

EPS and Arrhythmia

# Intrinsic Cardiac Autonomic Ganglionated Plexi within Epicardial Fats Modulate the Atrial Substrate Remodeling: Experiences with Atrial Fibrillation Patients Receiving Catheter Ablation

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**Background:** A recent study reported the close relationship between high dominant frequent (DF) sites [atrial fibrillation (AF) nest] and the intrinsic cardiac autonomic nervous system. The aim of this study was to investigate the correlation between the regional distribution of epicardial fat and the properties of the biatrial substrates in AF patients.

**Methods:** We studied 32 patients with paroxysmal (n = 23) and persistent (n = 9) AF. The epicardial fat volume around the left atrium (LA) was evaluated using 64-slice multidetector computed tomography and the topographic distribution of the fat volume was assessed. The biatrial DFs, voltages, and total activation times (TATs) were obtained during sinus rhythm.

**Results:** Out of the 8 divided LA regions, a significant linear correlation existed between the LA fat and mean DF values in the right upper anterior LA, left upper anterior LA, right lower anterior LA, right upper posterior LA, left upper posterior LA, and left lower posterior LA. There was no significant correlation between the regional LA fat distribution and regional LA peak-to-peak bipolar voltage and TAT. During a mean follow-up of  $17 \pm 8$  months, 22 of the 32 (69%) patients were free of AF. In the multivariate analysis, only the mean LA DF was found to be a significant predictor of recurrence.

**Conclusions:** There was a close association between the regional distribution of the LA epicardial fat and the atrial substrate manifesting high frequency during sinus rhythm (AF nest). Those nests were related to ablation outcome. Hence, epicardial fat may play a significant role in atrial substrate remodeling and thereby in the pathogenesis and maintenance of AF.

**Key Words:** Atrial fibrillation • Dominant frequency • Epicardial fat • Nest • Recurrence

## INTRODUCTION

Atrial fibrillation (AF) is the most common sustained clinical arrhythmia and is associated with an increased morbidity and mortality.<sup>1</sup> Increasing evidence supports the mechanistic links between inflammation and AF with a significant contribution from obesity.<sup>2</sup> Studies have focused on the relationship between the regional fat, cardiovascular risk and arrhythmias, especially AF, independent of the total adiposity.<sup>3,4</sup> Epicardial fat has an impor-

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tant endocrine and inflammatory function, and its close relationship to the myocardium and coronary arteries may play a central role in the pathogenesis of cardiovascular disease, mediated by its inflammatory properties.<sup>3,5</sup> Recent studies using multidetector computed tomography have described that the epicardial fat volume of the entire heart is significantly increased in patients with AF.<sup>6,7</sup> Also, the topographic distribution of the epicardial adipose tissue (EAT) surrounding the left atrium (LA) and the influence of catheter ablation on the outcome have been demonstrated by Tsao et al. and Chao et al.<sup>8,9</sup> The volume of EAT surrounding the LA is significantly increased in AF patients compared to the control.

In addition, the role of the intrinsic cardiac autonomic nervous system, has been shown to be a critical element responsible for the initiation and maintenance of AF,<sup>10,11</sup> and the autonomic inputs to the heart converge at several locations and are typically embedded in the epicardial fat pads and form ganglionated plexi (GP) that contain autonomic ganglia and nerves.<sup>12</sup> Previous studies have shown the self-perpetuation and progressive remodeling associated with AF and its important role in the maintenance of AF.<sup>13</sup> Therefore, significant interplay occurs between the inflammatory epicardial fat, autonomic modulation with a sympathovagal imbalance, and electroanatomical remodeling that may subsequently play an important role in the initiation and maintenance of AF. The abnormal atrial substrate can be identified by a high dominant frequency (DF) during sinus rhythm and it is named an AF nest.<sup>14</sup> An animal study by Chang et al. reported the AF nests were closely related to the intrinsic cardiac autonomic nervous system activity.<sup>15</sup> Therefore, there may be an important link between the regional distribution of epicardial fat and an abnormal biatrial substrate, which may play a significant role in the initiation and maintenance of AF. The aim of this study was to investigate the correlation between the regional distribution of epicardial fat and the signal characteristics of the corresponding epicardial fat region in AF patients.

## MATERIALS AND METHODS

### Study population

The study was comprised of 32 consecutive patients

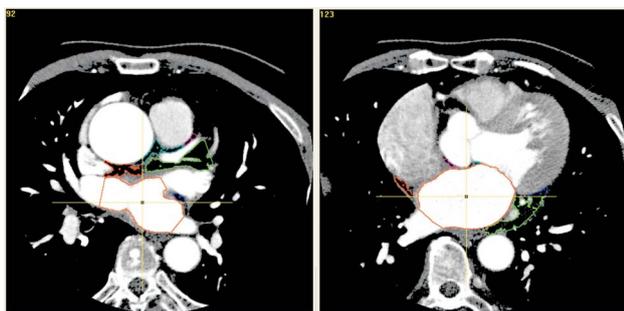
with symptomatic drug refractory AF who underwent radiofrequency catheter ablation of AF under the guidance of a 3D electroanatomical mapping system (Nav X, St. Jude Medical Inc., St. Paul, MN, USA). The mean age was  $58 \pm 10$  years old, and there were 27 males. Of those, 23 patients had paroxysmal AF and the remaining 9 had persistent AF.

