Epicardial Adipose Tissue in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Rationale: Epicardial Adipose Tissue (EAT) volume as determined by chest computed tomography (CT) is an independent marker of cardiovascular events in the general population. COPD patients have an increased risk of cardiovascular disease, however nothing is known about the EAT volume in this population.

Objectives: To assess EAT volume in COPD and explore its association with clinical and physiological variables of disease severity.

Methods: We measured EAT using low-dose CT in 171 stable COPD patients and 70 controls matched by age, smoking history and BMI. We determined blood pressure, cholesterol, glucose and HbA1c levels, microalbuminuria, lung function, BODE index, comorbidity index and coronary artery calcium score (CAC). EAT volume were compared between groups. Univariate and multivariate analyses explored the relationship between EAT volume and the COPD related variables.

Results: COPD patients had a higher EAT volume [143.7 (P25–75, 108.3–196.6) vs 129.1 (P25–75, 91.3–170.8) cm³, p = 0.02] and the EAT volume was significantly associated with CAC (r = 0.38, p < 0.001) and CRP (r = 0.32, p < 0.001) but not with microalbuminuria (r = 0.12, p = 0.13). In COPD patients, EAT volume was associated with: age, pack-years, BMI, gender, FEV1%, 6 MWD, MMRC and HTN. Multivariate analysis showed that only pack-years (B = 0.6, 95% CI: 0.5–1.3), BMI (B = 7.8, 95% CI: 5.7–9.9) and 6 MWD (B = −0.2, 95% CI: −0.3−−−−0.1), predicted EAT volume.

Conclusions: EAT volume is increased in COPD patients and is independently associated with smoking history, BMI and exercise capacity, all modifiable risk factors of future cardiovascular events. EAT volume could be a non-invasive marker of COPD patients at high risk for future cardiovascular events.

Introduction

Chronic obstructive pulmonary disease (COPD) and cardiovascular diseases (CVD) are two of the top causes of death worldwide [1]. COPD has been described as an independent risk factor for CVD [2] and the latter is a major cause of mortality in COPD, particularly in patients with mild to moderate disease [3–5].

Non-invasive CVD markers may be important to identify COPD patients who are at high risk to develop future cardiovascular events. Beyond the traditional non-invasive CVD risk factors, several other have been proposed for COPD patients, including C-Reactive Protein (CRP) [6], arterial stiffness evaluated by pulse wave velocity (PWV) [7], carotid intima-media thickness (IMT) [8], ankle-brachial index (ABI) [9], and microalbuminuria (MAB) [10].

Epicardial Adipose Tissue (EAT) is the visceral thoracic fat located between the myocardium and the visceral pericardium, and given its anatomical proximity to the heart, EAT can locally modulate the myocardium and coronary arteries [11]. Like other white adipose tissue loci, EAT could function as a lipid-storing depot, as an endocrine organ that secretes hormones, and as inflammatory tissue that secretes cytokines and chemokines [12]. EAT volume can be quantified by different non-invasive radiological techniques, such as echocardiography [13], magnetic resonance imaging (MRI) [14], and computed tomography (CT) [15]. Among available imaging modalities, volumetric quantification of EAT with multidetector computed tomography (CT) has been shown to be one of the most reliable and reproducible methods to assess the amount of EAT [16]. Furthermore, the large population based Multi-Ethnic Study of Atherosclerosis (MESA) Study recently confirmed that EAT volume measured with a chest CT, predicted incident coronary heart disease independent of conventional risk factors [17].
We hypothesized that patients with COPD would have larger EAT volumes than appropriately matched controls, and that the EAT volume in the COPD patients would relate to factors known to increase risk for cardiovascular events. To test this hypothesis we designed this cross sectional study that compared current and former smokers with and without COPD, matched for age, smoking history and body mass index (BMI).

Methods

Study Population

The institutional review board of the Clínica Universidad de Navarra approved this study. Participants were former and current smokers with and without COPD regularly seen in our pulmonary clinic (Figure 1). They all signed the consent form approved by the Human Review Board (Pamplona: “Comité de Ética de la Investigación, Universidad de Navarra IRB n°: 043/2006”). Subjects were consecutively enrolled from January 2002 to August 2012. COPD was defined by a history of smoking more than 10 pack-years and a post-bronchodilator FEV1/FVC less than 0.7. To be enrolled, COPD patients had to be clinically stable for 8 weeks prior to entry, and receiving optimal therapy according to international guidelines [18]. The non-COPD group was comprised of smokers and former smokers with a smoking history greater than 10 pack-years and without postbronchodilator airflow obstruction (FEV1/FVC > 0.7). All postbronchodilatation measurements were performed 15 minutes after the inhalation of 400 ug of albuterol. Exclusion criteria were uncontrolled co-morbidities such as malignancy or other confounding diseases. We recorded history of diabetes mellitus, hypertension, dyslipidemia, and the use of anti-hypertensive medications or statins. Blood pressure was measured following standard recommendations [19].

