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ROLE OF EPICARDIAL ADIPOSE TISSUE IN THE PATHOGENESIS OF CALCIFIC AORTIC STENOSIS

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Abstract

Background. The pathophysiologic mechanisms leading to aortic stenosis (AS) are characterized by early atherosclerosis and inflammation. Epicardial adipose tissue (EAT) represents a source of several pro-atherogenic inflammatory mediators involved in coronary atherogenesis. EAT thickness and inflammatory profile are increased in patients with coronary artery disease (CAD). Since the well recognized similarities between AS and CAD pathogenesis, we aimed to evaluate whether anatomic characteristics and pro-inflammatory profile of EAT are also modified in patients with severe isolated AS.

Methods and Results. We measured EAT thickness by echocardiography in 95 patients with isolated AS and 44 controls matched for age, gender and body mass index (BMI). In patients referred to aortic valve replacement, plasma, EAT and subcutaneous adipose tissue (SCAT) were collected and analyzed by human cytokine 27 multiplex immunoassay for the assessment of systemic and local inflammatory status. EAT thickness was significantly increased in patients with AS compared to controls. Overall, EAT showed a markedly increased inflammatory profile respect to SCAT. There was a significant relationship between EAT thickness and levels of EAT secreted inflammatory mediators.

Conclusions. Our results provide the first demonstration of EAT increased thickness and inflammatory status in patients with severe AS. Overall, these data raise the hypothesis of a potential role of EAT in AS pathogenesis.

Introduction

Aortic stenosis (AS) is the most prevalent form of valvular heart disease and becomes more prevalent with increasing age (1). It is widely recognized that aortic valve (AV) leaflets thickening and calcification are regulated by active processes which include chronic inflammation, lipoprotein deposition and activation of specific osteogenic and apoptotic signaling pathways (2). These phenomena lead to activation and differentiation of valvular interstitial cells into myofibroblasts and osteoblast-like cells, with consequent bone deposition (2–4).

Inflammation plays a crucial role in AV calcification both in early and advanced stages (5, 6). Similarly to the early stages of vascular atherosclerosis, the disruption of the endothelial continuity allows the infiltration of inflammatory cells and, importantly, of circulating lipids (7) that undergo oxidative modifications (8) and stimulate inflammatory activity and valve mineralization. Further, AS and coronary artery disease (CAD) share common risk factors (9-11), early pathogenesis (6), and clinical coexistence (12) with an high percentage (40% to 75%) of patients with severe AS and concomitant CAD. Thus, it is reasonable to hypothesize that the pathogenesis and progression of AS and CAD might be induced by similar stimuli, largely represented by the inflammatory activity intrinsic to the atherogenesis process.

Epicardial adipose tissue (EAT) is the visceral fat depot of the heart and it shares the same microcirculation with the myocardium. EAT has been shown to be predictive of cardiovascular events independently of conventional risk factors and body mass index (BMI) (13). The mechanisms by which EAT exerts its role in the atherogenesis seem to be related to the production and secretion of several inflammatory mediators (14-16). In particular, Mazurek et al. demonstrated in CAD patients that EAT secretes significant higher levels of cytokines and chemokines than subcutaneous adipose tissue (SCAT) irrespective of clinical variables and plasma concentrations of circulating biomarkers (14). EAT seems to be closely related to atherosclerosis in a mass-dependent way, and its volume is associated with the presence and severity of CAD (17) and coronary artery

calcification (18).

In this context, we sought to investigate whether EAT could also contribute to the inflammatory burden of AS. To this aim, we analyzed anatomic characteristics and secretory profile of EAT obtained from patients with isolated, calcific AS.

Methods

Study Population

In the present study, we enrolled 95 patients with severe, isolated, calcific AS. All subjects were referred to cardiac surgery for AV replacement and underwent a complete clinical and echocardiographic evaluation, and coronary angiography prior to intervention. Demographic and clinical characteristics of the study population are summarized in Table 1. Exclusion criteria were as follows: 1) presence of significant CAD at coronary angiography; 2) poor acoustic quality of parasternal view at echocardiography with inability to adequately visualize EAT; 3) presence of pathologic conditions associated with increased echocardiographic EAT thickness (metabolic syndrome, atrial fibrillation and history of CAD) (19-21); 4) chronic inflammatory diseases which could affect EAT thickness and/or systemic and local inflammatory profiles. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by our institution's human research committee. All patients provided written informed consent before their inclusion into the study.

Echocardiographic measurements

Echocardiograms were performed by a VIVID E9 (GE healthcare) machine according to standard technique. Echocardiographic images were recorded through an EchoPAC Clinical Workstation Software (GE Healthcare).

Measurements of EAT thickness were obtained from a parasternal long axis view. EAT thickness

was measured perpendicularly to the free wall of right ventricle, at end systole, in 3 cardiac cycles as previously described by Iacobellis et al (22). Echocardiographic parameters are reported in Table 1. Measurements of EAT thickness were performed offline by two independent echocardiographers. The average value from three cardiac cycles was used for the statistical analysis. The intra-observer and inter-observer correlation coefficients were tested.

EAT thickness was also measured in a control group of 44 healthy subjects, matched for age, gender and BMI screened by echocardiographic and clinical database of our Institution. In these control subjects, we excluded the presence of cardiovascular disease, cardiovascular risk factors, renal diseases, systemic inflammatory diseases or any cardiovascular drug therapy.

Tissues Collection

On the morning of surgery, a peripheral venous blood sample was drawn from all AS patients into EDTA tubes, and then centrifuged within 20 minutes at 1500g for 10 minutes at 4°C. Plasma was stored in aliquots at -80°C for all ELISA assays.