### Echocardiographic measurements

The LA diameter was measured during end systole in the parasternal long-axis view. An LA enlargement was defined as a diameter of  $> 4.0$  cm, measured during end systole. The left ventricular ejection fraction (LVEF) was measured using the Simpson method. An LVEF of  $< 50\%$  was considered as abnormal. All measurements were based on the recommendations for the chamber quantification consensus.<sup>16</sup>

### Computed tomography (CT) and epicardial fat measurement

A detailed analysis of the CT protocols has been previously described.<sup>17</sup> Briefly, the LA and pulmonary veins (PVs) were evaluated with an electrocardiographically gated, 64-slice multidetector CT scanner (Aquilion 64 CFX, Toshiba Medical System, Tokyo, Japan). All participants underwent contrast-enhanced CT scanning during sinus rhythm. Patients were instructed to hold their breath to acquire the images, which covered an area from the superior margin of the pulmonary hilum to the cardiac apex (collimation  $64 \times 0.5$  mm, gantry rotation time 350 ms, table speed 6.3 mm/rotation, tube voltage 120 kV, and effective tube current 545 mA). The acquisition time was 8 to 12 seconds depending on the heart rate. All CT images were analyzed offline with software developed by the Department of Biomedical Engineering, Chung Yuan Christian University (Chung-Li, Taiwan).<sup>18</sup> The volume of the epicardial fat was obtained using a semiautomated method and fat was recognized using threshold attenuation values of  $-50$  to  $-200$  HU. Axial images during the atrial end-diastole were used to trace the epicardial fat from the pulmonary artery to the coronary sinus. Figure 1 shows two examples of how the epicardial fat was measured. The total number of slices traced manually was 60 to 112 depending on the atrial size. All slices were verified for accuracy by 2 investigators. To understand the topographic distribution of the



**Figure 1.** Examples of the quantification of the epicardial fat surrounding the left atrium (LA). The black zone encircled by the green and red lines are the epicardial fat measured from the computed tomography scan.

epicardial fat, the periatrial space was equally divided into 8 regions. That is, the periatrial space was divided equally into halves in the x, y, and z planes. Region 1 was the space covering the right anterior-superior LA, region 2 the space covering the left anterior-superior LA, region 3 the space covering the right anterior-inferior LA, region 4 the space covering the left anterior-inferior LA, region 5 the space covering the right posterior-superior LA, region 6 the space covering the left posterior-superior LA, region 7 the space covering the right posterior-inferior LA, and region 8 the space covering the left posterior-inferior LA.

### Electrophysiological study and electroanatomic mapping

#### Signal recording

Each patient underwent an electrophysiological study and catheter ablation in the fasting, non-sedative state after written informed consent was obtained. After completing intact right atrium (RA) and LA geometry reconstructions, a sequential contact voltage map was constructed in all patients during sinus rhythm. For patients with persistent AF, IV dromicium was given for temporary sedation and external direct current cardioversion was performed to convert the patients to sinus rhythm to allow for mapping. More than one cardioversion was performed if AF recurred during the mapping. The bipolar electrograms were filtered between 32 to 300 Hz and recorded digitally. The electrodes of the coronary sinus catheter were used to provide the timing reference signal during the mapping procedure. The absolute peak was selected as the detection set-

ting to determine the point of activation in the waveform. An irrigated 4.0-mm tipped ablation catheter (Chilli II, EPT, Boston Scientific Corporation, Natick, MA, or CoolPath, St. Jude Medical, St. Paul, MN) was selected as the roving catheter. The roving signal was used to collect the local activation time (relative to the reference signal) and voltages while the roving catheter came in contact with the atrial wall as it was swiped throughout the atrium during sinus rhythm in both the LA and RA. The signals from the roving catheter were used to build a sequential map. After completion of the sequential map, the bipolar mapping points were collected and analyzed by the offline software.<sup>19</sup> For the purpose of a regional evaluation of the DF, the total activation time (TAT) and peak-to-peak (P-P) voltage points were designated as belonging to one of the eight LA areas corresponding to the epicardial distribution of fat around the LA.