Matching Process

Figure 1 shows the flow diagram of the matching process. From the initial sample of 420 former and current smokers we identified 70 subjects without COPD and 350 with COPD. For the purpose of this study we assigned former and current smokers without COPD to the control group, and matched each control patient with at least 2 COPD patients with similar age (±2 years), pack-years (±3 pack-years), and BMI (±3 units). Using the aforementioned criteria it was possible to properly match 70 controls with 171 COPD patients.

Clinical Variables

Lung volumes and spirometry were measured according to ATS/ERS guidelines [20].

The 6-min walking distance (6 MWD) was selected from the better of two walks separated by at least 30 minutes [21]. Dyspnea was evaluated by the modified Medical Research Council (MMRC) scale [22]. BMI was calculated in kg/m². The FEV1%, BMI, 6 MWD and MMRC values were integrated into the BODE index [23]. The Framingham Score was calculated as previously described [24].

Laboratory Methods

Morning fasting blood and spot-urine samples were collected simultaneously while at rest and before any other test. The microalbuminuria (MAB) or urinary albumin excretion was determined as the urinary albumin (milligrams) to creatinine (grams) ratio in the morning urine. Urine albumin concentration was determined by a standard turbidometric method (coefficient of variation 5.5%). Serum and urine creatinine concentrations were analyzed using the Jaffe reaction and quantified by a photometric

![Figure 1. Flow diagram of the study population.](doi:10.1371/journal.pone.0065593.g001)
method. Fasting serum levels of glucose, high sensitivity CRP, glycated haemoglobin (HbA1c) and cholesterol were also determined.

CT Image Acquisition and Reconstruction Protocol

All individuals underwent the CT examinations using a multidetector CT (Somatom Definition and Somatom Sensation 64, Siemens Healthcare, Forchheim, Germany). Low-dose chest CT was performed with 120 kV, 40 mAs, 32×0.6 mm detector collimation, 64×0.6 mm slice acquisition, 0.3 s gantry rotation time, and 1.4 pitch. Images were reconstructed with 5-mm slice thickness using soft-tissue convolution kernel (B31f).

Epicardial Adipose Tissue Quantification

The amount of EAT volume was assessed by two independent observers (JZ, GB) unaware of the clinical information, using a commercially available software tool (Volume, Siemens) based on attenuation-dependent segmentation methods (Fig. 2). Concordance coefficient between observers was 0.95; with 95% CI between 0.93–0.96. Observers manually traced the pericardium at its superior extent (the center of the right pulmonary artery as it crosses the mid-sagittal plane), at mid-ventricular level, and at the end of the pericardial sac, which defined the inferior extent of the pericardial volume. The software automatically interpolated between the user-defined traces. Automatically traced contours were manually adjusted to the pericardium if needed. Epicardial adipose tissue volume was defined as any fat tissue located within the pericardial sac. A predefined threshold of −195 to −45 HU was used to identify voxels corresponding to fat [17]. Figure 2 shows the CT of two matched patients participating in the study showing their EAT volume measurements.

Coronary Calcium Calcification Evaluation

Each of the four main coronary arteries was identified (left main, left anterior descending, circumflex, and right). Calcification in each artery was categorized as absent, mild, moderate, or severe and scored by the radiologist as 0, 1, 2, or 3, respectively. Calcification was classified as mild when less than one-third of the length of the entire artery showed calcification, moderate when between one-third and two-thirds, severe when more than two-thirds of the artery showed calcification. With four arteries thus scored, each subject received a CAC score ranging from 0 to 12 [25].

Statistical Analysis

Data are summarised as relative frequencies for categorical variables, mean (SD) for normally distributed variables and median (25th–75th percentile) for non-normal data. Comparisons between groups were performed using student t-test, Pearson Chi-square or U-Mann Whitney according to the variables type and distribution. A multivariate analysis including Charlson comorbidity index and COPD diagnosis explored the independent association with EAT in the entire population. Other variables with statistical significant difference between groups (lung function, 6 MWD, MMRC, CRP) were not included in the model because of their colinearity with COPD diagnosis.

