INTRODUCTION

Epicardial fat (EF) is metabolically active visceral adipose tissue positioned between the epicardial surface of the heart and the visceral surface of the pericardium. Recently, EF has emerged as a potential cardiovascular therapeutic target, having been shown to be an independent predictor of major cardiovascular adverse events (MACE) in the general population [1].

Previous studies have reported an association among epicardial fat volume (EFV), obstructive coronary artery disease [2], coronary artery calcium score (CACS) [3,4], and high-risk plaque measured on cardiac CT [5]. This relationship is thought to be a result of production of inflammatory mediators by EF and its...
proximity to the coronary arteries. Coronary microvascular dysfunction (CMD) is a precursor to coronary atherosclerosis and has been associated with an underlying inflammatory state [6,7]. Recent studies have described an association between EF and CMD in both healthy individuals and patients with metabolic syndrome [8,9]. However, it is unclear whether EF directly affects the coronary microcirculation prior to, and therefore in the absence of coronary atherosclerosis. Findings from other studies have so far been conflicting [10,11]. Understanding the impact of EF on coronary microcirculation is important as studies have now consistently linked CMD to MACE [12,13].

The purpose of this study was to investigate the relationship between EFV and CMD in patients with unobstructed coronary arteries on CT coronary angiogram (CTA), considering traditional cardiovascular risk factors and CACS.

**MATERIALS AND METHODS**

**Study design**

This was a prospective, cross-sectional, observational, single-centre study that recruited patients aged 30–80 years who were referred to cardiology clinics with chest pain suggestive of myocardial ischemia and subsequently underwent diagnostic CTA. Those with unobstructed coronaries on CTA were included in the study and underwent transthoracic echocardiography. Unobstructed coronary artery disease was defined as a quantitatively measured luminal stenosis <50%. Exclusion criteria were past history of ischemic heart disease and evidence of valvular heart disease, ≥50% luminal diameter narrowing, left ventricular hypertrophy, and left ventricular ejection fraction <55% on transthoracic echocardiography. The remaining patients underwent myocardial contrast echocardiography (MCE) to assess for CMD. We obtained information regarding cardiovascular risk factors [age, body mass index (BMI), hypertension, smoking status, and presence of diabetes]. Smoking status was inclusive of both current and past smokers. Blood samples were analysed for lipid profile (total cholesterol, low-density lipoprotein cholesterol, triglycerides, and high-density lipoprotein cholesterol).

**Calcium scoring and epicardial fat analysis**

This was performed using a 64-channel CT scanner (GE Lightspeed VCT; GE Medical Systems, Chicago, IL, USA). The mean heart rate during the scan was 64 beats/min. Calcium scoring and helical scanning were performed on all patients using prospective electrocardiograph (ECG) triggering at 75% of the R-R interval. Following this, a 20 mL bolus of contrast (Niopram 370®, Bracco, Milano, Italy) at 6 mL/s was injected, and the timing of peak contrast enhancement in the aortic arch was used to determine the timing of scan acquisition. The contrast-enhanced scan used 80 mL contrast injected at 6 mL/s, followed by a 50 mL at 6 mL/s saline flush, during a single expiration breath hold. Reconstructed CTA images were analysed on a dedicated 3-dimensional workstation (CardIQ Xpress; GE Medical Systems) with curved multiplanar reformation and short-axis cross-sectional viewing techniques. The CTA scan parameters were collimator 20 mm; slice thickness 0.625 mm; gantry rotation 350 ms; helical acquisition using a pitch of 0.16; tube current 455–515 mA with ECG tube current modulation; tube voltage range 100–140 kV; rotation time 350 ms. The estimated radiation dose per patient was 3.3 mSv. CACS was assessed using the Agatston et al. method [14]. Presence of calcium was defined as an Agatston score of 1 or greater. Epicardial adipose tissue was defined as adipose tissue surrounding the heart with pericardium as the outer boundary and attenuation from -195 Hounsfield units (HU) to -45 HU [15]. The cranial and caudal limits of EFV were defined based on the first CT slice showing the right pulmonary artery to the diaphragm. EFV was measured by manually tracing the pericardium every fourth slice in the regions of interest and then isolating from the intrathoracic structure according to overlying density (Fig. 1) [15,16]. Measurements were performed on non-contrast calcium score CT. The operators were blinded to clinical information. Intra-observer correlation for assessment of EFV ranged from R=+0.94 to R=+0.98.

