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Relationship between Epicardial Adipose Tissue and Coronary Vascular Function in Patients with Normal Myocardial Perfusion by ^{82}Rb PET/TC

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INTRODUCTION

Coronary flow reserve (CFR) is a powerful marker of the myocardial capacity to adapt blood supply to an increased myocardial work (1). A reduction CFR reflects not only the presence of coronary artery stenosis but also coronary vascular dysfunction (2).

Epicardial adipose tissue (EAT) defined as the adipose tissue located between the myocardium and pericardium, is the visceral fat storehouse of the heart and it is a metabolically active organ that generates a variety of bioactive molecules, which could significantly affect cardiac function (3). Indeed, EAT coats the coronary arteries without any intervening fascial barrier, thus sharing the same perfusion of the heart (4). The close anatomic relationship of epicardial adipose tissue to the adjacent myocardium may imply paracrine regulation by this small fat depot, although the relationship could not exclude also a systemic control (3).

Moreover, compared to other fat depots, EAT shows own unique characteristics such as higher density of smaller adipocytes suggesting specific metabolic properties with a greater rate of release of free fatty acids (5,6).

In addition, it has been demonstrated that EAT correlates with cardiac sympathetic denervation and it represents a source of catecholamine

production suggesting a potential role of EAT in cardiac response to sympathetic stimuli (7).

More recently, literature has proved that fat quantification may have prognostic implications in prediction of adverse cardiovascular events (8, 9). Despite a few evidences of a direct correlation between increased EAT and impaired CFR in small patients' population with suspected or known coronary artery disease (CAD) (10-12), the association between EAT and CFR parameters remains to be fully investigated.

AIM OF THE STUDY

We aimed to investigate the relationship between EAT and CFR in patients with suspected or known CAD referred to stress-rest ^{82}Rb PET/CT imaging showing normal myocardial perfusion imaging (MPI).

METHODS

Patients

For the purpose of the present investigation, we considered consecutive patients with normal stress-rest ^{82}Rb PET/CT perfusion selected from those referred to our Institution (Department of Advanced Biomedical Sciences, University Federico II, Naples, Italy) from December 2013 to June 2017 for the evaluation of suspected or known CAD as part of their diagnostic and treatment monitoring program.

As part of the baseline examination, clinical teams collected information on traditional cardiovascular risk factors including age, sex, hypertension, diabetes, hypercholesterolemia, smoking, family history of CAD, chest pain symptoms, and documented history of myocardial infarction and revascularization procedures

Hypertension was defined as a blood pressure >140/90 mmHg or the use of anti-hypertensive medication (13). Patients were classified as having diabetes if they were receiving treatment with oral hypoglycaemic drugs or insulin. Hypercholesterolemia was defined as total cholesterol level > 6.2 mmol/L or treatment with cholesterol lowering medication. A positive family history of CAD was defined by the presence of disease in first-degree relatives younger than 55 years in men or 65 years in women. Patients with atrial fibrillation, pacemaker

or prosthetic valve, severe loss of renal function, symptomatic asthma, and pregnancy were excluded.

The review committee of our institution approved the study, and all patients gave informed consent to the protocol.

PET/CT imaging protocol

As a routine preparation for ^{82}Rb cardiac PET/CT, patients were asked to discontinue taking nitrates for 6 hours, calcium channel blockers and caffeine-containing beverages for 24 hours, and b-blockers for 48 hours before their appointment.

Scans were acquired using a Discovery LS scanner (GE). Rest and stress cardiac PET/CT images were acquired as follows (14). Following a CT scout acquisition (120 kVp, 10 mA) for patient positioning, a CT transmission scan was acquired (140 kVp, 80 mA) for subsequent attenuation correction and for CAC scoring and EAT quantification. Rest and stress CT transmission scans were acquired at end-expiration breath-hold, and patients were instructed to breath normally during the PET acquisition. For both rest and stress, imaging 1110 MBq of ^{82}Rb was injected intravenously and a 6-min PET study was acquired. Pharmacologic stress was then induced by administration of dipyridamole (0.142 mg/kg/min for 4 min). Both rest and stress dynamic images were reconstructed into 20 time frames (12 × 8 s, 5 × 12 s, 1 × 30 s, 1 × 60 s and 1 × 60 s) using attenuation-weighted ordered-

subsets expectation maximization (two iterations, 24 subsets). CT-based attenuation, scatter, decay, and random corrections were applied to the reconstructed images. The heart rate, systemic blood pressure, and 12- lead ECG were recorded at baseline and throughout the infusion of dipyridamole. The rate–pressure product was calculated as heart rate multiplied by systolic blood pressure. Transaxial PET perfusion images were automatically reoriented into short-axis and vertical and horizontal long axis slices Regional myocardial perfusion was visually assessed, using standardized segmentation of 17 myocardial regions. Each myocardial segment was scored from normal (score = 0) to absent perfusion (score = 4). The summed stress score was obtained by adding the scores of the 17 segments of the stress images. A similar procedure was applied to the resting images to calculate the summed rest score and summed difference score was the difference between the stress and rest scores. Myocardial perfusion was considered abnormal when the summed stress score was ≥3 (15). Absolute MBF (in ml/min/gr) was computed from the dynamic rest and stress imaging series with commercially available software (Flow Quant, University of Ottawa Heart Institute). CFR was defined as the ratio of hyperaemic to baseline MBF and it was considered reduced when <2 (16). rate-pressure-product (RPP)-corrected CFR was calculated using baseline MBF corrected for RPP.

