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Gender differences in the association of epicardial adipose tissue and coronary artery calcification: EPICHEART study
EAT and coronary calcification by gender

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ABSTRACT

Background: The association of epicardial adipose tissue (EAT) and coronary artery calcification (CAC) seems to differ by gender. However, few studies have controlled for body size, and the ideal method for body size indexing has not been explored.
Objectives: To analyse the effect of gender related-body size and-body fat differences on the association of EAT with CAC.
Methods: This was a prospective cohort of 371 severe aortic stenosis patients (77 ± 8.5 year-old, 51% females) referred to cardiac surgery. Agatston calcium score, EAT volume and visceral abdominal fat (VAF) were obtained by computed tomography. Body composition was determined using bioelectrical impedance analysis. Body weight and height were measured to derive body mass index (BMI), body surface area (BSA), and body surface index (BSI). EAT volume was normalized for BSA, weight and height.
Results: Median CAC score was higher in men (887; IQR: 2010) than in women (279: IQR: 145) ($p < 0.01$). Similarly, men had higher volume of EAT than women (137 ± 65.6 *vs.* 106 ± 65.6 mL, $p < 0.01$), even when BSA- or height-indexed, but not if weight-indexed. EAT volume was associated with CAC adjusting for adiposity (BMI or BSI and VAF, or fat mass), but not with further adjustment for gender. In a stratified analysis, absolute- and indexed-volumes of EAT were independently associated with CAC in men while no association was found in women (gender-interaction $p < 0.05$).
Conclusions: In these high-risk patients, we demonstrated that EAT was associated with CAC score irrespective of body size, body fat and cardiovascular risk factors in men but not in women.

1. Introduction

The epicardial adipose tissue (EAT) is the visceral fat of the heart that, under physiological conditions, exerts metabolic (source of free fatty acid), thermogenic (similar to brown fat) and mechanical cardioprotective properties [1–3]. However, the excessive amount of EAT has been associated with coronary atherosclerosis [4–8], increased left ventricular mass [9,10], diastolic dysfunction [10,11], and atrial fibrillation [12]. Some studies showed that these associations remained significant after adjustment for measures of global adiposity reflected by body mass index (BMI) [4,5,7,13–17], but very few studies normalized the EAT volume for different body builds [6,18].

In small and large mammals, the heart size makes up approximately 0.6% of the body mass, and their heart mass was almost exactly proportional to the body mass with a slope regression of 0.98 [19,20]. Several methods can be used to normalize organs dimensions including indexing for height, weight, body surface area (BSA), BMI, and free-fat mass [21,22]. The challenge of scaling organ dimension for body size in studies of comparative physiology in mammals has generally been solved by using body weight. In humans, however, the problem of normalization is more complex as body weight varies markedly, mainly because of excessive amounts of body fat. For that reason, the body size correction by BSA, also, underestimates organs measures in the upper

Abbreviations: BMI, body mass index; BSA, body surface area; BSI, body surface index; CAC, coronary artery calcification; EAT, epicardial adipose tissue; VAF, visceral abdominal fat.

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range of its distribution, such as in the obese [21]. In biological processes, which the independent effect of obesity is in question, indexing for height would minimize body size differences without mitigating the pathophysiological role of obesity.

In this study we analysed the impact of body size and body fat gender-related differences on the association between EAT and CAC by comparing the association of CAC score and EAT absolute volume with the association of CAC score and body size indexed volumes of EAT. We normalized EAT volumes for patients' BSA, ideal BSA, weight and height, and in a subset of patients who underwent bioelectrical impedance analysis, we controlled for body size including free-fat mass in the regression models.