The associations of the other CVD risk parameters with EAT volume were estimated using Spearman correlation coefficients because of the non-normal distribution of EAT volume values. Linear regression analyses explored the association of each of the studied variables and EAT volume. A multiple linear regression model with EAT volume as the dependent variable and those parameters that showed statistical significance at the level of 0.05 in the univariate analysis was performed to estimate the independent association. Significance level was established as a two-tailed p-Value ≤0.05. Calculations were made with SPSS 15.0, Chicago, U.S.A.

Results

Seventy current and former smokers without COPD and 171 with COPD participated in the study and their characteristics are shown in Table 1. Although there were patients in every GOLD stage of the 2009 severity classification (I: 44%, II: 36%, III: 16% and IV: 4%), most of them were in GOLD stages I and II. Compared with controls and as expected for a previously matched population, patients and controls had similar age, gender distribution, pack-years history, smoking status, history of diabetes mellitus, cholesterol levels, history of hypertension, blood pressure values and Framingham score. As expected, patients with COPD had abnormal lung function, higher MMRC scores and lower 6 MWD. As previously described [6,10], other CVD risk markers such as CRP levels and MAB were higher in COPD. Surprisingly, although higher in COPD patients, CAC was not significantly different between the groups. Table S1 shows the characteristics of the COPD patients not selected in the matching process. In comparison to those selected, these patients had the following characteristics: greater age, more pack-years, were less likely to be actively smoking, higher comorbidity index, a similar degree of severity in terms of lung function, less exercise capacity and a higher EAT volume. A multivariate analysis exploring the association of EAT volume with COPD diagnosis after adjusting for Charlson comorbidity showed that the presence of COPD was a statistical significant predictor of EAT volume (β coefficient 28.86 95% CI: 7.6–50.1, p = 0.008).

The association of EAT volume with other CVD markers in COPD patients was: age (r = 0.26, p < 0.001), smoking history (r = 0.27, p < 0.001), BMI (r = 0.66, <0.001), arterial hypertension (r = 0.28, p = 0.001), diabetes mellitus (r = 0.19, p = 0.003), CRP (r = 0.32, <0.001), CAC (r = 0.38, <0.001) and MAB (r = 0.12, p = 0.14).

Table 2 shows the univariate analysis exploring the association of each of the studied parameters with EAT volume in COPD and...
in smokers without COPD. Statistical significance was found for age, pack-years, gender, BMI, FEV1%, 6 MWD, MMRC, hypertension, and use of anti-hypertensive medication. In smokers without COPD, we found statistically significant differences in pack-years, gender, BMI, 6 MWD, HDLc and glucose. Table 3 shows the multivariate analysis that determined the best predictors of EAT volume in COPD patients and in smokers without COPD, including those that showed statistical significance in the univariate analysis. Pack-years, BMI and 6 MWD were the best predictors of EAT volume in COPD, and only BMI in smokers without COPD.

Discussion

This study showed that in comparison with appropriately matched controls without COPD, Epicardial Adipose Tissue volume is greater in COPD patients. We also showed in these patients, that EAT volume is independently associated with modifiable CVD risk factors like smoking history, BMI and decreased exercise capacity.

Abnormal Visceral Abdominal Tissue (VAT) deposition is receiving increasing attention in patients with COPD for its potential pathogenic role in CVD in these high risk patients [26]. Previous studies have reported increased visceral fat deposition in

Table 1. Patients characteristics.