Coronary calcium score

For CAC scoring, rest CT axial reconstructions were transferred to a dedicated workstation (Vitrea Workstation, Toshiba Medical Systems, Tokyo, Japan) for post processing and subsequent analysis. Coronary calcification was defined as a plaque with an area of 1.03 mm^2 and a density ≥130 HU. CAC scores were calculated according to the method described by Agatston et al. (17). Experienced nuclear medicine physicians and radiologists analysed the CT studies, blinded to the PET results. CAC scores were calculated separately for calculated separately for the for the common trunk, left anterior descending, left circumflex and right coronary arteries and summed to provide a total CAC score.

Epicardial Adipose Tissue quantification

Epicardial adipose tissue (EAT) volume was quantified on non-contrast CT images, by Osirix software. The image processing started at the level of the pulmonary trunk and ended at the level of the inferior diaphragmatic surface of the heart to manually trace pericardial borders. The area outside the traced pericardium was excluded.

The range of attenuation for EAT segmentation was then set between −30 and −190 HU. This effectively excluded myocardium, coronary arteries, coronary calcium, the aorta, and blood pool as shown. Finally, images were checked and reviewed by operators to correct potential

errors and total EAT volume was calculated (18, 19). On unenhanced CT images, EAT thickness (mm) measurements were also performed along the right ventricular anterior free wall in a single sagittal slice having the greatest EAT thickness (12).

Statistical Analysis

Continuous variables are expressed as mean ± standard deviation (SD) and compared by the use of unpaired t-test. CAC scores and EAT volume were adjusted using the ln (CAC + 1) and ln (EAT) transformations to reduce the rightward skewness of the data and heteroscedasticity respectively.

Categorical variables are expressed as proportion and compared by use of chi-square test.

Patients were divided in to three groups according to tertile of EAT low-tertile (<86 cm^3); middle-tertile (≥86 cm^3 and <124 cm^3) and high-tertile (≥124 cm^3) respectively. Differences in hyperemic MBF and CFR across levels of EAT categories were assessed using ANOVA. A P value <0.05 was considered statistically significant.

Univariable and multivariable linear regression analyses were performed to examine the relationship between age, gender, cardiac risk factors, ln (CAC + 1) score, ln (EAT) volume and hyperemic MBF and CFR. We considered for the multivariable analysis only variables statistically significant at univariable analysis.

Univariable and multivariable logistic regression analyses were also performed to identify the variables associated with a reduced CFR.

The incremental value of imaging findings over clinical data in predicting reduced CFR was assessed considering variables in

hierarchical order. A P value <0.05 was considered statistically significant. Finally, the relationship between of EAT volume and CFR was evaluated by using Pearson coefficient.

All the analyses were performed using STATA version 14.0 for Windows (StataCorp LP, College Station, TX).

RESULTS

Between December 2013 to June 2017, 510 patients with suspected or known CAD were referred to our Institution to perform stress-rest ^{82}Rb cardiac PET/CT for the evaluation of suspected or known CAD as part of their diagnostic program. From the overall population, 240 patients showed abnormal perfusion and therefore they were excluded from the final analysis.

Baseline characteristics of the final population are described in Table 1. As illustrated, among 270 patients with normal MPI, 93 (35%) patients showed reduced CFR and 177 (65%) normal CFR. Compared to patients with normal CFR, those with impaired CFR were older ($P<0.05$). No other clinical differences were observed.

Imaging findings in the overall population are described in Table 2. Compared to patients with normal CFR, those with impaired CFR showed higher values of ln (CAC+1), $P<0.05$), EAT thickness ($P<0.01$) and ln (EAT) volume ($P<0.001$). Patients with reduced CFR did not show statistical difference of baseline MBF mean values as compared to those with normal CFR. However, they demonstrated a blunted response to pharmacological stressor ($P <0.001$).

Linear regression analyses considering hyperemic MBF as dependent variable are shown in Table 3. As shown, while age, sex, diabetes, hyperlipidemia, ln (CAC+1) and ln (EAT) volume were significantly

associated with hyperemic MBF at univariable analysis, at multivariable analysis only sex and ln (EAT) volume were inversely related with hyperemic MBF (β coefficient: -0.337 and -0.221; standard error 0.091 and 0.088; respectively, all P<0.005).