**MCE study and analysis**

Patients underwent MCE after having avoided all caffeine-containing products, beta-blockers, nitrates, and calcium antagonists in the previous 24 hours. MCE was performed using ultrasound machine iE33 (Phillips Medical Systems, Veenpluis, Best, Netherlands) and SonoVue (Bracco Research SA, Milano, Italy) for constant contrast infusion. Real-time echocardiographic images were recorded within 3–4 minutes in the apical 4-, 2-, and 3-chamber views with low-power settings at a mechanical index of 0.1. SonoVue was commenced at 60 mL/h through peripheral intravenous access with a Vueject infusion syringe pump (Bracco Research SA). Thereafter, the rate was maintained between 48 and 60 mL/h to maximise image quality. Machine settings were constant throughout study post-optimisation. Flash-impulse imaging using a high mechanical index of 1.0 was performed to achieve complete myocardial bubble destruction, after which 10 end-systolic frames were recorded in each apical view. Dipyridamole was infused at 0.56 mg/kg over a 4-minute period after acquisition of resting images. Poststress images were recorded within 3 to 4 minutes after an interval of 2 minutes. The entire sequence took approximately 14 minutes [17]. Quantitative analysis was performed offline using QLab V7.0 (Philips Medical Systems) as previously described, by individuals experienced in the performance and analyses of MCE imaging and blinded to patient demographic and CTA.
data [18]. Quantitative assessment of myocardial perfusion was performed for 10 consecutive end-systolic frames after microbubble destruction by placing a region of interest over the thickness of the myocardium. Plots of peak myocardial contrast intensity (linearly related to myocardial blood volume $A \text{ cm}^3$) versus pulsing interval (representing time) were automatically constructed to fit the mono-exponential growth function $y=A (1-e^{-\beta t})$, where $\beta$ is the instantaneous initial slope of the resulting curve and represents myocardial blood velocity ($\text{sec}^{-1}$), and the product of $A$ and $\beta$ is a reliable measure of myocardial blood flow (MBF) ($\text{cm}^3\cdot\text{sec}^{-1}$). Myocardial blood flow reserve (MBFR) is the ratio of post-dipyridamole (stress) MBF to baseline MBF, dividing the stress MBF by the baseline MBF for the same segment. A 16-segment model was used, excluding the basal segments in view of contrast attenuation and analysing the 10 remaining mid- and apical cardiac segments (Fig. 2). A segment was excluded if there was artefact, inadequate microbubble destruction, attenuation, or a wide variation in contrast intensity. Segmental MBFR was calculated by dividing peak MBF with resting MBF of the same segment. MBFR was the average MBFR of all segments. CMD was defined as MBFR <2 [19].

**Statistical analysis**

Statistical analysis was performed using a proprietary statistical package (STATA version 14.1, Stata Corp., College Station, TX, USA). Data were organized and trends reported using standard descriptive statistics. For normally distributed data, the mean and standard deviation (SD) were reported, and for non-normal data, the median and inter-quartile range (IQR) were reported. For simple proportions, number and percentage were reported. Where appropriate, bivariate comparisons were performed using a t-test for normally distributed data and a Wilcoxon rank-sum test or a two-sample analysis of proportion for non-normal distributed data. More detailed inferential analysis was conducted using multiple regression with MBFR initially as a continuous dependent variable followed by MBFR as a dichotomous variable (MBFR $\geq 2$ or <2). For predictor variables when significant, the regression slope ($\beta$) or odds ratio

![Fig. 1. Quantification of epicardial fat volume using CT coronary angiography. (A) showing axial view of the heart at mid-ventricular level with arrows indicating the pericardium. (B) showing the tracing of the epicardial fat along the pericardium and (C) showing isolation of the cardiac tracing from the intrathoracic structure. (D) isolation of the epicardial fat using digital subtraction corresponding to a density of -195 to -45 Hounsfield units.](image)
CVIA
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(OR) and 95% confidence interval (CI) and associated p-value were reported. The level of significance was set at p<0.05 for all analyses.

Ethics
This study complied with the Declaration of Helsinki and was approved by the local research ethics committee (H0102/78). All patients provided written informed consent.

RESULTS

Baseline participant characteristics
The baseline characteristics for all patients are listed in Table 1. We recruited 183 patients initially, of which complete data for MCE, EFV, and CACS for 134 patients were available for assessment. Forty-nine patients were removed from EFV analysis due to CT system hardware corruption. We assessed significance in patient demographics between the patients analysed in this study and the patients removed as a result of data corruption. This analysis revealed a higher prevalence of diabetes in the patients analysed -8% vs. 21% (p=0.03). The mean age of the study population was 59.2 (9.8) years, of whom 49% were male, 7% were diabetics, and 37% had hypertension. Of the 134 patients, 43 (32%) had a CACS of 0, 64 (48%) had a CACS of 1–100, 18 (13%) had a CACS of 101–400, and 9 (7%) had a CACS of >400. The measured mean EFV was 128 mm³ (IQR, 96–168).