| Patients characteristics | Smoker | COPD | P |
|--------------------------|--------|------|---|
| n                        | 70     | 171  |   |
| Age (X ± SD)              | 57±8   | 59±7 | 0.056 |
| Gender (%) male/female    | 80/20  | 80.5/19.5 | 1 |
| Pack-year (X ± SD)        | 39±16  | 42±17 | 0.31 |
| Current Smoker (%) yes/no | 58.6/41.4 | 55/45 | 0.67 |
| Framingham Score (%) Median(p25–p75) | 17.3 (10.4–20.8) | 17.8 (10.4–28.8) | 0.65 |
| Charlson Median(p25–p75)  | 0 (0–1) | 1 (1–2) | <0.0001 |
| BMI (X ± SD)              | 28.2±5.4 | 26.9±4.8 | 0.074 |
| FEV1/FVC (X ± SD)         | 75.5±4.8 | 56.8±10.5 | <0.001 |
| FEV1 liters (X ± SD)      | 3.05±0.8 | 2.18±0.8 | <0.001 |
| FEV1% (X ± SD)            | 99±15.6 | 73.6±21.9 | <0.001 |
| FVC % (X ± SD)            | 106±17 | 102.2±20.4 | 0.17 |
| TLC % (X ± SD)            | 94.7±13 | 107.7±14.3 | <0.001 |
| MMRC 0–4 (%)              | 80/186/1/4/0/0 | 44.4/31/15.8/8.2/0.6 | <0.001 |
| 6 MWD (X ± SD)            | 539.67±61.7 | 495.85±100 | 0.003 |
| BODE Quart 1–4 (%)       | 82.1/10.3/4.8/2.8 | 82.1/10.3/4.8/2.8 | 0.08 |
| Hypertension (%) yes/no   | 45.7/54.2 | 32.7/67.3 | 0.08 |
| Anti-hypertensive treatment (%) yes/no | 52.9/47.1 | 65.5/34.5 | 0.08 |
| SBP mmHg (X ± SD)         | 127.1±19.4 | 126.7±19.1 | 0.86 |
| DBP mmHg (X ± SD)         | 76.5±12.9 | 75.22±11.2 | 0.44 |
| DM (%) yes/no             | 18.6/81.4 | 14/86 | 0.43 |
| Glucose Median(p25–p75)   | 97 (89.5–108) | 98 (90.8–111) | 0.36 |
| HbA1c Median(p25–p75)     | 6 (5.7–6.9) | 6 (5.6–6.7) | 0.84 |
| Dyslipemia (%) yes/no     | 67/33 | 71/29 | 0.35 |
| Anti-hyperlipemia treatment (%)yes/no | 63/37 | 70/30 | 0.36 |
| Total Cholesterol Median(p25–p75) | 202 (185–227) | 201.5 (170–231.8) | 0.56 |
| LDL-C Median(p25–p75)     | 123 (104–149) | 122 (94.8–157.5) | 0.79 |
| HDL-C Median(p25–p75)     | 56 (45–66) | 51 (40–64) | 0.12 |
| Albumin/Creatinuria index Median(p25–p75) | 6.75 (4.4–12.6) | 11.8 (5.1–33.9) | 0.008 |
| EAT cm³ Median (p25–p75)  | 129.1 (91.3–170.8) | 143.7 (108.3–196.6) | 0.028 |
| Coronary Calcium Score Median (p25–p75) | 1 (0–2.25) | 2 (0–3) | 0.062 |
| CRP Median(p25–p75)       | 0.2 (0.1–0.4) | 0.3 (0.2–0.7) | 0.041 |
| Systemic corticosteroid treatm (%) yes/no | 1.4/98.6 | 4/1.959 | 0.44 |

n = Number of participants for each group; BMI = Body Mass Index; FEV1 = Forced Expiratory Volume in the fisrt second; FVC = Forced Vital Capacity; TLC = Total Lung Capacity; MMRC = Modified Medical research Council; 6 MWD = 6 Minutes Walk Distance; BODE index: BMI, Obstruction, Dyspnea, Exercise; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; DM = Diabetes Mellitus; LDL-C = Low Density Protein; HDL-C = High Density Protein; EAT = Epicardial Adipose Tissue CRP = C reactive Protein.
X ± SD = means ± Standard Desviation; y/n = Yes/No; p25–p75 = interquartile range.
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Epicardial Adipose Tissue in COPD

Table 2. Univariate analysis exploring the independent association of the studied variables with EAT volume in patients with COPD and in smokers.

| Variable                          | Coefficient | CI      | p       |
|-----------------------------------|-------------|---------|---------|
| **Univariate with EAT volume as the dependent variable in COPD** |             |         |         |
| Age                               | 2.7         | 2.5 to 2.9 | <0.001 |
| Pack-years                        | 1.3         | 0.7 to 2 | <0.001 |
| BMI                               | 9.6         | 7.5 to 11.6 | <0.001 |
| Gender (female vs male)           | -54.9       | -84 to -25.8 | <0.001 |
| FEV1%                             | -0.7        | -1.2 to -0.1 | 0.01   |
| 6 MWD                             | -0.3        | -0.4 to -0.1 | <0.001 |
| MMRC                              | 17.3        | 5.4 to 29.1 | 0.004  |
| HTN (yes vs no)                   | 37.9        | 13.1 to 62.7 | 0.003  |
| Charlson                          | 2.2         | -4.8 to 9.3 | 0.54   |
| BODE                              | 5.9         | -1.4 to 13.3 | 0.11   |
| Anti-Hypertensive treatment (yes vs no) | 44         | 19.8 to 68.2 | <0.001 |
| Anti-Hyperlipemia treatment (yes vs no) | 14.7     | -11.1 to 40.6 | 0.26   |
| Total Cholesterol                 | -0.1        | -0.4 to 0.2 | 0.58   |
| LDL-C                             | -0.1        | -0.4 to 0.2 | 0.46   |
| HDL-C                             | -0.6        | -1.4 to 0.1 | 0.08   |
| DM (yes vs no)                    | 29.8        | -4.3 to 64.0 | 0.08   |
| Glucose                           | 0.3         | -0.1 to 0.7 | 0.14   |
| HbA1c                             | 2.6         | -9.1 to 14.5 | 0.65   |