2. Methods

2.1. Study population

This was a prospective cohort of 371 symptomatic severe aortic stenosis patients referred to surgical aortic valve replacement at our institution. All patients were recruited as part of the EPICHEART (The influence of EPICardial adipose tissue in HEART disease) study, which is a translational study investigating the mechanisms that underly the association of epicardial adipose tissue with heart disease, including coronary artery disease, cardiac remodeling and atrial fibrillation after cardiac surgery in high cardiovascular risk patients. The study protocol was approved by the Institutional Ethical Committee, and all patients provided written informed consent. We defined severe aortic stenosis as aortic valve area of <1 cm [2] or 0.6 cm [2]/m [2] by transthoracic echocardiography, and patients were excluded from the study if they had coexisting moderate to severe aortic valve regurgitation or moderate to severe mitral valve disease, bicuspid aortic valve, left ventricular dilatation (end-diastolic volume index >75 mL/m [2]) or left ventricular ejection fraction <55% [23], stage 3 to 5 chronic renal failure defined as glomerular filtration rate GFR estimated by Cockcroft-Gault formula adjusted for body surface area <30 mL/min/1.73m [2] [24], moderate to severe chronic obstructive pulmonary disease defined as forced expiratory volume in one second <50% according to the 2011 Global Initiative for Chronic Obstructive Pulmonary Disease guidelines [25], or active malignancy (*i.e.* with no evidence of recurrence and no longer receiving active treatment) [26].

2.2. Computed tomography scan protocol and CAC score analysis

All patients underwent non-contrasted cardiac multidetector computed tomography (Somatom Sensation Cardiac 64, Siemens, Forchheim, Germany) 1 to 3 months before cardiac surgery to CAC score scanning and fat depots analyses. A coronary artery calcium scan imaging was performed using a prospectively ECG triggered scanning protocol (tube voltage of 120 kV, tube current of 190 mA, gantry rotation of 330 ms, collimation of 24 × 1.2 mm, pitch of 0.2; and image reconstruction of 3 mm). CAC was reported as the Agatston score and was calculated using a detection threshold of 130 Hounsfield Unit (HU) with semi-automated software (Syngo Calcium Scoring, Siemens Medical Solutions). In addition, an abdominal single slice acquisition was performed between L4 and L5-S1 with the following radiographic factors: 120 kV and 216 mA s with 5 mm thickness resulting in an estimated radiation exposure of 0.06 mSv.

2.3. EAT volume and abdominal fat areas analyses

Epicardial fat volumes and abdominal fat areas were measured with an offline workstation (SyngoVolume, Siemens Medical Solutions). We used a predefined image display setting [window with, −150 to −50 HU] to identify voxels that correspond to adipose tissue. EAT volumes were measured with a semiautomatic segmentation technique. The pericardium was manually traced for every 10 mm from the right pulmonary artery to the diaphragm to determine the region of interest. Total abdominal fat area was the sum of adipose tissue presented in the examined abdominal slice; a cursor pointer was used to trace the visceral abdominal fat (VAF) area by delineating the abdominal wall muscular layer. Subcutaneous abdominal fat was obtained by subtracting VAF from total abdominal fat. All body fat depots were normalized to body surface area. Intra – and interrater reliabilities for each fat measurement were evaluated in 55 random patients; the intraclass correlation coefficients' are shown in Supplemental Table 1.

2.4. Anthropometric evaluation

Anthropometric measurements were performed according to the reference manual *International Society for the Advancement of Kinanthropometry* (ISAK, 2011). All participants should be barefoot and wearing light clothing during the evaluation. Weight was measured using an electronic digital scale, with weighing accuracy of 0.1 kg (model 764: Seca gmbh & co, Germany), and height was measured with a precision of 1 mm (model 764, Seca gmbh & co., Germany). The BMI was calculated by dividing body weight (kg) by height (m [2]). The body surface index (BSI) was determined by the division of the subject's weight with the BSA which was calculated using the equation of DuBois (BSA = weight $^{0.425}$ × height $^{0.725}$ × 0.007184) [27]. Ideal body weight was determined using the formula of Lemmens et al. (Ideal body weight = 22 × height [2]) [28], and the ideal BSA was calculated on the basis of the calculated ideal body weight.