The results of logistic regression analyses with reduced CFR as dependent variable are depicted in Table 4. As presented, although age, ln (CAC+1) and ln (EAT) volume resulted significant predictors of reduced CFR, at multivariable analysis, only age and ln (EAT) volume were independently associated with reduced CFR (hazard ratio 1.055 and 1.913 and 95% confidence interval 1.025-1.086 and 1.021-3.585 respectively, all P<0.005).

Interestingly, although body mass index (BMI) was significantly related to ln (EAT) volume (r= 0.192 P value <0.005), this parameter did not result a predictor neither of CFR nor of hyperemic MBF.

Accordingly, at incremental analysis, while a model including clinical data and ln (CAC+1) score did not show significant difference as compared to a model including only clinical data (global chi-square from 23.3 to 23.5, P=0.65), the addition of ln (EAT) volume to clinical data significantly increased the global chi-square of the model (from 23.3 to 28.5, P<0.05) demonstrating incremental power of EAT in predicting reduced CFR (Figure 1).

To test the relationship of EAT with CFR and with hyperemic MBF, patients were divided in to three groups according to tertile of EAT: low-tertile (n= 90); middle-tertile (n= 90) and high-tertile (n= 90) respectively and global hyperemic MBF and CFR scatter plots in relation to EAT tertile groups are illustrated in Figure 2. As shown both global hyperemic MBF (2A) and CFR (2B) decreased with increasing EAT amount (P value for trend <0.05 in 2A and <0.001 in 2B).

In addition, to assess a potential correlation between CAC score and EAT, patients were considered according to evidence of measurable CAC. The correlation of EAT volume and CFR in relation to documented CAC is represented in Figure 3. As shown, a significant correlation was observed between ln (EAT) volume and CFR ($r = -.229$, P value <0.05) in patients with CAC score =0 (n=114) while such a relationship was not significant in patients with documented CAC (n=156) ($r = -.101$, P value N.S.) suggesting that EAT may have a stronger role in predicting reduced CFR in patients without coronary artery calcium load.

Representative examples of similar age patients with normal MPI and different EAT volume values according to CFR results are depicted in Figure 4.

DISCUSSION

It has been demonstrated that CFR provides independent prognostic power for outcome prediction in patients with suspected and known CAD (1,20). Therefore, there is great interest in early identification of reliable markers of impaired CFR. To our knowledge, this is the first study investigating the relationship between not only CFR but also hyperemic MBF and EAT in a large population of patients with suspected and known CAD and normal MPI.

The studied population only included patients with normal MPI. Indeed, we hypothesized that a potential relationship of EAT and coronary vascular function may have stronger diagnostic implications at initial stage of coronary vascular impairment, allowing early therapeutic interventions.

In our population, CFR and hyperemic MBF were independently associated with

the two unchangeable cardiac risk factors, by means age and sex, and with ln(EAT) volume. However, this latter variable demonstrates incremental power in predicting coronary vascular dysfunction over clinical data and documented coronary calcium burden.

Cardiac fat depot can be measured with different modalities and parameters. While the evaluation of EAT thickness on echocardiography shows good reproducibility but it is affected by the

lack of information about the total epicardial fat load, cardiac CT and magnetic resonance, allow more accurate volumetric quantification with higher reproducibility (21). Therefore, although both ln (EAT) volume and EAT thickness resulted predictors of reduced CFR and hyperemic MBF, we only included ln (EAT) volume in multivariable models.

Our findings are substantially in agreement with few previous studies including smaller populations with different clinical characteristics (10,12).

Otaki and coworkers (9) demonstrated that EAT volume is a significant predictor of reduced CFR in a population of 85 patients with suspected CAD referred to invasive coronary angiography on the basis of stress-rest cardiac ^{82}RB PET/CT perfusion results. Moreover, Alam et al (12) found, in a smaller cohort of patients only without obstructive CAD and normal MPI, an inverse relationship between EAT thickness and CFR

Our results, obtained in a larger size population, may suggest to extend clinical investigations regarding both CFR and hyperemic MBF from patients with suspected CAD and abnormal perfusion to those with suspected or known CAD and normal MPI.

Noteworthy, in our study, even if CAC score resulted a predictor of reduced CFR, in a multivariable model including clinical data and EAT volume, it did not show an independent association with impaired

coronary vascular function. As described, while a significant correlation was observed between ln (EAT) volume and CFR in patients without coronary calcium, such a relationship was not significant in patients with documented CAC suggesting that EAT may contribute to development of coronary vascular dysfunction before coronary artery calcium accumulation.