From a subgroup of 10 patients, we obtained EAT and SCAT samples during cardiac surgery for AV replacement. Adipose tissue biopsy samples (average 0.1 to 0.5 g) were obtained before the initiation of cardiopulmonary bypass. EAT biopsies were taken near the proximal right coronary artery, while SCAT samples were obtained from the chest. Conditioned media from EAT and SCAT biopsies were isolated as described by Mazurek et al (14). Briefly, the tissue was weighted, cut into small pieces, and transferred into a 12-well plate. According to tissue weight, serum-free DMEM (1 mL medium/0.1 g tissue) was added to the well and incubated at 37°C in a CO2 incubator. After 24h, medium was collected and centrifuged at 14,000 g to remove debris and analyzed for cytokines and growth factors content, as described below.

Cytokine and growth factor assay

Plasma and conditioned media from EAT and SCAT were screened for the concentration of Interleukin (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-

17, basic Fibroblast Growth Factor (FGF), Eotaxin, Granulocyte-Colony Stimulating Factor (G-CSF), Granulocyte–Macrophage Colony Stimulating Factor (GM-CSF), Interferon (IFN)-γ, Interferon gamma-Induced Protein (IP)-10, Monocyte Chemoattractant Protein (MCP)-1, Macrophage Inflammatory Protein 1-alpha/beta (MIP-1α, MIP-1β), Platelet-Derived Growth Factor (PDGF), Regulated on Activation Normal T-cell Expressed and Secreted RANTES, Tumor Necrosis Factor (TNF)-α, and Vascular Endothelial Growth Factor (VEGF), using the Bio-Plex multiplex Human Cytokine and Growth factor kits (Bio-Rad) according to the manufacturer's protocol.

Statistical Analysis

Continuous variables are presented as mean± standard deviation (SD) and compared by the use of Student t test (normally distributed) or as median ± interquartile range value and compared by the use of Mann-Whitney U test (not normally distributed), as appropriate. Normality of data distribution was evaluated using the Kolmogorov-Smirnov test. Not normally distributed continuous variables were natural log transformed. Categorical variables are expressed as proportion and compared by use of $\chi 2$ test. Associations between EAT thickness and EAT inflammatory profile was determined with the Pearson correlation coefficient.

All data were collected in an Excel database and analyzed by SPSS version 19.0 (SPSS, Inc., Chicago, Illinois). Statistical significance was accepted at $p < 0.05$.

Results

Patient Characteristics

Table 1 illustrates demographic and clinical characteristics of the study population. The mean age was 72.5 years and 60% of patients were females. As regard to common atherosclerotic risk factors, 42% of patients showed dyslipidemia, 29% were diabetics, 66% were hypertensives and 26% were smokers. 8.4 % of patients had glomerular filtration rate (GFR) < 60% ml/min. Use of

aspirin and statins was reported in 40% and 37.9% of patients respectively. Almost 50% of patients assumed beta-blockers and ACE-inhibitors/sartans.

At echocardiography, all patients showed echocardiographic findings of severe aortic stenosis (AV area 0.8±0.2 cm^2 with a mean gradient of 44.2±19.6 mmHg) and left ventricular (LV) diastolic dysfunction. Mean LV ejection fraction was 64%, thus indicating a globally preserved LV systolic function. Patients of control group had a mean age of 70.41±10.6 years and a BMI of 26.4±2.4. The percentage of females was 54.5% (n. 24 subjects).

EAT thickness

EAT thickness was markedly increased in patients with AS respect to control subjects matched for age, gender and BMI (9.85±2.78 vs 4.91±1.27 mm; $p<0.0001$) (Figure 1). Intra and inter-observer reproducibility for echocardiographic EAT thickness assessment was excellent (0.962 and 0.951, respectively).

Demographic and clinical variables were also evaluated after study population stratification above and below EAT thickness median value (Table 2). There were no differences between the two groups for age, BMI, GFR and atherosclerotic risk factors. Interestingly, as regard to cardiovascular therapy, only statin use was significantly higher in patients below the EAT thickness median value. Although both subgroups showed preserved LV systolic function, patients below the EAT thickness median value presented higher LV ejection fraction.

Inflammatory secretory profile of Epicardial Adipose Tissue

EAT secretome was characterized by higher levels of inflammatoty mediators than those observed in SCAT (Figure 2). In fact, of 27 factors analyzed we found that levels of IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-15, IL-17, eotaxin, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, TNF-α, VEGF, were significantly increased in EAT secretome compared to SCAT. Noteworthy, IL-1β, IL-1ra, IL-4, IL-10, IL-12, eotaxin, IFN-γ, TNF-α,VEGF and PDGF showed

more than two fold increase, and IL-6, IL-8, G-CSF, IP-10, MIP-1α and MIP-1β more than fivefold increase in EAT compared to SCAT secretome (Table 3). Only IL-13 levels were higher in SCAT than in EAT. Interestingly, after patients stratification above and below EAT thickness median value, no differences were found between the two groups in plasma inflammatory mediators levels, except for PDGF and VEGF. Contrarily, in EAT secretome, 22 of 27 measured inflammatory mediators levels were significantly higher in patients above the EAT thickness median value (Table 4).

Since the above mentioned observation of a larger use of statins in patients below EAT thickness median value, we tested differences, in plasma and EAT secretome, in inflammatory mediators levels between patients taking and non taking statins (Table 5). In plasma, we found that only IL-6 and MIP-1α were significantly lower in statin users than in non users. Contrarily, in EAT secretome, all inflammatory mediators, except for PDGF, IL-13, G-CSF, GM-CSF, RANTES and VEGF, were significantly reduced in patients on statin therapy.

EAT thickness and EAT inflammatory profile

We tested the relationship between EAT echocardiographic thickness and its inflammatory profile. Interestingly, we found a close direct correlation between EAT thickness and levels of secreted inflammatory mediators. In particular, EAT thickness significantly correlated with levels of IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-17, basic FGF, eotaxin, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF and TNF-α (Table 6).