#### Signal analysis

##### DF

The signals for the frequency analysis and electrogram morphology analysis were both exported from the NavX mapping system and analyzed by the Matlab computer program (MathWorks Inc., Natick, MA, USA). Spectral analysis was performed on the single discrete bipolar electrogram during sinus rhythm (unrectified, Hanning window, and 1 second in duration). Then the data were exported to an external computer program including single discrete electrograms during sinus rhythm and the baseline on both sides of the discrete electrogram. The fast Fourier transform analysis was performed using a Hanning window function on each segment from all recording sites in the LA. The DF was defined as the frequency with the maximum power in the frequency range. Higher frequencies were mapped toward the purple end of the NavX color spectrum, as a real time built-in function. AF nests were defined as sites with a DF of more than 70 Hz.<sup>14</sup> To ensure the reliability of the DF detection, the lowest noise signal was chosen for the analysis. Further, to avoid an excessively high density in some regions, any mapping site with a distance of less than 0.5 mm to the nearest neighbouring site was excluded. The spectral analysis was performed before the catheter ablation in all patients.

### Voltage and total activation time

Scar was defined as an absence of any voltage or a bipolar P-P voltage amplitude  $\leq 0.05$  mV, indistinguishable from noise. A low voltage region was defined as an amplitude of  $\leq 0.5$  mV.<sup>20</sup> The TAT of the atrial electrogram was measured from the earliest atrial activation site to the latest atrial activation site.

### Relationship between the epicardial fat, DF, voltage and TAT

The relationship between the epicardial fat and DF was evaluated as follows: (i) patient-by-patient to explore the correlation between the mean epicardial fat, DF, P-P voltage and TAT for biatrial maps; (ii) region-by-region to explore the correlation between the epicardial fat, DF, P-P voltage and TAT at each sample location in every patient.

### Catheter ablation

After mapping, provocation of AF was performed in each patient before the catheter ablation procedure.<sup>21,22</sup> All patients then underwent circumferential PV isolation with an endpoint of isolation confirmed by either the elimination or dissociation of the PV potentials. Ablation of the PVs was performed using a delivered power of 30 Watts with an irrigation rate of 17 mL/min. Additional substrate modification by linear ablation (roof line or mitral isthmus ablation) or targeting regions of complex fractionated atrial electrograms was performed in patients with AF episodes persisting for  $> 48$  hours and structural heart disease. Cavotricuspid isthmus ablation with an endpoint of bidirectional isthmus block was performed in every patient during the procedure. The endpoint of the substrate modification was either electrophysiologically confirmed linear conduction block established via pacing maneuvers or the elimination of local fractionation. Substrate modification was performed using a delivered power of 25-30 Watts with an irrigation rate of 17 mL/min.

### Follow-up of AF recurrence

After discharge, all patients underwent follow-up (2 weeks after the catheter ablation, then every 1-3 months) at our cardiology clinic or with the referring physician, and antiarrhythmic drugs were prescribed for 8 weeks to prevent the early recurrence of paroxysmal AF ( $< 1$

month after the ablation). When the patients experienced symptoms suggestive of a tachycardia after the ablation, 24-hour Holter monitoring or cardiac event recordings were performed to define the cause of the clinical symptoms. AF recurrence was defined as an episode lasting more than 1 minute and confirmed by electrocardiograms 3 months after the ablation (blinking period).

### Statistical analysis

The analysis of the data was performed using SPSS statistical analysis software (SPSS Inc., Chicago, Illinois, USA). The results were expressed as the mean  $\pm$  SD or number (percent). The correlations were analyzed by a correlation coefficient to assess the relationship between the regional distribution of the epicardial fat volume and the parameters of the biatrial substrate. A Chi-square test was used for categorical variables and a Pearson's correlation for continuous variables. A univariate analysis of various clinical variables was performed with a two-sample t test to determine the predictors of recurrence after the catheter ablation of AF. Variables selected to be tested in the multivariate analysis (age-adjusted Cox proportional model) were those with a  $p < 0.1$  in the univariate models. A value of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

### Patient characteristics

The patient characteristics of the entire study are summarized in Table 1. The average duration of paroxysmal and persistent AF was  $34 \pm 28$  months and  $116 \pm 62$  months, respectively. Prior to the ablation, 56.3% were on one antiarrhythmic drug, 21.9% on two or more antiarrhythmic drugs, 12.5% on an antiarrhythmic drug and beta blockers, and 9.3% only received beta blockers and no other antiarrhythmic drugs.