2.5. Bioelectrical impedance analysis

In a subset of 77 patients body composition by bioelectrical impedance analysis was performed. A minimum 4 h of fasting and 24 h of caffeine abstinence were required, and participants should withdraw any jewelry and metallic objects. The body composition was obtained by Kyle et al.'s bioelectrical impedance analysis formula [29], and given in percentage of fat free mass and percentage of fat mass. The values of resistance and reactance were measured at 800 uA and 50 kHz with an impedance analyzer bioelectrical impedance analysis 101 (Physiological Data Analyzer System, Akerne, Florence, Italy), with the subject lying in supine position and the electrodes fixed on the right hand and right foot, as described by Lucaski et al. [30]

2.6. Cardiovascular risk factors assessment

Traditional cardiovascular risk factors and ongoing medical treatments were recorded. Hypertension was defined as systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or antihypertensive treatment Diabetes was defined as fasting plasma glucose >126 mg/dl, treatment with a hypoglycemic agent or insulin. Dyslipidemia was defined as previously detected elevation of serum total cholesterol (>200 mg/dl), triglycerides (>200 mg/dl) or LDL-cholesterol (>150 mg/ dl) – known for at least 1 year – or use of lipid-lowering agents. Smoking history was classified as current smokers, former smokers, and no history of smoking, assessed by interview; current smoking status was defined as smoking at least 1 cigarette per day in the past year.

2.7. Statistical analysis

Continuous variables were reported as mean ± SD for normally distributed data or median and quartiles (Q1; Q3) for non-normally distributed data. Discrete variables were given in frequency and percent. Differences between genders were compared using T-student test for normally distributed variables, Mann-Whitney *U* Test for non-normal distributed variables, and Chi-square test or Fisher-exact test were used as appropriate to compare categorical variables. We used propensity score analysis to evaluate if male and female differ regarding their background covariates (age, BMI, arterial hypertension, dyslipidemia, diabetes mellitus, smoking status, use of statins, aspirin, or hypoglycaemic drugs) that can affect the dependent variable we were interested in (*i.e.* CAC score). Subsequently, we compared how EAT volumes are distributed across gender controlling for age, body fat, and cardiovascular risk factors; adjusted means with their standard error were then graphically represented. Given the non-normal distribution of CAC score, and as previously suggested by Tanami et al. [13], we assessed the association of absolute and indexed volumes of EAT with CAC score by median regression analysis; therefore, we provided the unstandardized B-coefficients which predict the median CAC score change per unit of EAT volume increment. We, first, analysed the univariate association of EAT with CAC score; then, we added measures of adiposity (BMI or BSI, and VAF), and finally, we further adjusted for age, gender other traditional cardiovascular-risk factor and medical drugs that modulate CAC progression. The models were the following: 1) univariate analysis with the EAT volumes as sole predictors, 2) multivariable-analysis adjusted for BMI or BSI, 3) multiple-analysis adjusted for BMI or BSI, and VAF, 4) multivariable -analysis adjusted BMI or BSI, VAF, age, and gender, 5) cardiovascular-risk factors-adjusted model; and 6) cardiovascular-risk factors and -drugs adjusted-model. To assess the effect of EAT on CAC by gender and rule out modelling effects of gender and EAT due to their association, we further investigated the association of absolute and indexed volumes of EAT with CAC score stratified by gender including a test for interaction. We based our sample size calculations on the results provided by Wang et al. [31] and Sarin et al. [32], using DerSimonian and Laird random-effects models, the pooled EAT volume mean difference between subjects with and without coronary calcification was 19.2 mL (95% confidence intervals [CIs']: 12.2–26.3). Therefore, we estimated that at least 260 patients were needed to detect EAT volume mean differences between patients with CAC score equal zero and CAC score > 0 with beta-value of 20% and alpha-value of 0.05. The analysis was performed using STATA software (version 13.1, StataCorp LP, Texas, US). *P*-values are 2-sided, and values <0.05 indicated statistical significance.

3. Results

Sample characteristics (unadjusted) by gender are shown in Table 1. The 371 elderly patients with severe aortic stenosis consisted of 51% women who were older than men (79 ± 8.4 *vs.* 76 ± 8.5 year-old, $p < 0.01$). The propensity scores given the multiple covariates contribution in CAC score were normally distributed and similar (*p*-value of 0.338) between gender. The histograms for the propensity scores distributions for female and male are shown in Supplemental Fig. 1 – A and B, respectively. Although BMI did not differ between male and female, body fat distributed differently by gender: VAF was significantly higher in men, and in contrast, subcutaneous abdominal fat was significantly higher in women. Supplemental Fig. 2 illustrates age and

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