| **Univariate with EAT volume as the dependent variable in Smokers without COPD** |             |         |         |
| Age                               | 1.2         | -0.3 to 2.6 | 0.12   |
| Pack-years                        | 1.2         | 0.4 to 1.9 | 0.04   |
| BMI                               | 7.3         | 5.2 to 9.4 | <0.001 |
| Gender (female vs male)           | -46.9       | -78 to -15.8 | 0.04  |
| FEV1%                             | -0.4        | -1.3 to 0.4 | 0.3    |
| 6 MWD                             | -0.4        | -0.7 to -0.05 | 0.03  |
| MMRC                              | 24.1        | -3.2 to 51.3 | 0.08  |
| HTN (yes vs no)                   | 25          | -1.5 to 51.5 | 0.06  |
| Charlson                          | 16          | -2.5 to 35.5 | 0.08  |
| Anti-Hypertensive treatment (yes vs no) | 22       | -4.7 to 48.7 | 0.11  |
| Anti-Hyperlipemia treatment (yes vs no) | 22.9   | -5 to 50.7 | 0.11  |
| Total Cholesterol                 | -0.2        | -0.5 to 0.2 | 0.3    |
| LDL-C                             | -0.3        | -0.7 to 0.1 | 0.21   |
| HDL-C                             | -1.4        | -2.3 to -0.6 | 0.002 |
| DM (yes vs no)                    | 34.8        | -1.6 to 71.2 | 0.06  |
| Glucose                           | 0.7         | 0.1 to 1.2 | 0.025  |
| HbA1c                             | 2.8         | -17.7 to 23.4 | 0.78  |

BMI = Body Mass Index; FEV1% = Forced Expiratory Volume in the first second percent; 6 MWD = Six Minutes Walk distance; MMRC = Modified Medical Research Council Dyspnea Scale; BODE = Body Mass Index, Obstruction, Dyspnea, Exercise; HTN = Hypertension; LDL-C = Low Density Protein Cholesterol; HDL-C = High Density Protein Cholesterol; DM = Diabetes Mellitus; HbA1c = glycylated haemoglobin.

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non-obese mild to moderate COPD patients evaluated with CT [27,28]. Epicardial Adipose Tissue is the visceral white deposit located between the myocardium and the visceral pericardium that has metabolically active properties due to its anatomical and functional contiguity with the myocardium [12,13]. Biochemical and thermogenic cardio-protective properties under physiological conditions have been associated with EAT. However, in pathological circumstances, EAT can affect the myocardium and the coronary arteries through vasocrine or paracrine secretion of pro-inflammatory cytokines [29]. EAT has been associated with BMI, VAT, metabolic risk factors, insulin resistance, and coronary artery disease [13,30,31,32]. Recently, it has been shown that EAT volume can independently predict a higher risk of future incident coronary heart disease in community-based adults without a history of CVD [17]. Therefore EAT volume measured on a chest CT has been postulated as a non-invasive tool to identity high-risk CVD patients in whom specific therapies could be implemented.
inflammatory cytokines (adiponectin, leptin, TNFα, IL-1β, MCP-1, PAI-1, IL-8, IL-6, resistin, angiotensinogen, VEGF) [29], and the direct release of free fatty acids into the vasa vasorum that are then transported downstream into the arterial wall [13]. Therefore, as occurs in the general population [32], the significant association between EAT volume and CAC scores is expected. MAB levels were also increased in the COPD cohort of this study [40]. Interestingly, MAB was not associated with EAT volume. A potential explanation could be that MAB is a measure of general systemic endothelial dysfunction [41], whereas EAT represents a local phenomenon affecting primarily the coronary arteries.

The present study also shows that EAT volume in COPD patients is significantly associated with important CVD risk factors (age, pack-years, BMI, hypertension), and clinical or physiological descriptors of COPD (age, BMI, FEV1%, MMRC, 6 MWD). The multivariate model found that the best predictors of EAT volume were smoking history as expressed in pack-years, the BMI and inversely to the 6 MWD. Interestingly in current or former smokers without COPD, EAT volume was only predicted by BMI as previously reported [16]. Our findings have important implications in the daily management of high-risk COPD patients since all of them are modifiable CVD risk factors. There is evidence in that EAT deposition is dynamic and that it could be modified with different interventions such as weight loss achieved by diet [42] or bariatric surgery [30], and by physical activity [43]. Although not yet studied, it is likely that smoking cessation would have similar consequences. Strategies aiming at decreasing BMI will decrease their EAT volume and probably decrease their CVD risk. Perhaps one of the most interesting findings of this study was the indirect association between EAT volume and 6 MWD. We know that 6 MWD reflects daily exercise activity in COPD patients [44]. It is possible that patients with greater 6 MWD do more exercise and as a result have lower EAT volumes.