### Correlation between the epicardial fat and electrophysiological properties of the LA and RA

#### *Substrates (patient-by-patient)*

The total amount of epicardial fat varied from 15.5 to 69.9 cm<sup>3</sup> in patients with AF, and the mean amount of

**Table 1.** Baseline clinical characteristics

Number of patients (n)	32
Gender (male n, %)	27 (84)
Age (years)	58 ± 10
BMI (kg/m <sup>2</sup> )	25 ± 3
Hypertension (n, %)	18 (56)
Diabetes (n, %)	4 (13)
Coronary artery disease (n, %)	9 (28)
Congestive heart failure (n, %)	2 (6)
Total cholesterol (mg/dl)	185 ± 35
Triglycerides (mg/dl)	122 ± 65
2D echocardiographic features	
Left atrial diameter (mm)	39.8 ± 4.6
Left ventricle end diastole dimension (mm)	50.7 ± 4.0
Left ventricle end systole dimension (mm)	28.8 ± 5.9
Left ventricular ejection fraction (%)	59.0 ± 9.5
Paroxysmal/persistent AF (n, %)	23 (72)/9 (28)

2D, two dimensional; AF, atrial fibrillation; BMI, body mass index.

epicardial fat did not significantly differ between the patients with paroxysmal and persistent AF ( $27.9 \pm 11.9 \text{ cm}^3$  vs.  $29.7 \pm 9.2 \text{ cm}^3$ ;  $p = 0.7$ ), respectively. A statistical analysis revealed that there was no correlation between the mean epicardial fat volume and sinus rhythm LA mean DF ( $p = 0.23$ ,  $R = 0.22$ ), RA mean DF ( $p = 0.48$ ,  $R = 0.13$ ), LA P-P voltage ( $p = 0.55$ ,  $R = 0.35$ ), RA P-P voltage ( $p = 0.4$ ,  $R = 0.15$ ), LA TAT ( $p = 0.44$ ,  $R = 0.14$ ), and RA TAT ( $p = 0.45$ ,  $R = 0.14$ ) in the patient-by-patient analysis, respectively.

#### Substrates (region-by-region)

The regional distribution of the epicardial fat volume, DF, P-P voltage, and TAT in all AF patients is reported in Table 2. The regional epicardial fat was distributed unevenly around the LA. Most of this fat was located in region 1 (29.5% of the total epicardial fat), region 2 (28.0% of the total epicardial fat), and region 8 (15.6% of the total epicardial fat). The other epicardial fat distributions were in an order of decreasing frequency as follows: region 6 (9.4% of the total epicardial fat), region 5 (7.6% of the total epicardial fat), and region 7 (6.9% of the total epicardial fat). During the analysis, it was also found that fat was significantly greater in the superior region (regions 1, 2, 5 and 6) of the LA versus inferior (regions 3, 4, 7 and 8,  $p < 0.001$ ), with no significant difference in the anterior (regions 1, 2, 3 and

**Table 2.** Regional distribution of the fat, DFs, P-P Voltage, and total activation time in the LA

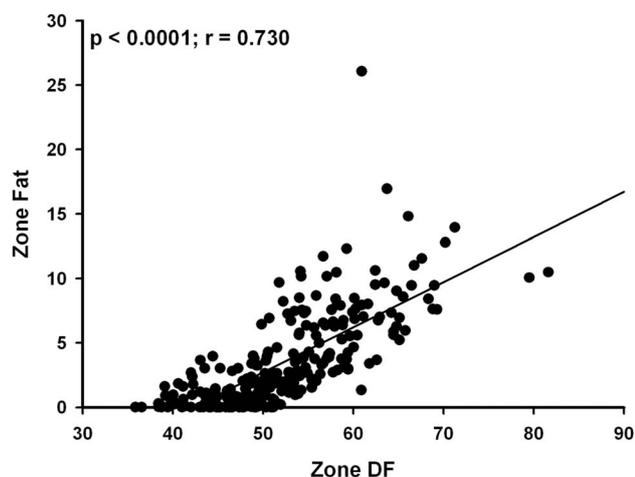
LA regions	Epicardial fat (cm <sup>3</sup> )	DF (Hz)	Voltage (mV)	Activation time (ms)
Region 1	8.26 ± 2.54	61.99 ± 5.97	1.90 ± 0.67	99.28 ± 53.20
Region 2	7.84 ± 4.12	59.34 ± 6.58	2.39 ± 1.02	71.59 ± 46.80
Region 3	0.54 ± 0.68	46.35 ± 4.14	2.01 ± 0.71	78.72 ± 51.86
Region 4	0.31 ± 0.74	45.54 ± 4.48	2.43 ± 0.75	62.31 ± 34.16
Region 5	2.12 ± 0.94	49.91 ± 6.36	2.23 ± 0.90	76.16 ± 35.80
Region 6	2.62 ± 2.07	50.49 ± 5.11	2.30 ± 0.80	60.22 ± 31.81
Region 7	1.95 ± 1.03	49.53 ± 4.24	2.22 ± 0.56	67.06 ± 36.99
Region 8	4.36 ± 2.31	54.80 ± 5.75	2.83 ± 0.94	56.34 ± 33.62

DF, dominant frequently; LA, left atrium; P-P, peak to peak.

4) and posterior (regions 5, 6, 7 and 8) regions in all the study patients.