Discussion

The present study analyzed, for the first time, morphologic characteristics and inflammatory profile of EAT in patients with isolated AS. Major findings of the present study are: 1) patients with AS had an increased EAT thickness compared to controls; 2) EAT of patients with AS showed an

increased secretion of cytokines, chemokines, and growth factors compared to SCAT; 3) there was a close relationship between EAT thickness and its secretion of inflammatory mediators.

EAT thickness in Aortic Stenosis

EAT represents the true visceral fat depot of the heart. Under normal physiologic conditions, EAT plays several protective functions for the heart by absorbing the excess of fatty acids, serving as a local energy source in conditions of increased metabolic demand, and, as brown fat, protecting the myocardium against hypothermia (23). The anatomic proximity to the heart, the absence of fascial boundaries, and the presence of a common microcirculation underlie the close interaction between the myocardium and its visceral fat depot (23). However, it is known that EAT may also play an unfavorable activity for the heart. In fact, it is a source of several pro-inflammatory and pro-atherogenic cytokines and chemoattractant factors, which can biologically influence the myocardium and epicardial coronary arteries through paracrine or vasocrine actions (15, 24, 25). It has been described that EAT could influence, through an exalted secretion of inflammatory mediators, the progression and the stability of atherosclerotic coronary plaques. Accordingly, in the last years, several evidence have indicated that EAT thickness represents a specific marker of visceral adiposity which is strongly associated with the presence and severity of atherosclerotic CAD (26-28). Of note, our study provides the first evidence that EAT thickness is also increased in patients with isolated AS. This observation further supports previous evidence indicating that CAD and AS share common pathogenetic mechanisms. In this regard, histologic studies have demonstrated that, in the early AS disease, the biological processes show similar characteristics to those observed in the coronary atherosclerotic plaque (6). Furthermore, several studies have reported that early, pre-stenotic lesions of AV are associated with a 50% increase in the rate of coronary events (29, 30).

In our study, we used echocardiography for the assessment of EAT thickness. This method has a good correlation with magnetic resonance EAT measurements, and with anthropometric and

metabolic parameters (22). Further, echocardiographic measurement of EAT thickness is widely diffused and has an excellent reproducibility (27, 28, 31), that has been also confirmed in the present study.

Proinflammatory Properties of Epicardial Adipose Tissue in Aortic Stenosis

Several evidence address the 'atherogenicity' of EAT to secretion of epicardial inflammatory cytokines, such as TNF-α, MCP-1, IL-6, IL-1β, plasminogen activator inhibitor-1, resistin, and others (14,15,24). Previous evidences have indicated that in patients with significant CAD, EAT secretome shows significantly higher levels of chemokines, such as MCP-1, and several inflammatory cytokines, as IL-1, IL-6, IL-6sR, and TNF-α compared to SCAT (14). Interestingly, it has been shown that local levels of these inflammatory mediators do not correlate with plasma concentrations of circulating cytokines and are independent of several clinical variables, such as obesity, and diabetes (14).

The present study evaluated the secretory characteristics of EAT and SCAT secretome of patients with AS. We found that several inflammatory and pro-atherogenic mediators were significantly increased in EAT compared to SCAT. In particular, IL-6 showed more than a fivefold increase in EAT respect to SCAT. The involvement of IL-6 in AS has been recently described by El Husseini et al. (32). These authors demonstrated that high expression of IL-6 promotes the mineralization process of AV leaflets, which is partially reversed by IL-6 inhibition (32). Further, it has been described that pro-inflammatory stimulation promotes the transition of AV interstitial cells toward inflammatory cells resulting in a significantly increased production of IL-6, IL-8 and MCP-1 (33-35). As regard to IL-1β, we found a 2.5 fold increase in EAT compared to SCAT secretome. Histologic studies of stenotic AV leaflets have demonstrated increased levels of this potent pro-inflammatory cytokine which promotes matrix metalloproteinase expression and cell proliferation in calcific AV and has been implicated in the pathogenesis of several inflammatory diseases (34, 35). TNF-α, that showed 2.2 fold increase in EAT vs SCAT in our study, has also been detected in

human calcified valves (36). This pleiotropic cytokine has been shown to accelerate the calcification of human AV interstitial cells obtained from patients with calcific AS (37). Overall these findings, indicating a potent pro-inflammatory activation of EAT in patients with isolated AS, together with the strong association observed between EAT thickness and AS, support the hypothesis of an involvement of cardiac visceral fat in inflammatory and atherogenic phenomena occurring in the AV and promoting its degeneration and calcification. To further strengthen this hypothesis there is the observation that EAT, but not plasma, shows a more evident pro-inflammatory phenotype in patients exhibiting a larger amount of cardiac visceral fat.

Relationship between EAT thickness and pro-inflammatory profile

In the present study, we have demonstrated for the first time a significant direct correlation between EAT thickness and levels of pro-inflammatory mediators in the EAT secretome of AS patients. Interestingly, the majority of measured inflammatory mediators (22 of 27) showed a highly significant correlation with EAT thickness assessed by echocardiography. To further support the link between EAT thickness and its inflammatory activity in AS, there is the observation in our study population that the use of statins was significantly associated with both reduced thickness and pro-inflammatory activity of EAT. These data are in line with the results of a recent sub-analysis of a large randomized clinical trial of hyperlipidemic post-menopausal women reporting that intensive lipid-lowering therapy induces significant EAT regression together with an attenuation of inflammation indexes (38).

Study Limitations

Since EAT was measured and explanted in patients with advanced AV disease, it remains unknown whether an increase of EAT mass together with an exalted inflammatory state of this tissue might be present at the initial stages of AV degeneration.