Characteristics of participants with coronary microvascular dysfunction
CMD was present in 54 (40%) patients, while 80 patients (60%) had no CMD (MBFR ≥2). Table 1 shows baseline characteristics of patients subdivided into those with CMD and without CMD. Participants with CMD were significantly older, had a higher BMI, and had a history of diabetes. We divided participants into 4 groups based on CACS: CACS 0, CACS 1–100, CACS 101–400, and CACS >400. Patients with CMD had significantly higher CACS (CACS >100) compared to those without CMD (Table 1), while a significant proportion of patients with

![Fig. 2. Myocardial blood flow analysis with regions of interest and time-intensity curves. Showing method used for quantitative analysis of myocardial segments: apical 4 chamber (A), apical 2 chamber (B), apical 3 chamber (C). Coloured software-constructed replenishment curves below each apical view correspond to each region of interest manually drawn.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>MBFR &lt;2 (n=54)</th>
<th>MBFR ≥2 (n=80)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.2±9.8</td>
<td>61.7±9.4</td>
<td>57.5±9.8</td>
<td>0.010</td>
</tr>
<tr>
<td>Male</td>
<td>66 (49)</td>
<td>27 (50)</td>
<td>39 (49)</td>
<td>0.510</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10 (7)</td>
<td>8 (15)</td>
<td>2 (3)</td>
<td>0.020</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5±3.8</td>
<td>28.5±0.5</td>
<td>26.9±0.4</td>
<td>0.020</td>
</tr>
<tr>
<td>Smoking history</td>
<td>57 (43)</td>
<td>26 (48)</td>
<td>31 (39)</td>
<td>0.180</td>
</tr>
<tr>
<td>Hypertension</td>
<td>50 (37)</td>
<td>24 (44)</td>
<td>26 (33)</td>
<td>0.110</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.3±1.10</td>
<td>5.15±0.17</td>
<td>5.33±0.12</td>
<td>0.380</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.1±1.00</td>
<td>3.02±0.17</td>
<td>3.24±0.11</td>
<td>0.250</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4 [1.2, 1.7]</td>
<td>1.44 [1.23, 1.71]</td>
<td>1.38 [1.09, 1.74]</td>
<td>0.650</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>1.3 [1.0, 1.8]</td>
<td>1.40 [1.00, 1.80]</td>
<td>1.30 [0.90, 1.80]</td>
<td>0.300</td>
</tr>
<tr>
<td>Epicardial fat volume (mm³)</td>
<td>128 [96, 168]</td>
<td>147 [111, 178]</td>
<td>118 [92, 155]</td>
<td>0.010</td>
</tr>
<tr>
<td>CACS (HU)</td>
<td>43 (32)</td>
<td>11 (20)</td>
<td>32 (40)</td>
<td>0.010</td>
</tr>
<tr>
<td>1–100</td>
<td>64 (48)</td>
<td>23 (43)</td>
<td>41 (51)</td>
<td>0.360</td>
</tr>
<tr>
<td>101–400</td>
<td>18 (13)</td>
<td>13 (24)</td>
<td>5 (6)</td>
<td>0.003</td>
</tr>
<tr>
<td>&gt;400</td>
<td>9 (7)</td>
<td>7 (13)</td>
<td>2 (3)</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Values are given as mean±standard deviation, median [interquartile range], or n (%). MBFR: myocardial blood flow reserve, BMI: body mass index, LDL: low-density lipoprotein, HDL: high-density lipoprotein, CACS: coronary artery calcium score, HU: Hounsfield units
no CMD had a CACS of 0 (p=0.01). EFV was significantly higher in the group with CMD than in those with no CMD (p=0.01).

**Relationship between epicardial fat volume and coronary microvascular dysfunction**

In univariate regression analysis, EFV and MBFR had a correlation coefficient of R=−0.22, with significant regression slope (β=−0.002, p=0.012) but a poor model fit (R²=0.04). Table 2 lists the explanatory variables used in the regression analysis. The initial β and p-values represent the fully saturated multivariable model using the listed variables, while the final β and p-values in the final model contain only the corresponding significant predictors. Multivariable linear regression analysis using MBFR as a continuous outcome variable revealed only age (β=−0.012, p=0.011) and CACS (β=−0.0003, p=0.023) to be associated with MBFR. Subsequent multiple logistic regression analysis using a cut off MBFR of <2 or ≥2 showed that CACS (β=−0.0003, p=0.004) was independently associated with impaired MBFR as shown in Table 3.