Age and gender were the other patients’ characteristics that predict EAT volume. Interestingly, Dagvasumberel et al. [45] recently showed that in 90 patients from Japan with moderate to severe coronary artery disease (CAD), EAT volume was higher in men and strongly associated with coronary atherosclerosis. These authors also found that age was among the predictors of EAT volume in their study patients. Therefore, the present study confirms the consistency of these CVD risk factors as predictors of EAT volume as had previously been shown in a different high-risk population.

### Table 3. Multivariate analysis exploring the independent association of the studied variables with EAT volume in patients with COPD and in smokers.

| Variable | Coefficient | CI    | p   |
|----------|-------------|-------|-----|
| Age      | 1           | −0.41 to 2.1 | 0.06 |
| Pack-years | 0.6         | 0.04 to 1.3  | 0.035 |
| Gender (female vs male) | −21.4 | −44.1 to 1.2 | 0.06 |
| BMI      | 7.8         | 5.7 to 9.9   | <0.001 |
| 6 MWD    | −0.2        | −0.3 to −0.1 | <0.001 |

(*) Variables included in the model: Age, Pack-years, Gender, BMI, FEV1%, 6 MWD, MMRC, HTN

### Table 3. Multivariate analysis with EAT as the dependent variable in Smokers without COPD

| Variable | Coefficient | CI    | p   |
|----------|-------------|-------|-----|
| BMI      | 9.6         | 6.7 to 12.5 | <0.001 |

(*) Variables included in the model: Pack-years, BMI, 6 MWD, HDL-C, Glucose.

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The reason why VAT deposition is increased in COPD patients is unclear and was not within the scope of the present work. The report that other chronic inflammatory diseases like rheumatoid arthritis, Crohn’s disease and psoriasis have increase deposition of VAT, suggest a link between inflammatory and VAT deposition [26]. The finding that the Fat Mass and Obesity (FTO) gene has been positively associated with BMI in COPD patients, suggests a potential genetic origin since this gene has been associated with differential fat deposition in the visceral o subcutaneus stores [46]. In obese non-COPD patients, increased visceral relative to subcutaneous fat has been associated with hypertriglycerideremia and a decreased rate of glucose utilization [47]. Several reports propose the link between intermittent hypoxemia and visceral fat deposition in animal models [48,49], suggesting the potential role of hypoxic stress in VAT deposition in patients with COPD, although this has not been demonstrated yet in this population.

There are several limitations to this study. Firstly, it was performed in patients from a pulmonary clinic, and with specific clinical and physiological characteristics limited by the randomisation criteria. Therefore conclusions may not apply to COPD patients in general. Secondly, most of the patients participating in this study had mild to moderate disease. Whether the findings can be extrapolated to patients with more severe disease remains unknown. However, there is plenty of evidence that the highest risk for CVD in COPD patients occurs precisely in those with mild to moderate disease [3,50]. Thirdly, this is a cross sectional study and therefore the association could be viewed as either a cause or a consequence. Also the sample size of the present work could viewed as a potential limitation, although this is the first report on VAT deposition in COPD patients. Large longitudinal studies should confirm that VAT volume is an independent risk factor for CVD events in COPD patients.

In conclusion, this is the first study showing that VAT volume is greater in patients with COPD and that it is independently associated with important modifiable CVD risk factors. If confirmed this information could have important implications in the overall management strategy of patients with COPD.

**Supporting Information**

Table S1 Characteristics of the COPD patients not selected in the matching process. (DOCX)

**Author Contributions**

Conceived and designed the experiments: JZ GB JPT. Performed the experiments: JZ JG GB IC ABA AC JPT. Analyzed the data: JZ JG GB JPT. Contributed reagents/materials/analysis tools: JZ JG GB JPT. Wrote the paper: JZ JG GB IC ABA AC JPT. Patient recruitment: JZ JG IC ABA AC JPT. Guarantor of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article: JPT.