The emerging concept that EAT evaluation may have not only a role in identification of reduced CFR but it could also be considered a prognostic tool and a therapeutic target, has been supported by recent literature (8,22-24). Indeed, different studies highlighted the potential interplay between inflammatory stimuli released from epicardial adipose tissue and CFR before clinical manifestations of CAD (22-28). Recently, Goeller et al (22) found that increased EAT volume was associated with presence of coronary calcification, serum levels of plaque inflammatory markers as well as with major cardiac events, suggesting that dysfunctional EAT may be linked to early plaque development inflammation and adverse cardiac outcome regardless to coronary calcium evidence. A positive correlation between EAT volume index, the degree of myocardial fibrosis, and LV systolic function impairment was also found in 40 volunteers without diabetes mellitus or hypertension (29).

Interestingly, we found that although BMI was significantly related with EAT volume, it was not associated with CFR. Similarly, Ding et al. (25) found that neither BMI nor waist circumference were associated with calcified coronary plaque despite their being significantly correlated with fat depot. Whilst, Taguchi and coworkers (28) observed that pericardial fat accumulation is more closely associated with CAD morbidity than any other body fat distribution. Thus, our results in agreement with previous studies (27-30), support the growing idea that anthropometric measures of adiposity such as BMI and body surface area (BSA) may not fully reflect the cardiovascular risk associated to obesity.

As previously suggested by Iacobellis et al. (31), the contiguity of EAT to the myocardium and coronary arteries, allows local paracrine interactions, rendering EAT most likely a stronger correlate to left ventricular (LV) mass than general measures of adiposity such as BMI. Furthermore, there is growing evidence that EAT is a metabolically active organ and an important source of pro- and anti-inflammatory mediators and cytokines (32) such as tumour necrosis factor-α, leptin and interleukins 6 and 17, which are believed to exert local effects on the underlying coronaries and myocardium. Indeed, these inflammatory markers have been implicated in the pathogenesis of LV remodelling

and further highlight the importance of EAT as an important and early marker for cardiovascular risk.

Therefore, in the light of large body literature and according to our results, the perspective of specifically targeting the cardiac fat depot for more effective CAD prevention seems very promising.

It remains to be estabilished whether any changes in EAT profile could be the result, rather than the cause, of underlying cardiac disease. Indeed, as suggested by Antonopoulos et al. (33) a reverse (inside-out) signaling from the heart to EAT may lead to changes in the EAT phenotype and secretory profile to protect the heart from oxidative damage. Therefore, a deeper understanding of cardiac fat role as heart caretaker rather than cardiac damage trigger could have strong therapeutic impact.

Some limitations of our study should be acknowledged. First, our study was a single center investigation.

Moreover, 38% of studied patients have history of previous revascularization. This characteristic could represent a limitation in the assessment of relation between coronary calcium burden and EAT for the understanding of different contribution of each variable in the coronary vascular dysfunction development. However, as described, a significant correlation was observed between ln (EAT) volume and CFR

in patients without evidence of coronary calcium, suggesting that EAT may have a stronger role in coronary vascular dysfunction onset before coronary artery calcium accumulation.

Yet, it has been demonstrated that not only the EAT volume but also the EAT density has prognostic implication in prediction of cardiac events (22). Though, EAT density data were not available for the studied population. Therefore, in further studies it could be interesting to investigate the relationship of EAT density and coronary vascular function, as well as outcome.

CONCLUSION

In patients with suspected or known CAD and normal MPI EAT is strongly associated with reduced CFR and hyperemic MBF confirming that visceral fat depot may directly influence coronary vascular function.

Thus, EAT evaluation may play a major role in the identification of coronary vascular dysfunction even in patients with normal MPI. In particular, it could be useful to consider EAT in patients with to no evidence of coronary calcium burden.

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FIGURE LEGEND

Figure 1 Incremental analysis for prediction of reduced coronary flow reserve (CFR). A model including clinical data and ln (CAC+1) score did not show significant difference as compared to a model including only clinical data (global chi-square from 23.3 to 23.5, P=0.65) did not show. Conversely the addition of ln (EAT) volume to clinical data significantly increased the global chi-square of the model (from 23.3 to 28.5, P<0.05).

Figure 2 Global hyperemic myocardial blood flow (MBF) (A) and coronary flow reserve (CFR) (2B) scatter plots in relation to epicardial adipose tissue (EAT) tertile groups (P for trend <0.05 in A and <0.001 in B).

Figure 3 Correlation between ln (EAT) volume and coronary flow reserve (CFR) values in patients with CAC score =0 (dark navy dots and line), (r= −0.229, P<0.05) and in patients with CAC score > 0 (maroon dots and line), (r= −0.101 P=N.S.).

Figure 4 Representative examples of patients with similar age and normal MPI with different EAT volume values according to CFR results. The patient described in panel A shows low EAT volume and normal CFR. Conversely, as illustrated in panel B, high values of EAT volume were consistent with reduced CFR.