**DISCUSSION**

This study evaluated the association among EFV, CACS, and MBFR in a cohort of patients presenting with chest pain and unobstructed coronary arteries. Although increasing EFV was negatively associated with MBFR in univariate analysis, it was not associated with MBFR after consideration of traditional cardiovascular risk factors and calcium score. This suggests that previous apparent associations between EFV and CMD may have been confounded by the presence of other factors known to be associated with microvascular function.

EF is a biologically active fat depot surrounding coronary arteries. Some studies have shown it to be associated with severity of coronary artery disease [20]. This is thought to occur through production of pro-inflammatory and pro-atherogenic mediators by EF that influence vascular wall composition and tone [21]. Given the close anatomical proximity between EF and coronary vasculature as well as their shared microcirculation, there is an ongoing interest in the role of EF as a pathophysiological culprit in the development of CMD. Importantly, the prognosis of patients with CMD is not benign. There is growing evidence that CMD is associated not only with higher incidence of MACE and cardiac mortality [22], but also worsening of anginal symptoms leading to impaired quality of life [23].

Previous studies investigating EFV and CMD have yielded conflicting results. In a pilot study involving a small cohort of healthy volunteers, Gaborit et al. [9] showed an independent association between EFV and MBF measured by cold pressor testing. Compared to our study, these healthy volunteers were free of traditional cardiovascular risk factors. In a separate study of patients with metabolic syndrome, EF thickness was measured by transthoracic echocardiography, and CMD was determined by measurement of peak diastolic flow velocities in the left anterior descending coronary artery. Although EF thickness was shown to be an independent predictor of CMD, contrary to our study presence or absence of CAD was not determined, and a functional study was not performed to rule out ischemia [8]. More recently, in a retrospective study, Otaki et al. [24] showed a correlation between impaired myocardial flow reserve and indexed EFV measured with non-contrast CT in a group of 85 patients without known coronary artery disease who initially underwent rest-stress Rb-82 positron emission tomography (PET)/CT and subsequent invasive coronary angiography. That study may have been subject to selection bias as these patients were selected based on need for invasive coronary an-
giography and included patients with epicardial artery stenosis [24]. Therefore, the presence of severe epicardial artery stenosis rather than CMD could have contributed to the lower diastolic peak velocities and abnormal perfusion found on PET. In a cohort of patients with non-obstructive coronary artery disease, Alam et al. [10] showed an association between EF thickness and volume with impaired myocardial flow reserve measured by PET. However, quantification of EF thickness at a single point has significant limitations as it may not accurately represent the total EFV [25]. More recently, in agreement with the findings from the studies above, Nappi et al. [26] showed a link between reduced myocardial perfusion reserve and adipose tissue volume measured in 270 patients with suspected CAD but normal myocardial perfusion. However, in contrast to these studies but in agreement with our findings, Bakkum et al. [11] failed to show any association between EFV measured by non-contrast CT and CMD in a cohort of 208 obese patients with chest pain and non-obstructive coronary artery disease. Similarly, Brinkley et al. [27] also failed to show an association between EF and coronary vasoreactivity in a group of asymptomatic patients with no prior cardiovascular disease. In a larger cohort of patients (n=380) with known or suspected CAD, Tanami et al. also failed to find any association between EFV measured by CT with presence and severity of CAD or with myocardial perfusion abnormalities.

Our study also showed that a greater CACS score was linked to CMD independent of traditional cardiovascular risk factors in patients with non-obstructive CAD, which accords with previous studies [28,29]. The presence of CMD and coronary calcification reflect different stages of atherosclerosis. It has been hypothesized that loss of coronary vasoreactivity predisposes to coronary endothelial damage via shear stress through increased blood velocity and hence potentiation of vascular injury with subsequent formation of coronary atheroma [30].

**Study limitations**

There are certain limitations to this study that must be considered. First, this was a single-centre study, patients were recruited based on referral for CTA, and there was potential for selection bias. Second, a significant number of patients were excluded due to data corruption, which could have introduced additional bias. However, on comparison of patient demographics, the only significant difference was a higher incidence of diabetes in patients who were excluded, with similar MBFR and CACS in the groups.

**Conclusion**

Although increasing EFV was associated with MBFR in univariate analysis, this was not the case when traditional cardiovascular risk factors and calcium score were considered. This suggests that previous studies reporting associations between EFV and CMD may have been confounded by other factors known to be associated with microvascular function.

**Conflicts of Interest**

The authors have no potential conflicts of interest to disclose.

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**REFERENCES**