Unfortunately, we could not obtain EAT samples from controls for comparisons. EAT from patients with CAD is expected to show a pro-inflammatory profile, thus it cannot reflect a normal status. Moreover, ethical reasons do not allow to extract EAT samples from other patients categories. Although our study demonstrates a strong association between the presence of AS and thickness and inflammatory status of EAT, the demonstration of a mechanistic role of cardiac visceral fat in the AS pathogenesis is still so far.

Conclusions

This study demonstrates that patients with isolated severe AS have an increased EAT thickness. Importantly, increased EAT thickness also reflects an increased local inflammatory status. Further studies are needed in order to confirm these data and explore a potential role of EAT in the development of AV diseases.

References

1. Lindroos M, Kupari M, Heikkilä J, Tilvis R. Prevalence of aortic valve abnormalities in the elderly: an echocardiographic study of a random population sample. *J Am Coll Cardiol* 1993;21:1220–1225.

2. Rajamannan NM, Evans FJ, Aikawa E, et al. Calcific aortic valve disease: not simply a degenerative process: a review and agenda for research from the national heart and lung and blood institute aortic stenosis working group. *Circulation* 2011;124:1783–1791.

3. Jian B, Narula N, Li QY, Mohler ER 3rd, Levy RJ. Progression of aortic valve stenosis: TGF-beta1 is present in calcified aortic valve cusps and promotes aortic valve interstitial cell calcification via apoptosis. *Ann Thorac Surg* 2003;75:457-65.

4. Galeone A, Brunetti G, Oranger A, et al. Aortic valvular interstitial cells apoptosis and calcification are mediated by TNF-related apoptosis-inducing ligand. *Int J Cardiol* 2013;169:296-304.

5. Olsson M, Dalsgaard CJ, Haegerstrand A, Rosenqvist M, Rydén L, Nilsson J. Accumulation of T lymphocytes and expression of interleukin-2 receptors in nonrheumatic stenotic aortic valves. *J Am Coll Cardiol* 1994;23:1162-70.

6. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation* 1994;90:844-53.

7. O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. *Arterioscler Thromb Vasc Biol* 1996;16:523–532.

8. Olsson M, Thyberg J, Nilsson J. Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves. *Arterioscler Thromb Vasc Biol* 1999;19:1218 –1222.

9. Deutscher S, Rockette HE, Krishnaswami V. Diabetes and hypercholesterolemia among patients with calcific aortic stenosis. *J Chronic Dis* 1984;37:407– 415.
10. Hoagland PM, Cook EF, Flatley M, Walker C, Goldman L. Case-control analysis of risk factors for presence of aortic stenosis in adults (age 50 years or older). *Am J Cardiol* 1985;55:744 –747.
11. Pohle K, Maffert R, Ropers D, et al. Progression of aortic valve calcification: association with coronary atherosclerosis and cardiovascular risk factors. *Circulation* 2001;104:1927–1932.
12. Goel SS, Ige M, Tuzcu EM, et al. Severe aortic stenosis and coronary artery diseasedimplications for management in the transcatheter aortic valve replacement era: a comprehensive review. *J Am Coll Cardiol* 2013;62:1–10.
13. Ding J, Hsu FC, Harris TB, et al. The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr* 2009; 90: 499–504.
14. Mazurek T, Zhang L, Zalewski A, et al. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation*. 2003 Nov 18;108:2460-2466.
15. Kremen J, Dolinkova M, Krajickova J, et al. Increased subcutaneous and epicardial adipose tissue production of proinflammatory cytokines in cardiac surgery patients: possible role in postoperative insulin resistance. *J Clin Endocrinol Metab* 2006;91: 4620-4627.
16. Baker AR, Silva NF, Quinn DW, et al. Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol* 2006; 5:1.
17. Kim SH, Chung JH, Kwon BJ, Song SW, Choi WS. The associations of epicardial adipose tissue with coronary artery disease and coronary atherosclerosis. *Int Heart J* 2014;55:197-203.

18. Torres T, Bettencourt N, Mendonça D, et al. Epicardial adipose tissue and coronary artery calcification in psoriasis patients. *J Eur Acad Dermatol Venereol*. 2014 Apr 21. doi: 10.1111/jdv.12516.
19. Iacobellis G, Sharma AM. Epicardial adipose tissue as new cardio-metabolic risk marker and potential therapeutic target in the metabolic syndrome. *Curr Pharm Des* 2007;13:2180-2184.
20. Iacobellis G, Zaki MC, Garcia D, Willens HJ. Epicardial Fat in Atrial Fibrillation and Heart Failure. *Horm Metab Res* 2014;46:587-590.
21. Ahn SG, Lim HS, Joe DY, Kang SJ, Choi BJ, Choi SY. Relationship of epicardial adipose tissue by echocardiography to coronary artery disease. *Heart* 2008;94:e7.
22. Iacobellis G, Willens HJ. Echocardiographic epicardial fat: a review of research and clinical applications. *J Am Soc Echocardiogr* 2009;22:1311-1319.
23. Iacobellis G, Corradi D, Sharma AM. Epicardial adipose tissue: anatomic, biomolecular and clinical relationships with the heart. *Nat Clin Pract Cardiovasc Med* 2005;2:536-543.
24. Cheng KH, Chu CS, Lee KT, et al. Adipocytokines and proinflammatory mediators from abdominal and epicardial adipose tissue in patients with coronary artery disease. *Int J Obes (Lond)* 2008;32:268-274.
25. Iacobellis G, Pistilli D, Gucciardo M, et al. Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease. *Cytokine* 2005; 29:251-255.
26. Eroglu S, Sade LE, Yildirir A, et al. Epicardial adipose tissue thickness by echocardiography is a marker for the presence and severity of coronary artery disease. *Nutr Metab Cardiovasc Dis* 2009;19:211-217.
27. Sade LE, Eroglu S, Bozbaş H, et al. Relation between epicardial fat thickness and coronary flow reserve in women with chest pain and angiographically normal coronary arteries. *Atherosclerosis* 2009;204:580-585.

