clarify the mechanisms involved. Third, we did not compare the pathological analyses and IB-IVUS findings. However, there was no significant pathological lipid core in our sampling sites of non-stenotic coronary arteries. Finally, we were unable to obtain clinical information for the cadavers other than the direct cause of death. Therefore, we cannot exclude the possibility that they underwent anti-inflammatory and/or immunosuppressive treatments that may have affected systemic inflammation.

Conclusions
In fresh cadavers, lipid-rich coronary plaques are associated with adventitial VV and local inflammation in adjacent EAT. This study suggests that local inflammation of the EAT is associated with coronary plaque progression via the VV.

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Conflict of Interest
None declared.

References
1. Barger AC, Beewkes R 3rd, Lainey LL, Silverman KJ. Hypothesis: Vasa vasorum and neovascularization of human coronary arteries: A possible role in the pathophysiology of atherosclerosis. N Engl J Med 1984; 310: 175–177.
2. Shi Y, O’Brien JE, Fard A, Mannion JD, Wang D, Zalewski A. Adventitial myofibroblasts contribute to neointimal formation in injured porcine coronary arteries. Circulation 1996; 94: 1655–1664.
3. Narula J, Finn AV, Demaria AN. Picking plaques that pop. J Am Coll Cardiol 2005; 45: 1970–1973.
4. Moreno PR, Purushothaman KR, Zias E, Sanz J, Fuster V. Neovascularization in human atherosclerosis. Curr Mol Med 2006; 6: 457–477.
5. Sacks HS, Fain JN. Human epicardial adipose tissue: A review. Am Heart J 2007; 153: 907–917.
6. Tanaka K, Sata M. Roles of perivascular adipose tissue in the pathogenesis of atherosclerosis. Front Physiol 2018; 9: 3.
7. Hirata Y, Tabata M, Kurobe H, Motoki T, Akaike M, Nishio C, et al. Coronary atherosclerosis is associated with macrophage polarization in epicardial adipose tissue. J Am Coll Cardiol 2011; 58: 248–255.
8. Berry C, L’Allier PL, Grégoire J, Lespérance J, Levesque S, Ibrahim R, et al. Comparison of intravascular ultrasound and quantitative coronary angiography for the assessment of coronary artery disease progression. Circulation 2007; 115: 1851–1857.
9. Nissen SE, Nicholls D, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, et al. Effect of very high-intensity statin therapy on progression of coronary atherosclerosis: The ASTEROID trial. JAMA 2006; 295: 1556–1565.
10. Tanaka S, Noda T, Iwama M, Tanihata S, Kawasaki M, Nishigaki K, et al. Long-term changes in neointimal hyperplasia following implantation of bare metal stents assessed by integrated backscatter intravascular ultrasound. Heart Vessels 2013; 28: 415–423.
11. Kawasaki M, Takatsu H, Noda T, Sano K, Ito Y, Hayakawa K, et al. In vivo quantitative tissue characterization of human coronary arterial plaques by use of integrated backscatter intravascular ultrasound and comparison with angiographic findings. Circulation 2002; 105: 2487–2492.
12. Kanda Y. Investigation of the freely available easy-to-use software “EZR” for medical statistics. Bone Marrow Transplant 2013; 48: 452–458.
13. Patel VB, Shah S, Verma S, Oudit GY. Epicardial adipose tissue as a metabolic transducer: Role in heart failure and coronary artery disease. *Heart Fail Rev* 2017; 22: 889–902.

14. Gaborit B, Sengenes C, Ancel P, Jacquier A, Dutour A. Role of epicardial adipose tissue in health and disease: A matter of fat? *Compr Physiol* 2017; 7: 1051–1082.

15. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev* 1997; 18: 4–25.

16. Greenberg DA, Jin K. Vascular endothelial growth factors (VEGFs) and stroke. *Cell Mol Life Sci* 2013; 70: 1753–1761.

17. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemotactic protein-1 (MCP-1): An overview. *J Interferon Cytokine Res* 2009; 29: 313–326.

18. Cahill PA, Redmond EM. Vascular endothelium: Gatekeeper of vessel health. *Atherosclerosis* 2016; 248: 97–109.

19. Ragno YI, Striukova EV, Muravish IS, Polonskaya YV, Volkov AM, Kuruguzov AV. Association of some hemostasis and endothelial dysfunction factors with probability of presence of vulnerable atherosclerotic plaques in patients with coronary atherosclerosis. *BMC Res Notes* 2019; 12: 336.