Table 1

Baseline characteristics of patients according to CFR results

	All Patients (n=270)	Reduced CFR (n=93)	Normal CFR (n=177)	P value
Age (years)	62.31±11.1	66.66±9.1	60.03±11.4	<0.001
Male gender, n (%)	188 (69)	66 (71)	121 (68)	0.659
Body mass index (kg/m^2)	28.22±5.8	28.76±4.3	27.94±6.3	0.266
Diabetes, n (%)	77 (29)	27 (29)	50 (28)	0.892
Hypertension, n (%)	231 (86)	81 (87)	150 (85)	0.602
Hypercholesterolemia, n (%)	192 (71)	72 (77)	120 (68)	0.097
Smoking history, n (%)	121 (45)	40 (43)	81 (46)	0.666
Family history of CAD, n (%)	149 (55)	56 (60)	93 (53)	0.228
Previous revascularization, n (%)	104 (38)	40 (43)	64 (36)	0.272
Previous myocardial infarction, n (%)	75 (28)	26 (27)	49 (28)	0.962

Values are expressed as mean value ± standard deviation or as number (percentage) of subjects. CFR: coronary flow reserve; CAD: coronary artery disease.

Table 2

Imaging test results according to CFR results

	All Patients (n=270)	Reduced CFR (n=93)	Normal CFR (n=177)	P value
ln (CAC+1)	4.15±2.98	4.67±2.94	3.88±2.97	<0.05
EAT thickness (mm)	6.14±2.24	6.63±2.39	5.89±2.12	<0.01
ln (EAT) volume	4.60±0.48	4.73±0.41	4.52±0.50	<0.001
Rest MBF (mL/min/g)	0.79 ±0.26	0.83±0.30	0.78±0.24	0.063
Hyperemic MBF (mL/min/g)	1.81±0.69	1.29±0.53	2.08±0.61	<0.001

Values are expressed as mean value ± standard deviation or as number (percentage) of subjects. CFR: coronary flow reserve; CAD: coronary artery disease; EAT: epicardial adipose tissue; MBF: myocardial blood flow

Table 3

Linear regression analyses with hyperaemic MBF as dependent variable

	Univariable analysis			Multivariable analysis		
	b coefficient	SE	P value	b coefficient	SE	P value
Age	-.012	.002	.004	-.004	.004	.235
Male gender	-.442	.088	.000	-.337	.091	.000
Body mass index	-.011	.007	.132			
Diabetes	-.195	.093	.036	-.058	.091	.526
Hypertension	.006	.120	.957			
Hypercholesterolemia	-.239	.092	.010	-.116	.091	.206
Smoking history	-.102	.073	.232			
Family history of CAD	.091	.085	.286			
Previous revascularization	-.155	.086	.074			
Previous myocardial infarction	-.078	.094	.407			
ln (CAC+1)	-.054	.014	.000	-.018	.015	.235
EAT thickness	-.054	.019	.004			
ln EAT volume	-.359	.085	.000	-.221	.088	.013

SE: standard error; MBF: myocardial blood flow; CAD: coronary artery disease; CAC: coronary artery calcium EAT: epicardial adipose tissue;

Table 4

Logistic regression analyses with reduced CFR as dependent variable

	Univariable analysis		**Multivariable analysis**	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	1.064 (1.036-1.093)	<0.001	1.055 (1.025-1.086)	<0.001
Male gender	0.884 (511.-1.530)	0.659		
Body mass index	1.025 (.980-1.071)	0.288		
Diabetes	1.039 (.597-1.809)	0.893		
Hypertension	1.215 (.585-2.526)	0.602		
Hypercholesterolemia	1.629 (.912-2.907)	0.099		
Smoking history	.894 (.539-1.484)	0.666		
Family history of CAD	1.367(.821-2.275)	0.229		
Previous revascularization	1.333 (.798-2.224)	0.272		
Previous myocardial infarction	1.205(.725-2.004)	0.472		
ln (CAC+1)	1.094(1.004-1.191)	0.039	1.012 (.921-1.112)	0.802
EAT thickness	1.160 (1.036-1.300)	0.01		
ln EAT volume	2.690 (1.472-4.915)	0.001	1.913 (1.021-3.585)	<0.05

CAD: coronary artery disease; CAC: coronary calcium score; EAT epicardial adipose tissue