28. Jeong JW, Jeong MH, Yun KH, Oh SK, Park EM, Kim YK. Echocardiographic epicardial fat thickness and coronary artery disease. *Circ J* 2007; 71:536-539.
29. Vandeplas A, Willems JL, Piessens J, De Geest H. Frequency of angina pectoris and CAD in severe isolated valvular aortic stenosis. *Am J Cardiol* 1988;62:117–120.
30. Rapp AH, Hillis LD, Lange RA, Cigarroa JE. Prevalence of CAD in patients with aortic stenosis with and without angina pectoris. *Am J Cardiol* 2001;87:1216–1217.
31. Iacobellis G,Willens HJ, Barbaro G, Sharma AM. threshold values of highrisk echocardiographic epicardial fat thickness. *Obesity (Silver Spring)* 2008;16:887-892.
32. El Husseini D, Boulanger MC, Mahmut A, et al. P2Y2 receptor represses IL-6 expression by valve interstitial cells through Akt: implication for calcific aortic valve disease. *J Mol Cell Cardiol* 2014;72:146-156.
33. Nadlonek N, Lee JH, Reece TB, et al. Interleukin-1 Beta induces an inflammatory phenotype in human aortic valve interstitial cells through nuclear factor kappa Beta. *Ann Thorac Surg* 2013;96:155-162.
34. Kaden JJ, Dempfle CE, Grobholz R, et al. Interleukin-1 beta promotes matrix metalloproteinase expression and cell proliferation in calcific aortic valve stenosis. *Atherosclerosis* 2003; 170:205–211.
35. Dinarello CA. Interleukin-1 beta and the autoinflammatory diseases. *N Engl J Med* 2009; 360:2467–2470.
36. Galeone A, Brunetti G, Oranger A, et al. Aortic valvular interstitial cells apoptosis and calcification are mediated by TNF-related apoptosis-inducing ligand. *Int J Cardiol* 2013;169:296-304.
37. Yu Z, Seya K, Daitoku K, Motomura S, Fukuda I, Furukawa K. Tumor necrosis factor-α accelerates the calcification of human aortic valve interstitial cells obtained from patients with calcific aortic valve stenosis via the BMP2-Dlx5 pathway. *J Pharmacol Exp Ther* 2011;337:16-23.

38. Alexopoulos N, Melek BH, Arepalli CD, et al. Lipid-Lowering Therapy on Epicardial Adipose Tissue in Hyperlipidemic Post Menopausal Women. *J Am Coll Cardiol* 2013;61:1956–1961.

Table 1. Demographic and clinical characteristics of the study population

Age, years	72.52±11.22
Gender Female, % (n)	60.0% (57)
BMI	27.66±4.61
Dyslipidemia, % (n)	42.1% (40)
Diabetes, % (n)	29.5% (28)
Hypertension, % (n)	66.3% (63)
Smokers, % (n)	26.3% (25)
GFR<60 ml/min	8.4% (8)
Medications	
Aspirin, % (n)	40.0% (38)
Statin, % (n)	37.9% (36)
ACE-I/ARBs, % (n)	53.7% (51)
BBs, % (n)	51.6% (49)
Echocardiographic characteristics	
LVEF, %	64.35±12.33
E/A	3.16±15.75
E Dec, msec	248.50±87.77
E/e’	15.43±5.96
AV area, cm^2	0.8±0.2
Mean gradient, mmHg	44.27±19.66
Mass, gr	180.50±45.02
Mass / BSA, gr/m^2	99.36±27.66
RWT	0.44±0.10
EAT thickness, mm	9.85±2.78

BMI, Body Mass Index; GFR, Glomerular Filtration Rate; ACE-I, Angiotensin Converting Enzym inhibitors; ARB, Angiotensin Receptor Blockers; BBs, Beta-blockers; LVEF, Left Ventricular Ejection Fraction; AV, Aortic Valve; BSA, Body Surface Area; RWT, Relative Wall Thickness; EAT, Epicardial Adipose Tissue.

Table 2. Demographic and clinical characteristics of AS patients stratified according to EAT thickness median value

	Pts below EAT median value (n = 46)	**Pts above EAT median value (n = 49)**	**p value**
Age, mean ± SD	73.33±8.73	71.78±13.15	0.504
Gender Female, % (n)	66.7% (30)	54% (27)	0.294
BMI, mean ± SD	28.34±4.74	27.04±4.45	0.178
Dyslipidemia, % (n)	48.9% (22)	36.0% (18)	0.503
Diabetes, % (n)	35.6% (16)	24% (12)	0.482
Hypertension, % (n)	68.9% (31)	64% (32)	0.798
Smokers, % (n)	24.4% (11)	28% (14)	0.628
Medications			
Aspirin, % (n)	48.9% (22)	32% (16)	0.257
Statins, % (n)	51.1% (23)	26% (13)	0.043
ACE-I/ARBs, % (n)	62.2% (28)	46% (23)	0.341
BBs, % (n)	64.4% (29)	40% (20)	0.134
Echocardiographic characteristics			
LVEF	67.36±9.89	61.78±13.66	0.03
E/A	5.08±21.90	1.12±0.62	0.334
E Dec	270.30±79.42	226.01±91.45	0.41
E/e’	16.11±6.52	14.70±5.32	0.348
Mean gradient (mmHg)	44.41±18.74	44.15±20.69	0.951
Mass (gr)	186.48±47.01	174.82±42.86	0.256
Mass / BSA	99.20±30.93	99.51±24.58	0.961
RWT	0.46±0.07	0.42±0.11	0.084
EAT (mm)	7.58±1.51	11.90±1.92	<0.0001

BMI, Body Mass Index; GFR, Glomerular Filtration Rate; ACE-I, Angiotensin Converting Enzym inhibitors; ARB, Angiotensin Receptor Blockers; BBs, Beta-blockers; LVEF, Left Ventricular Ejection Fraction; AV, Aortic Valve; BSA, Body Surface Area; RWT, Relative Wall Thickness; EAT, Epicardial Adipose Tissue.