A comparison of the regional fat distribution with the type of AF revealed that there was a statistically significant difference in the total regional fat between paroxysmal and persistent AF ( $3.2 \pm 3.0 \text{ cm}^3$  vs.  $4.1 \pm 3.2 \text{ cm}^3$ ;  $p = 0.02$ ), respectively. A region-by-region analysis of the epicardial fat revealed that region 1 had a statistically significant difference between paroxysmal and persistent AF ( $7.9 \pm 1.9 \text{ cm}^3$  and  $9.2 \pm 3.7 \text{ cm}^3$ , respectively;  $p = 0.004$ ). There was no significant difference in the other regions according to the type of AF.

We then analysed the correlation between the regional epicardial fat and regional DF. There was a significant linear correlation between the summated regional epicardial fat and regional DF of all the regions ( $p < 0.001$ ;  $R = 0.73$ ) in all AF patients as well as in paroxysmal and persistent AF patients ( $p < 0.001$ ;  $R = 0.73$  and  $p < 0.001$ ;  $R = 0.68$ , Figure 2), respectively. The region-by-region analysis showed that there were statistically significant linear correlations between the epicardial fat and DF in region 1 ( $p = 0.02$ ,  $R = 0.41$ ), region 2 ( $p = 0.03$ ,  $R = 0.38$ ), region 3 ( $p = 0.01$ ,  $R = 0.44$ ), region 5 ( $p < 0.001$ ,  $R = 0.71$ ), region 6 ( $p = 0.05$ ,  $R = 0.36$ ), and region 8 ( $p = 0.004$ ,  $R = 0.49$ , Figure 3), respectively. No significant region-by-region difference was found between paroxysmal and persistent AF. There were also significant linear correlations between the distribution of the regional epicardial fat and DF in the anterior ( $p < 0.001$ ,  $R = 0.78$ ), posterior ( $p < 0.001$ ,  $R = 0.57$ ), superior ( $p < 0.001$ ,  $R = 0.68$ ), and inferior ( $p < 0.001$ ,  $R = 0.67$ ) regions of the LA, respectively (Figure 4). Figure 5 shows



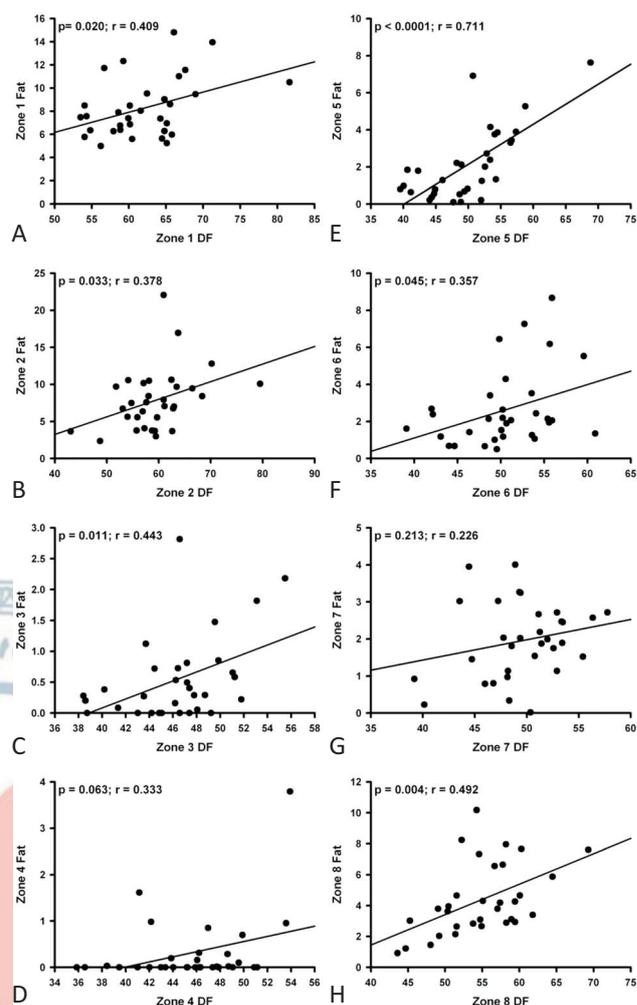
**Figure 2.** Comparison of the cumulative regional distribution of the epicardial fat and DFs in the LA. DF, dominant frequency; LA, left atrium.

an example in a paroxysmal AF patient. Panels A and B are the CT LA anatomies with epicardial fat surrounding the atrium especially at the lower anterior and posterior LA. 3D mapping revealed that the high DF (purple color, indicating AF nest) sites were located mostly at the corresponding sites. There was no correlation between the regional distribution of the P-P voltage and TAT and that of the epicardial fat.