**References**

1. Murray CJ, Lopez AD. (1997) Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet; 349: 1490–1504.
2. Sin DD, Man SF. (2005) Chronic obstructive pulmonary disease as a risk factor for cardiovascular morbidity and mortality. Proc Am Thorac Soc.; 2: 8–11.
3. Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, et al. (2007) Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med; 356: 756–769.
4. Sin DD, Anthonisen NR, Soriano JB, Agusti AG. (2006) Mortality in COPD: role of comorbidities. Eur Respir J.; 29: 1245–1257.
5. Ikemoto H, Yokoyama A, Kihara H, Ishikawa N, Haruta K, et al. (2009) Airflow limitation in smokers is associated with subcutaneous adiposopathy. Am J Respir Crit Care Med.; 179: 35–40.
6. Sin DD, Man SF. (2003) Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular disease? The potential contribution of systemic inflammation in chronic obstructive pulmonary disease. Circulation.; 107: 1514–9.
7. McAllister DA, Maclay JD, Mills NL, Mair G,Miller J, et al. (2007) Arterial stiffness is independent of obesity and hyperinsulinemia in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med.; 176: 1208–14.
8. van Gestel YR, Flu WJ, van Knijn JP, Hocke SE, Bars JJ, et al. (2010) Association of VAT with carotid wall intima-media thickness in vascular surgery patients. Respir Med.; 104: 712–6.
9. Barg RR, Ahmed FS, Carr JJ, Hoffmann EA, Jiang R, et al. (2012) Subclinal atherosclerosis, airflow obstruction and emphysema: the MESA Lung Study. Eur Respir J.; 39: 846–54.
10. Casanave C, de Torres JP, Navarro J, Aguirre-Jaline A, Toledano P, et al. (2010) Microalbuminuria and hypoxemia in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med.; 182: 1004–10.
11. Iacobellis G, Malavazos AE, Coetzee MM. (2011) Epididymal fat: from the bedside to the molecular aspects to clinical practice. The Int J of Bioch Cell Biol.; 1631–1654.
12. Sacks HS, Fair JN. (2007) Human epididymal adipose tissue: a Review. Am Heart J.; 153: 967–97.
13. Iacobellis G, Ripandell MC, Asaad F, Vecchi E, Tiberii C, et al. (2003) Echocardiographic epididymal adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. J Clin Endocrinol Metab.; 88: 5163–70.
14. Keseci K, Craver MJ, Veldhuizen B. (2000) Epididymal adipose tissue imaged by magnetic resonance imaging: an important risk marker of cardiovascular disease. Heart; 92: 962.
15. Abbbara S, Desai JC, Cury RC, Butler J, Nieman K, et al. (2006) Mapping epididymal fat with multi-detector computed tomography to facilitate percuta- neous transapical arrhythmia ablation. Eur J Radiol.; 57: 417–22.
16. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, et al. (2008) Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. Circulation.; 117: 605–13.
17. Ding J, Hsu FC, Harris TB, Liu Y, Kritchevsky SB, et al. (2009) The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr.; 90: 499–504.
18. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, et al. (2012) Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease, GOLD Executive Summary. Am J Respir Crit Care Med.; 185: 736–48.
19. The Seventh Report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure. (2003) U. S. Department of Health and Human Services. National Institutes of Health. NHLBI NIH Publication No. 03-5233. December 2003.
20. American Thoracic Society Statement. Lung function testing; selection of reference values and interpretative strategies. (1991) Am Rev Resp Dis.; 144: 1202–1218.
21. ATS Statement: Guidelines for the Six-Minute Walk Test. (2002) Am J Respir Crit Care Med.; 166: 111–117.
22. Mahler D, Weels C. (1988) Evaluation of clinical methods for rating dyspnea. Chest.; 93: 580–586.
23. Celli BR, Cote C, Marin JM, Casanova C, Montes de Oca M, et al. (2004) The Body Mass Index, Airflow Obstruction, Dyspnea, Exercise Performance (BODE) index in chronic obstructive pulmonary disease. N Engl J Med.; 350: 1005–1012.
24. D’Agostino RB Sr, Vasan RS, Pennica MJ, Wolf PA, Cobain M, et al. (2008) General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation.; 117: 749–757.
25. Shemesh J, Henschke C, Shabah D, Yip R, Farooqi AO, et al. (2010) Ordinal scoring of coronary artery calcifications on low-dose CT scans of the chest is predictive of death from cardiovascular diseases. Radiology; 257: 541–548.
26. van den Borst B, Gosker HR, Schols AMWJ. (2012) Excessive visceral fat accumulation in advanced chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis.; 6: 423–50.