20. Maimaituxun G, Shimabukuro M, Fukuda D, Yagi S, Hirata Y, Iwase T, et al. Local thickness of epicardial adipose tissue surrounding the left anterior descending artery is a simple predictor of coronary artery disease: New prediction model in combination with Framingham Risk Score. *Circ J* 2018; 82: 1369–1378.

21. Dagvasurenberel M, Shimabukuro M, Nishiuchi T, Ueno J, Takao S, Fukuda D, et al. Gender disparities in the association between epicardial adipose tissue volume and coronary atherosclerosis: A 3-dimensional cardiac computed tomography imaging study in Japanese subjects. *Cardiovasc Diabetol* 2012; 11: 106.

22. Hirata Y, Yamada H, Kusunose K, Iwase T, Nishio S, Hayashi S, et al. Clinical utility of measuring epicardial adipose tissue thickness with echocardiography using a high-frequency linear probe in patients with coronary artery disease. *J Am Soc Echocardiogr* 2015; 28: 1240–1246.

23. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, et al. Role of adiponectin in preventing vascular stenosis the missing link of adipo-vascular axis. *J Biol Chem* 2002; 277: 37487–37491.

24. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. *J Biol Chem* 2004; 279: 1304–1309.

25. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, et al. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res* 2004; 94: e27–e31.

26. Komura N, Hibi K, Kusama I, Otsuka F, Mitsuhashi T, Endo M, et al. Plaque location in the left anterior descending coronary artery and tissue characteristics in angina pectoris: An integrated backscatter intravascular ultrasound study. *Circ J* 2010; 74: 142–147.

27. Slager CJ, Wentzel J, Gijsen FJH, Schuurbiers JCH, van der Wal AC, van der Steen AFW, et al. The role of shear stress in the generation of rupture-prone vulnerable plaques. *Nat Clin Pract* 2005; 2: 401–407.

28. Taruya A, Tanaka A, Nishiguchi T, Matsu T, Ozaki Y, Kashiwagi M, et al. Vasa vasaorum restructuring in human atherosclerotic plaque vulnerability: A clinical optical coherence tomography study. *J Am Coll Cardiol* 2017; 69: 2469–2477.

29. Nishimiya K, Matsumoto Y, Uzuka H, Ohyama K, Hao K, Tsuambura R, et al. Focal vasa vasaorum formation in patients with focal coronary vasospasm: An optical frequency domain imaging study. *Circ J* 2016; 80: 2252–2254.

30. Shimokawa H. 2014 Williams Harvey Lecture: Importance of coronary vasomotion abnormalities–from bench to bedside. *Eur Heart J* 2014; 35: 3180–3193.

31. Kandabashi T, Shimokawa H, Miyata K, Kunihiro I, Kawano Y, Fukata Y, et al. Inhibition of myosin phosphatase by upregulated Rho-kinase plays a key role for coronary artery spasm in a porcine model with interleukin-1β. *Circulation* 2000; 101: 1319–1323.

32. Shimokawa H, Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 2005; 25: 1767–1773.

33. Shimokawa H, Ito A, Fukumoto Y, Kadokami T, Nakaik R, Sakata M, et al. Chronic treatment with interleukin-1β induces coronary intimal lesions and vasospastic responses in pigs in vivo: The role of platelet-derived growth factor. *J Clin Invest* 1996; 97: 769–776.

34. Maiellaro K, Taylor WR. The role of the adventitia in vascular inflammation. *Cardiovasc Res* 2007; 75: 640–648.

35. Tanaka K, Nagata D, Hirata Y, Tabata Y, Nagai R, Sata M. Augmented angiogenesis in adventitia promotes growth of atherosclerotic plaque in apolipoprotein E-deficient mice. *Atherosclerosis* 2011; 215: 366–379.

36. Ohota M, Kawasaki M, Ismail TF, Hattori K, Serruys PW, Ozaki Y. A histological and clinical comparison of new and conventional integrated backscatter intravascular ultrasound (IB-IVUS). *Circ J* 2012; 76: 1678–1686.

37. Iwama M, Tanaka S, Noda T, Segawa T, Kawasaki M, Nishigaki K, et al. Impact of tissue characteristics on luminal narrowing of mild angiographic coronary stenosis: Assessment of integrated backscatter intravascular ultrasound. *Heart Vessels* 2014; 29: 750–760.