### Ablation results

PV isolation was performed in all patients. In addition to the PV isolation, LA substrate modification was performed with a LA roof line alone in 9.4%, mitral isthmus alone in 3.1%, both an LA roof line and mitral isthmus in 34.4% and CFAE ablation in 25% of the patients. The mean procedure time was  $136.9 \pm 61.5$  minutes. A vasovagal response was observed in 34.4%, and an AF inducibility test was positive in 25% of the cases.

In the patients who underwent LA substrate modification in addition to the PVI, the epicardial fat volume was found to be higher in those with a CFAE ablation than in those without the CFAE ablation ( $35.9\text{cm}^3$  vs.  $25.9\text{cm}^3$ ;  $p = 0.02$ ), as shown in Figure 6. There was no difference between the epicardial fat in those with and without a roof line ablation ( $29.8\text{cm}^3$  vs.  $28.3\text{cm}^3$ ;  $p = 0.7$ ), with and without a mitral isthmus ablation ( $28.7\text{cm}^3$  vs.  $21.4\text{cm}^3$ ;  $p = 0.5$ ), with and without a roof line and mitral isthmus ablation together ( $31.9\text{cm}^3$  vs.  $26.6\text{cm}^3$ ;  $p = 0.3$ ), with and without a vasovagal response ( $16.5\text{cm}^3$  vs.  $14.5\text{cm}^3$ ;  $p = 0.2$ ), and with and without a

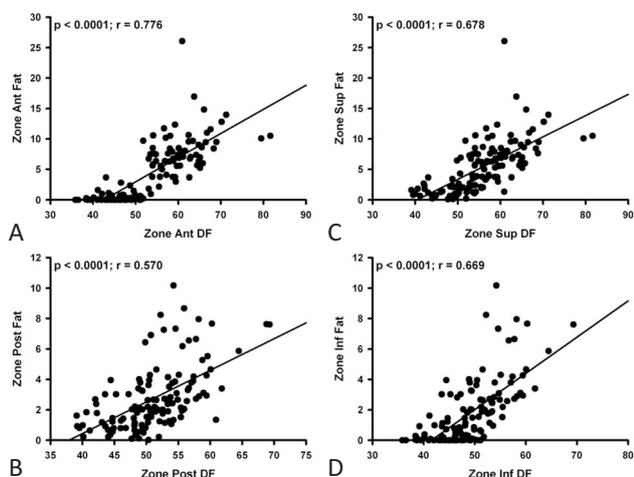


**Figure 3.** Comparison of the epicardial fat and DFs in the 8 regions of the LA. Region 1 covers the space of the right anterior-superior LA, Region 2 the space of the left anterior-superior LA, Region 3 the space of the right anterior-inferior LA, Region 4 the space of the left anterior-inferior LA, Region 5 the space of the right posterior-superior LA, Region 6 the space of the left posterior-superior LA, Region 7 the space of the right posterior-inferior LA, and Region 8 the space of the left posterior-inferior LA. The abbreviations are as in Figure 2.

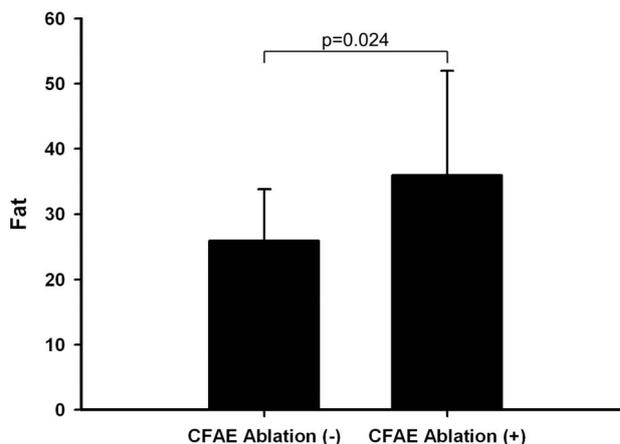
positive AF inducibility ( $33.4\text{cm}^3$  vs.  $26.8\text{cm}^3$ ;  $p = 0.3$ ), respectively.

### Follow-up

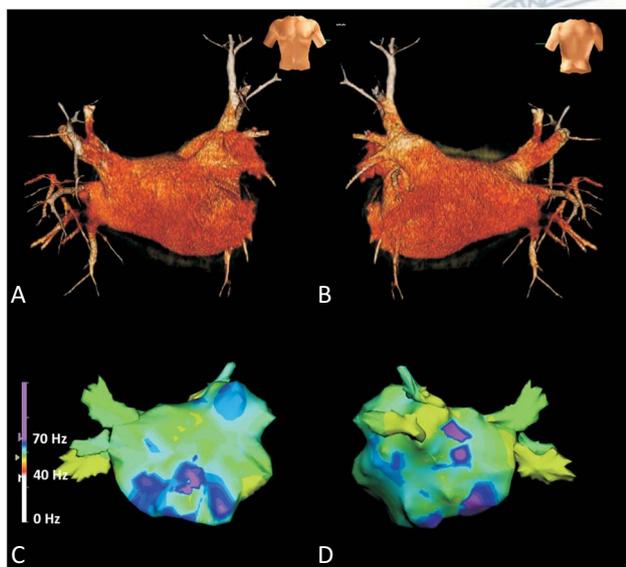
During a mean follow-up of  $17 \pm 8$  months, 22 (69%) of the 32 patients were free of AF (Table 3). The patients with a recurrence of AF had a larger LA diameter ( $p < 0.001$ ), longer duration of AF ( $p = 0.004$ ), higher incidence of coexisting structural heart disease ( $p = 0.01$ ) and persistent AF ( $p = 0.01$ ), high LA DF ( $p = 0.02$ ), and