Figure 1

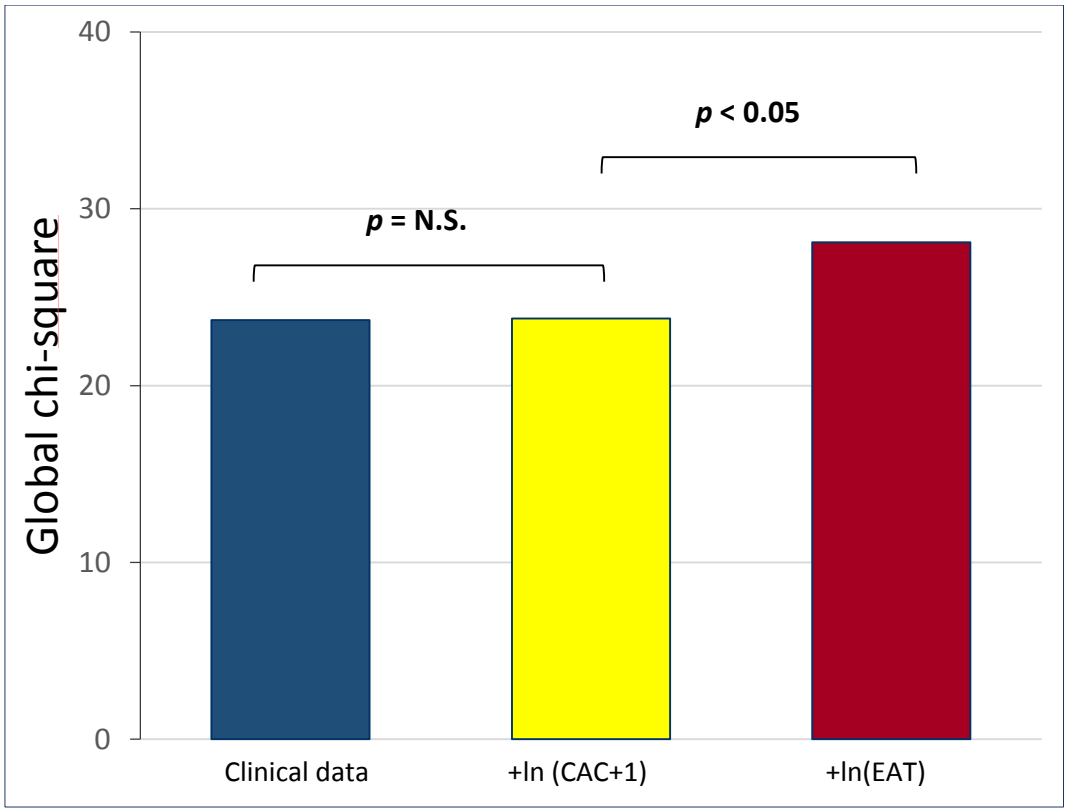
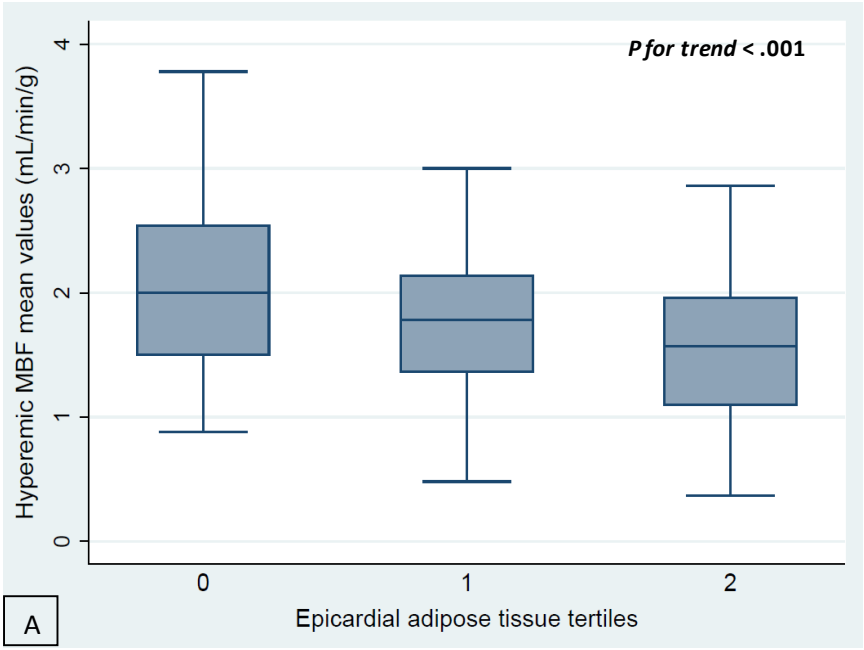

40
30
20
10
0
Global chi-square
p < 0.05
p = N.S.
Clinical data
+ln (CAC+1)
+ln(EAT)

Figure 2


P for trend < .001
Hyperemic MBF mean values (mL/min/g)
0
1
2
3
4
0
1
2
Epicardial adipose tissue tertiles
A