Table 3. Fold Increase in Secretion of Inflammatory Markers in Epicardial/Subcutaneous Adipose Tissue

	Fold Increase (95% CI)	**p value**
PDGF	2.642 (1.386 - 3.898)	.074
IL-1β	2.508 (1.476 - 3.540)	.007
IL-1ra	3.605 (2.478 - 4.732)	.005
IL-2	1.368 (0.979 - 1.758)	.036
IL-4	2.484 (1.395 - 3.572)	.009
IL-5	1.519 (1.128 - 1.910)	.011
IL-6	6.191 (0.657 - 11.724)	.005
IL-7	1.083 (0.811 - 1.354)	.838
IL-8	11.065 (-0.321 - 22.451)	.012
IL-9	1.771 (1.136 - 2.406)	.013
IL-10	2.480 (1.602 - 3.357)	.007
IL-12	2.541 (1.738 - 3.344)	.007
IL-13	0.363 (0.190 - 0.537)	.005
IL-15	1.457 (1.092 - 1.822)	.007
IL-17	1.909 (1.669 - 2.149)	.008
Eotaxin	2.108 (1.270 - 2.947)	.012
FGF basic	1.173 (0.749 - 1.597)	.878
G-CSF	9.870 (6.002 - 13.738)	.005
GM-CSF	1.170 (0.990 - 1.350)	.093
IFN-γ	2.173 (1.686 - 2.661)	.005
IP-10	16.373 (1.678 - 31.068)	.005
MCP-1	1.718 (1.010 - 2.425)	.017
MIP-1α	6.546 (4.121 - 8.971)	.009
MIP-1β	5.351 (3.276 - 7.427)	.009
RANTES	1.112 (0.757 - 1.468)	.721
TNF-α	2.271 (1.844 - 2.698)	.005
VEGF	2.320 (1.594 - 3.046)	.009

Table 4. Plasma and EAT secretory profile of AS patients stratified according to EAT thickness median value

	PLASMA			EAT		
	Pts below EAT median value (n = 46)	**Pts above EAT median value (n = 46)**		**Pts below EAT median value (n = 46)**	**Pts above EAT median value (n = 46)**	
	pg/ml mean±SD	**pg/ml mean±SD**	**P value**	**pg/ml mean±SD**	**pg/ml mean±SD**	**P value**
PDGF	2212.1 ±995.7	1107.0±625.1	.010	34.1±1.8	67.0±32.8	.056
IL-1β	4.8 ±2.2	4.8±1.5	.963	47.8±10.5	84.4±6.3	.000
IL-1ra	525.1±304.9	447.4±242.3	.541	685.2±137.6	1436.1±163.6	.000
IL-2	30.2±41.8	24.2±38.1	.748	17.9±1.6	81.4±6.9	.000
IL-4	4.6±1.7	3.5±1.3	.129	2.5±0.9	6.8±1.0	.000
IL-5	3.5±1.4	4.2±2.5	.340	1.5±0.5	3.3±1.1	.010
IL-6	43.0±34.8	27.6±16.3	.255	52114±28322.	112394.1±18003.4	.004
IL-7	17.6±8.1	18.0±17.1	.938	2.7±0.6	5.3±0.8	.000
IL-8	61.6±32.6	44.3±14.1	.172	7222.9±3785.8	134151.5±14886.3	.000
IL-9	26.4±13.4	28.4±22.9	.792	6.3±1.8	18.0±1.8	.000
IL-10	50.3±59.4	35.0±29.4	.503	33.4±3.0	229.8±99.6	.002
IL-12	56.3±47.4	41.7±51.6	.504	32.6±8.2	84.1±48.7	.048
IL-13	15.2±7.1	11.4±7.9	.246	13.2±3.0	12.7±2.7	.792
IL-15	74.8±27.6	132.3±56.3	.093	322.6±54.7	388.4±50.5	.083
IL-17	110.5±58.4	128.1±128.6	.655	41.7±15.3	240.7±34.6	.000
Eotaxin	119.6±44.9	124.0±97.7	.888	26.0±7.2	177.3±15.6	.000
FGF basic	95.6±33.1	135.6±103.1	.176	205.7±39.8	389.0±104.7	.006
G-CSF	111.3±46.3	131.8±62.6	.382	3548.6±615.5	85329.2±81013.9	.054
GM-CSF	119.7±83.5	124.6±130.6	.913	612.5±21.5	620.9±103.6	.864
IFN-γ	159.2±80.4	147.6±38.4	.707	134.5±18.4	371.6±42.7	.000
IP-10	1635.3±1125.0	1116.5±603.5	.241	179.9±140.5	3809.0±2601.8	.014
MCP-1	152.4±55.9	146.0±117.3	.859	3709.8±190.5	18155.8±2274	.000
MIP-1α	8.4±6.5	9.8±7.3	.646	30.7±4.3	638.6±122.3	.000
MIP-1β	226.9±238.4	148.4±58.0	.375	368.3±93.7	1646.2±174.7	.000
RANTES	26332±26881	12707.6±10742. 5	.187	233.4±150.7	301.3±98.2	.423
TNF-α	51.9±23.4	80.8±64.4	.129	63.9±9.6	207.8±17.6	.000
VEGF	187.6±102.0	64.6±44.4	.004	465.1±198.3	1143.4±805.6	.105