27. Mazzurk T, Zhang L, Zalewski A, Mannion JD, Duhl JT, et al. (2003) Human epididymal adipose tissue is a source of inflammatory mediators. Circulation.; 108: 2459–60.
28. Furutate R, Ishii T, Wakabayashi R, Motegi T, Yamada K, et al. (2011) Excessive visceral fat accumulation in advanced chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis.; 6: 423–50.
29. Willem HJ, Byers P, Chirinos JA, Labrador E, et al. (2007) Effects of weight loss after bariatric surgery on epicardial fat measured using echocardiography. Am J Cardiol.; 99: 1292–5.
30. Iacobellis G, Leonetti F J. (2005) Epididymal adipose tissue and insulin resistance in obese subjects. Clin Endocrinol Metab.; 90: 6300–2.
32. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, et al. (2008) Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. Circulation.; 117: 605–13.
33. Mahabadi AA, Massaro JM, Rosito GA, Levy D, Murabito JM, et al. (2009) Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study. Eur Heart J.; 30: 830–6.
34. Aquini A, Macner W. (2012) The COPD control panel: towards personalised medicine in COPD. Thorax. 2012 Nov 1. [Epub ahead of print].
35. National Lung Screening Trial Research Team, Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, Fagerstrom RM, Gareen IF, Gatsounis G, Marcus PM, Sicks JD. (2011) Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med.; 365: 395–409.
36. de Torres JP, Marin JM, Casanova C, Cote C, Carrio S, et al. (2011) Lung cancer in patients with chronic obstructive pulmonary disease: incidence and predicting factors. Am J Respir Crit Care Med.; 184: 913–9.
37. Nishimura M, Makita H, Kagai K, Konno S, Nasuhara Y, et al. (2012) Annual change in pulmonary function and clinical phenotype in chronic obstructive pulmonary disease. Am J Respir Crit Care Med.; 185: 44–52.
38. Martinez-Garcia MA, Soder-Catalatha JJ, Donat Sanz Y, Catalán Serra P, Agramunt Lerma M, et al. (2011) Factors associated with bronchiectasis in patients with COPD. Chest.; 140: 1130–7.
39. Wells JM, Washko GR, Han MK, Abbas N, Nath H, et al. (2012) N Engl J Med.; 367: 913–21.
40. Casanova C, de Torres JP, Navarro J, Aguierre-Jaime A, Toledo P, et al. (2010) Microalbuminuria and hypoxemia in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med.; 182: 1004–10.
41. Diercks GF, Van Boven AJ, Hilleghe JL, de Jong PE, Rouleau JL, et al. (2002) The importance of microalbuminuria as a cardiovascular risk indicator: a review. Can J Cardiol; 18: 525–35.
42. Nakazato R, Rajani R, Cheng YY, Shumilovich H, Nakanishi R, et al. (2012) Weight change modulates epicardial fat burden: a 4-year serial study with non-contrast computed tomography. Atherosclerosis.; 220: 139–44.
43. Kim MK, Tomita T, Kim MJ, Sasai H, Maeda S, et al. (2009) Aerobic exercise training reduces epicardial fat in obese men. J Appl Physiol.; 106: 5–11.
44. Pitta F, Troosters T, Spruit MA, Probst VS, Decramer M, et al. (2005) Characteristics of physical activities in daily life in chronic obstructive pulmonary disease. Am J Respir Crit Care Med.; 171: 972–7.
45. Dayyasumberel M, Shimahaku M, Nishimura T, Ueno J, Takao S, et al. (2012) 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.; 11: 106.
46. Samaras K, Botello NK, Chinthim DJ, Lord RV. (2010) Subcutaneous and visceral adipose tissue FTO gene expression and adiposity, insulin action, glucose metabolism, and inflammatory adipokines in type 2 diabetes mellitus and in health. Obes Surg.; 20: 108–13.
47. Klimacikova E, Roussel B, Kovacikova Z, Kovalcikova M, Sildova-Vitkova M, et al. (2011) Macrophage gene expression is Related to obesity and the metabolic syndrome in human subcutaneous fat as well as in visceral fat. Diabetologia.; 54: 876–87.
48. Almendros I, Farré R, Planas AM, Torres M, Bonisigue MR, et al. (2011) Tissue oxygenation in brain, muscle, and fat in a rat model of sleep apnea: differential effect of obstructive apneas and intermittent hypoxia. Sleep.; 34: 1127–33.
49. Nakagawa Y, Kishida K, Kihara S, Yoshida R, Fumahashi T, et al. (2011) Nocturnal falls of adiponectin levels in sleep apnea with abdominal obesity and impact of hypoxia-induced dysregulated adiponectin production in obese murine mesenteric adipose tissue. J Atheroscler Thromb.; 18: 240–7.
50. Divo M, Cote C, de Torres JP, Casanova C, Marin JM, et al. (2012) Comorbidities and risk of mortality in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med.; 186: 155–61.