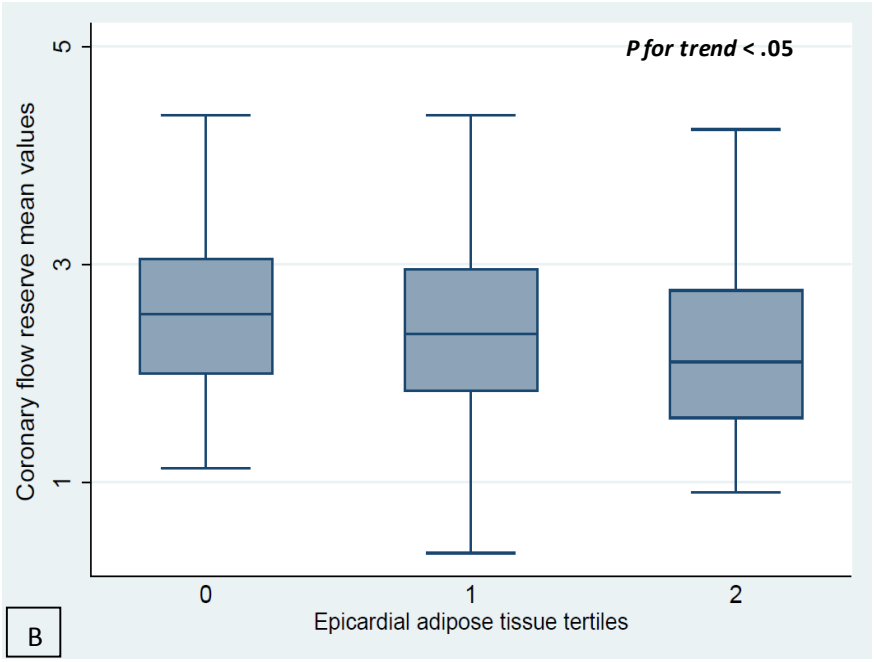

P for trend < .05
Coronary flow reserve mean values
1
3
5
0
1
2
Epicardial adipose tissue tertiles
B