EAT, Epicardial Adipose Tissue; AS, Aortic Stenosis

Table 5. Differences of inflammatory mediators levels in patients taking vs non taking statins in plasma and EAT secretome

Plasma	P value	**EAT secretome**	P value
PDGF	.994	PDGF	.056
IL-1β	.366	IL-1β	.000
IL-1ra	.962	IL-1ra	.000
IL-2	.251	IL-2	.000
IL-4	.503	IL-4	.000
IL-5	.532	IL-5	.010
IL-6	.021	IL-6	.004
IL-7	.812	IL-7	.000
IL-8	.414	IL-8	.000
IL-9	.723	IL-9	.000
IL-10	.145	IL-10	.002
IL-12	.499	IL-12	.048
IL-13	.721	IL-13	.792
IL-15	.511	IL-15	.008
IL-17	.753	IL-17	.000
Eotaxin	.761	Eotaxin	.000
FGF basic	.613	FGF basic	.006
G-CSF	.583	G-CSF	.054
GM-CSF	.930	GM-CSF	.0864
IFN-γ	.369	IFN-γ	.000
IP-10	.592	IP-10	.014
MCP-1	.679	MCP-1	.000
MIP-1α	.011	MIP-1α	.000
MIP-1β	.108	MIP-1β	.000
RANTES	.631	RANTES	.423
TNF-α	.091	TNF-α	.000
VEGF	.004	VEGF	.105

Table 6 . Correlation between EAT thickness and EAT inflammatory profile

	Epicardial fat	
	Spearman	**p value**
PDGF	.728*	.017
IL-1β	.887**	.001
IL-1ra	.936**	.000
IL-2	.790*	.020
IL-4	.801**	.005
IL-5	.954**	.000
IL-6	.691*	.027
IL-7	.991**	.000
IL-8	.837**	.010
IL-9	.911**	.000
IL-10	.960**	.000
IL-12	.636*	.048
IL-13	.110	.762
IL-15	.538	.108
IL-17	.838**	.002
Eotaxin	.850**	.007
FGF basic	.917**	.000
G-CSF	.813**	.004
GM-CSF	.275	.441
IFN-γ	.813**	.004
IP-10	.881**	.001
MCP-1	.711*	.048
MIP-1α	.813**	.004
MIP-1β	.869**	.001
RANTES	.575	.082
TNF-α	.869**	.001
VEGF	.569	.086

Figure Legends

Figure 1. Epicardial adipose tissue **(**EAT) thickness, measured by echocardiography, in patients with aortic stenosis (AS) and in control subjects matched for age, gender and body mass index. *p<0.001 AS vs Controls.

Figure 2. Inflammatory mediators levels in epicardial adipose tissue (EAT) and subcutaneous adipose tissue (SCAT) secretomes in patients with aortic stenosis (AS).