**Figure 4.** Comparison of the epicardial fat and DF distribution in the anterior, posterior, superior, and inferior regions of the LA. DF, dominant frequent; LA, left atrium.



**Figure 6.** Comparison of the epicardial fat with the CFAE ablation in addition to the PV isolation in AF patients. AF: atrial fibrillation, CFAE, complex fractionated electrograms; PV, pulmonary vein.



**Figure 5.** Regional distribution of the epicardial fat and high DFs in the LA. Panels A and B are the superimposed antero-posterior and postero-anterior images of the LA showing the epicardial fat (light yellow color surrounding the LA) distribution on the cardiac CT. Panels C and D are the 3D LA antero-posterior and posterior-anterior anatomies with a high DF distribution (purple color) constructed by the NavX system. The abbreviations are as in Figure 2.

high LA epicardial fat volume ( $p = 0.02$ ) compared to those without a recurrence in the univariate analysis. During the multivariate analysis using an age-adjusted Cox proportional hazard model, only the LA DF ( $p = 0.04$ ) was found to be significantly associated with an AF recurrence (Table 4).

**Table 3.** Clinical and electrophysiological characteristics of the patients with and without recurrences of AF

	Recurrence (n = 10)	No recurrence (n = 22)	p value
Age (yrs)	60.1 ± 11.6	56.6 ± 8.8	0.36
Gender, M/F	9/1	18/4	> 0.99
Structural heart disease (n)	7	4	0.01
LA diameter (mm)	43.8 ± 5.3	37.9 ± 2.9	< 0.001
LVEF (%)	59.0 ± 6.7	59.1 ± 10.7	0.9
AF duration (months)	96.0 ± 61.4	39.1 ± 27.1	0.004
Persistent AF (n, %)	6, 66.67	3, 33.33	0.01
LA DF (Hz)	47.7 ± 4.0	43.0 ± 5.5	0.02
LA Fat volume (cm <sup>3</sup> )	34.9 ± 14.7	25.5 ± 7.7	0.02
LA voltage (mV)	1.8 ± 0.7	2.1 ± 0.6	0.24
LA total activation time (ms)	98.8 ± 22.5	112.0 ± 45.5	0.4

AF, atrial fibrillation; DF, dominant frequency; LA, left atrial; LVEF, left ventricular ejection fraction.

**Table 4.** Predictor of AF recurrence after an age-adjusted Cox proportional hazard model

	Adjusted hazard ratio (95% CI)	p value
Structural heart disease	1.53 (0.09-5.00)	0.68
LA diameter	1.26 (0.96-1.65)	0.10
AF duration	1.00 (0.98-1.02)	1.00
Persistent AF	4.44 (0.008-6.49)	0.23
LA DF	1.49 (1.03-2.18)	0.04
LA fat volume	0.96 (0.90-1.03)	0.27

AF, atrial fibrillation; DF, dominant frequency; LA, left atrial.

## DISCUSSION

### Main findings

This study analysed the epicardial fat and signal electrogram characteristics in AF patients during SR within the atria and described the significant relationship between the epicardial fat and DF. The major findings are as follows:

- (i) These variables did not demonstrate a relationship on a patient-by-patient basis in any of the AF patients as well as in paroxysmal and persistent AF analysed separately.
- (ii) A region-by-region analysis showed that epicardial fat was greater in persistent AF versus paroxysmal AF and an exploration of their spatial relationship demonstrated the direct relationship between the epicardial fat and DF with high DF sites in close proximity to sites with greater epicardial fat regardless of the type of AF.
- (iii) Also, a direct relationship was seen between the epicardial fat and CFAE ablation, done in patients requiring substrate modification in addition to the PVI.
- (iv) No significant correlation was found between the epicardial fat and RA DF.
- (v) During a follow-up of  $17 \pm 8$  months, the LA DF during sinus rhythm was found to be a significant predictor of recurrence.