Figure 3

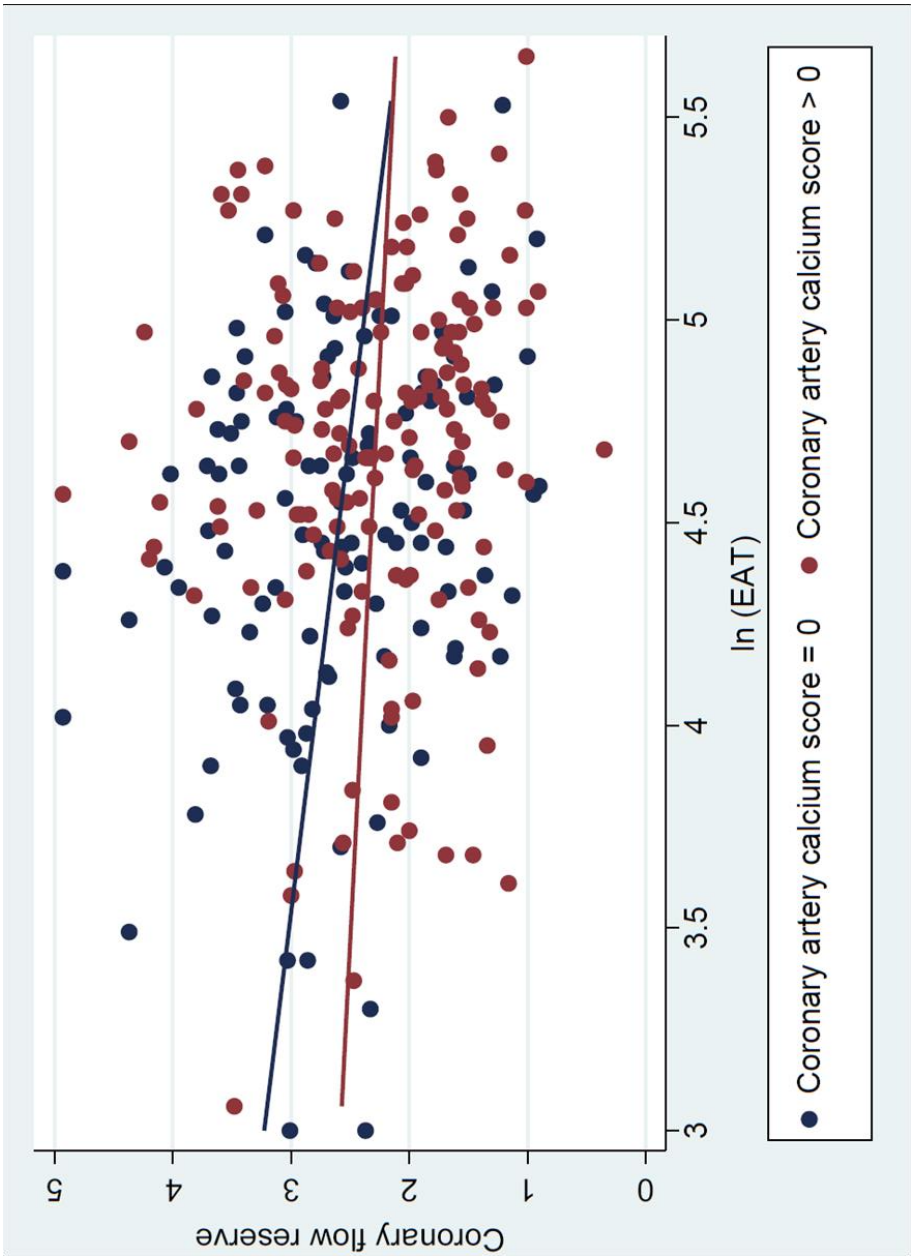
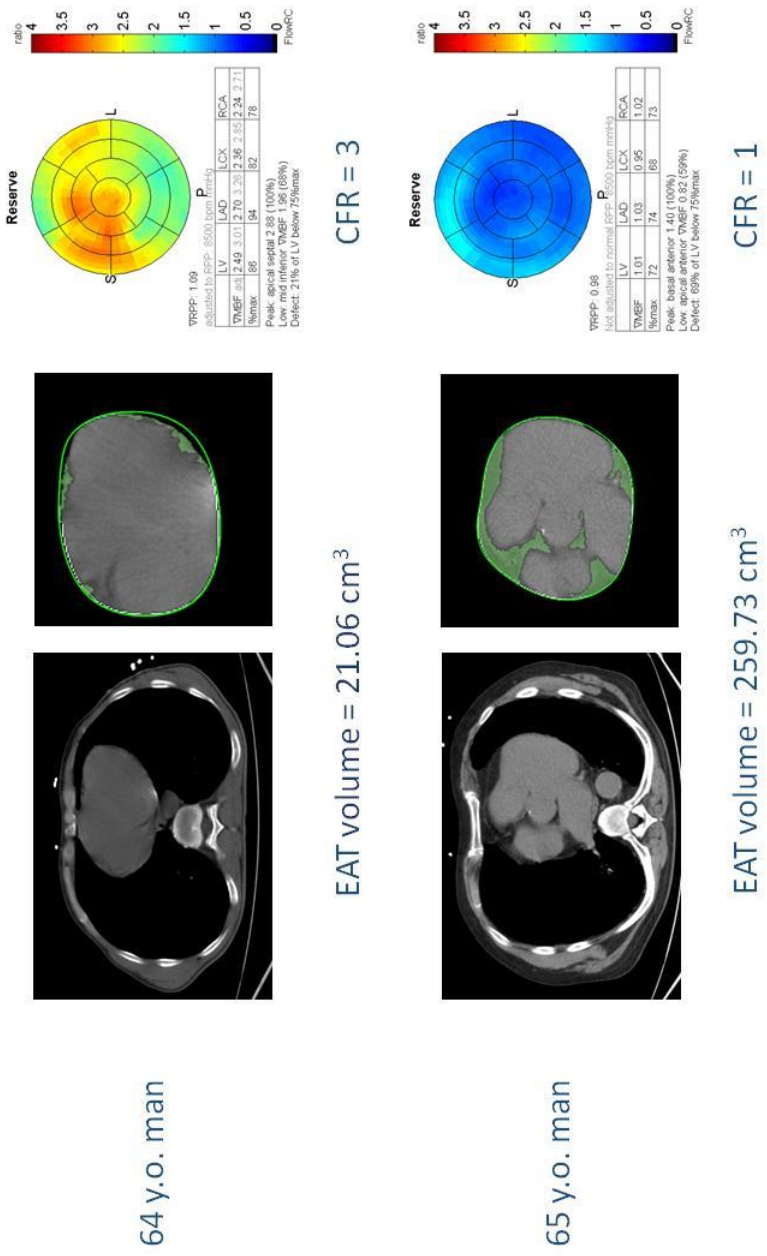

Coronary flow reserve
0
1
2
3
4
5
3
3.5
4
4.5
5
5.5
ln (EAT)
Coronary artery calcium score = 0
Coronary artery calcium score > 0

Figure 4


Reserve
ratio
4
3.5
3
2.5
2
1.5
1
0.5
0
FlowRC
L
S
RPP: 1.69
adjusted to RPP: 8500 bpm.mmHg
LV
LAD
LCX
RCA
MBF adj
2.49
3.01
2.70
3.26
2.36
2.85
2.24
2.71
%max
86
94
82
78
Peak: apical septal 2.93 (100%)
Low: mid inferior MBF 1.96 (68%)
Defect: 21% of LV below 75%max
CFR = 3
EAT volume = 21.06 cm3
64 y.o. man
Reserve
ratio
4
3.5
3
2.5
2
1.5
1
0.5
0
FlowRC
L
S
RPP: 0.98
Not adjusted to normal RPP: 8500 bpm.mmHg
LV
LAD
LCX
RCA
MBF
1.01
1.03
0.95
1.02
%max
72
74
68
73
Peak: basal anterior 1.40 (100%)
Low: apical anterior MBF 0.82 (59%)
Defect: 69% of LV below 75%max
CFR = 1
EAT volume = 259.73 cm3
65 y.o. man