Figure 1

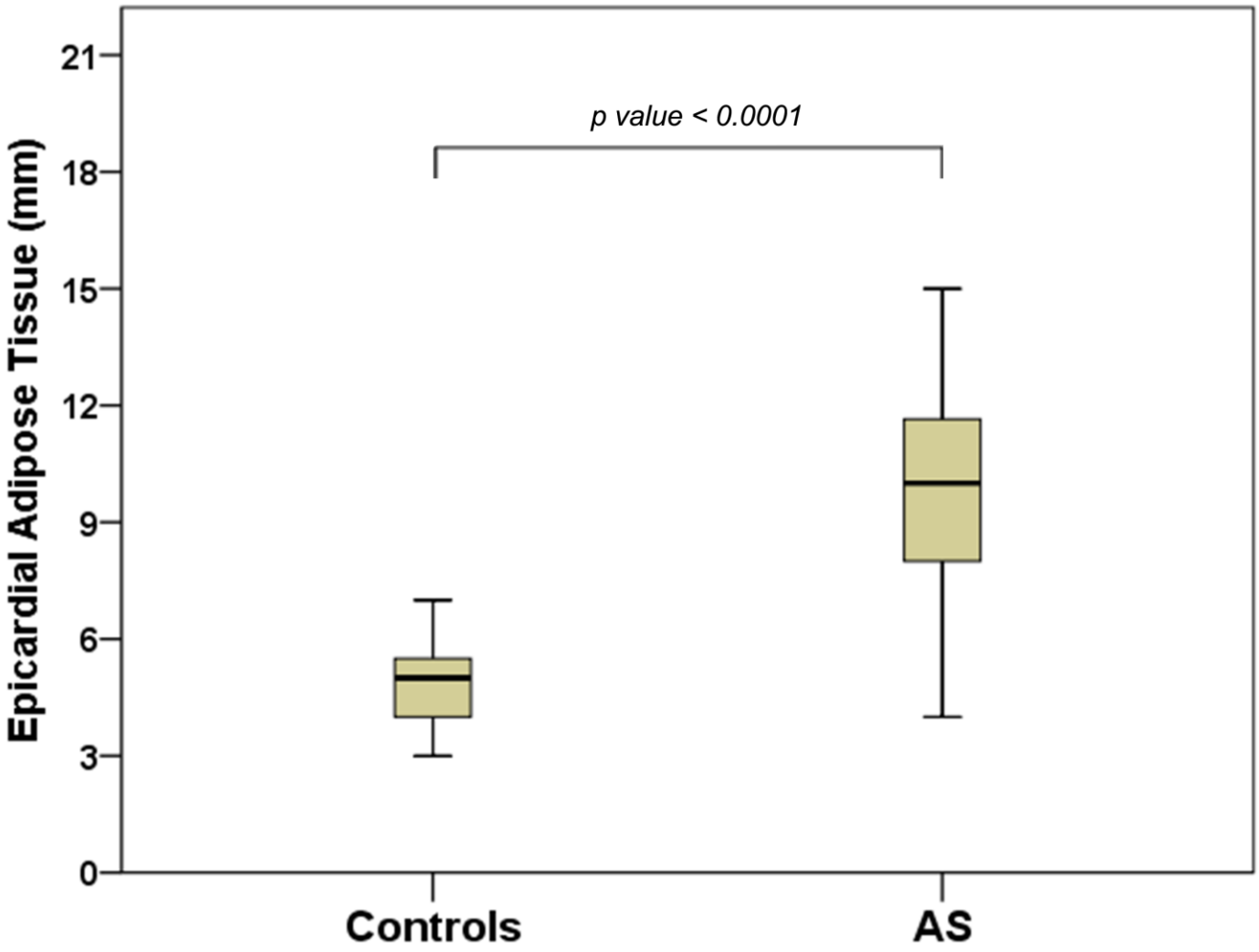
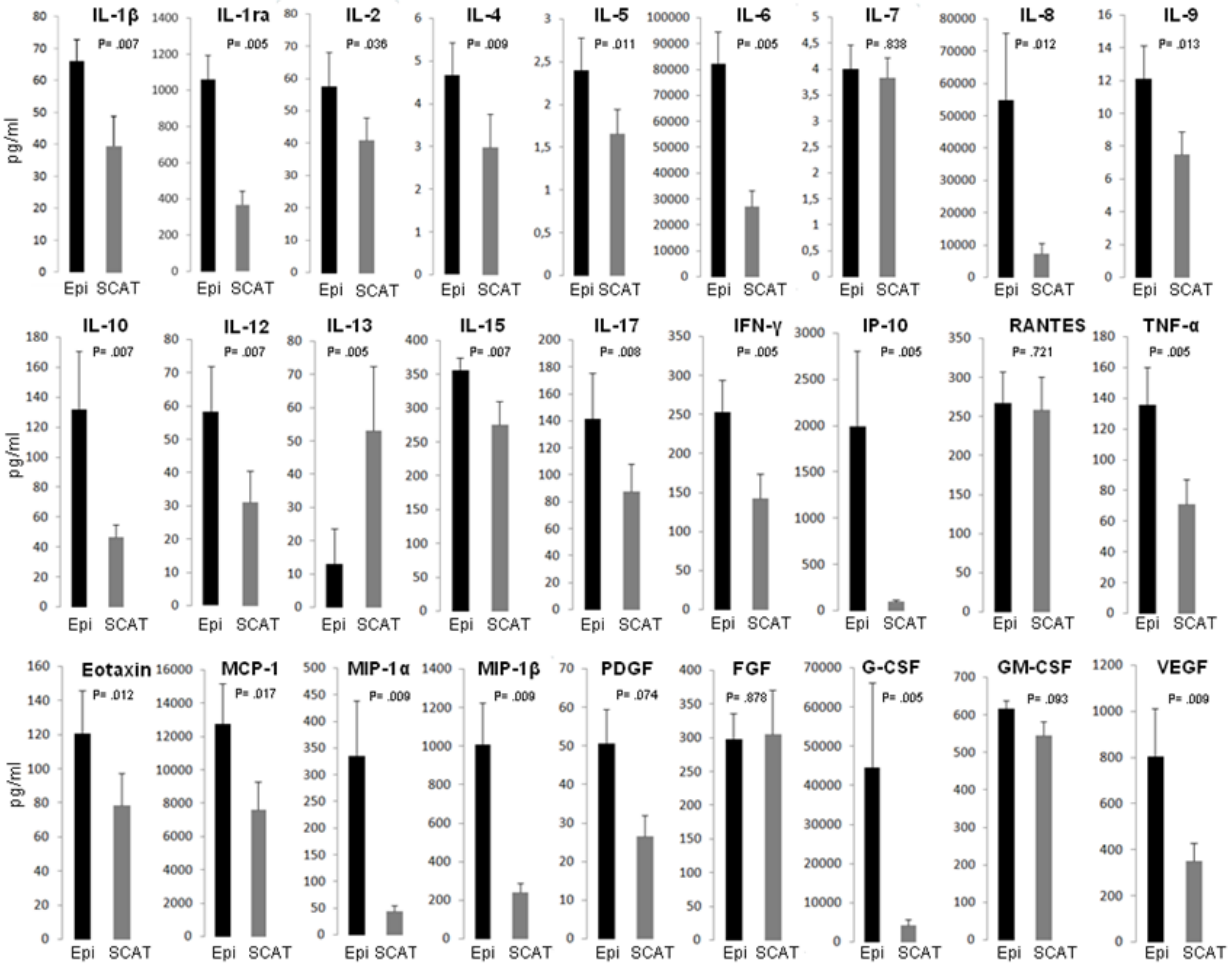

p value < 0.0001
Epicardial Adipose Tissue (mm)
21
18
15
12
9
6
3
0
Controls
AS

Figure 2


IL-1β P= .007
IL-1ra P= .005
IL-2 P= .036
IL-4 P= .009
IL-5 P= .011
IL-6 P= .005
IL-7 P= .838
IL-8 P= .012
IL-9 P= .013
IL-10 P= .007
IL-12 P= .007
IL-13 P= .005
IL-15 P= .007
IL-17 P= .008
IFN-γ P= .005
IP-10 P= .005
RANTES P= .721
TNF-α P= .005
Eotaxin P= .012
MCP-1 P= .017
MIP-1α P= .009
MIP-1β P= .009
PDGF P= .074
FGF P= .878
G-CSF P= .005
GM-CSF P= .093
VEGF P= .009
pg/ml
Epi
SCAT