### Epicardial fat: a key element of AF

It has been increasingly recognized that epicardial fat deposits differ in regards to the metabolic activity and proinflammatory mediators secreted.<sup>23</sup> This distinct fat is a source of several inflammatory mediators, including interleukin-1 $\beta$ , interleukin-6, tumour necrosis factor- $\alpha$ , and monocyte chemoattractant protein-1. Paracrine interactions of these cytokines and mediators contribute to the perivascular inflammation and development of coronary artery disease.<sup>3-5,23-25</sup> In addition, the expression of adiponectin, a protective adipokine, is significantly lower in the epicardial adipose tissue of patients with coronary artery disease than in healthy controls.<sup>24</sup> Further, LA biopsies from patients with AF have shown evidence of inflammatory cells in the atrial tissue, and a local inflammatory response may have a role in the development of AF.<sup>9,25</sup>

### DF: an important link in AF

Previous studies have indicated that the positive inducibility of AF after circumferential PV isolation requires additional LA substrate modification for a better clinical outcome.<sup>21,26</sup> The regions of an abnormal atrial substrate can be identified by multiple rapid deflections, fractionated electrograms, and low electrogram voltage during sinus rhythm.<sup>19,26-28</sup> These areas with fibrillatory myocardium play a significant role in the perpetuation of AF and indicate an atrial substrate abnormality. Our present study revealed that areas with a high DF were localized to around areas with a high fat volume, and a high DF represents an abnormal atrial substrate and stable source, which thereby may contribute to rapid repetitive activations with a fractionated wavefront. This not only promotes AF, but also predicts recurrence after catheter ablation of AF.

### Association of epicardial fat with AF

Animal models have demonstrated that parasympathetic nerve activity within the fat pads promotes the inducibility of AF. Large collections of cardiac ganglia are associated with epicardial fat, forming islands of adipose tissue on the myocardium. These GPs constitute a neural network composed of interconnecting neurons in this neural network, bilateral autonomic inputs to the heart appear to come together at many "convergence points" before giving rise to selective innervations that control important cardiac physiology functions. Four of the major left atrial GPs are located around the antrum of the pulmonary veins. Po et al. named these the superior left GP (SLGP), anterior right GP (ARGP), inferior left GP (ILGP), and inferior right GP (IRGP).<sup>29</sup> A histological examination has revealed adipocytes surrounding the cardiac ganglia resembling "raisins-in-bread". Stimulation of the ICANS by applying high-frequency electrical stimulation to the GP or by injecting parasympathomimetics into the GP at the fat pads has drawn attention to the critical role of the ICANS in the dynamics of AF initiation and maintenance.<sup>30,31</sup> Vagal activation seems to be more involved than sympathetic activation in promoting AF, probably because vagal innervation is more heterogeneous than that of the sympathetic nervous system.<sup>32</sup> The importance of a high epicardial fat volume and its potential role in initiating autonomic remodeling by activation of local stretch receptors, inflam-

matory cytokines and pathogenesis of AF, has been highlighted in previous studies.<sup>5,33,34</sup> In addition, a recent animal study also demonstrated that LA AF nest sites during sinus rhythm are primarily located near the PV-LA junction close to the GPs, indicating that the cardiac autonomic nervous system might play an important role in the mechanism of LA AF nest sites.<sup>15</sup>

Our study highlighted that the signal electrograms with high DFs were recorded in close proximity to the fat pads at the PV-atrial junction. The postulated mechanisms by which the epicardial fat may play a crucial role in the genesis of AF are: first, the parasympathetic activity of the GPs within the fat shortens the atrial effective refractory period and action potential duration, which results in a decreased wavelength of the atrial excitation wavefronts, widens the window of vulnerability, and allows both late-coupled and early coupled premature stimulations, which may initiate multiple rotors to initiate AF. The resultant rapid atrial activation due to triggered activity may result in a high DF around epicardial fat sites. Secondly, a high DF around fat may be due to fractionation of the atrial electrograms, as a sympathovagal imbalance may cause a break up of a uniform wavefront and lead to the contamination of the atrial electrograms. These findings, therefore, may explain the important role of high volumes of epicardial fat in the neural mechanisms of AF.

### Clinical and research implications

The hyperactive state of the ICANS plays a significant and crucial role not only in the initiation, but also in the maintenance of AF. GPs constituting the ICANS are localized in the fat pads especially around the junction of the PV-LA. Our study highlighted two important facts. First, the large volumes of epicardial fat constitute a critical factor in the pathogenesis of AF, and by its crucial link to high DFs (AF nests), it underscores the neural mechanism of AF besides its proinflammatory effect. Second, the spectral analysis of the signal electrogram may not only reveal the abnormal atrial substrate, but may also identify areas with parasympathetic innervation. This in turn, may lead to targeting areas with high DFs around the GPs lying close to fat pads, as potential targets for AF ablation in addition to the PV isolation.

However, a more extensive understanding of the GP distribution in the epicardial fat may be crucial to further improve the outcome of AF ablation. Further stu-

dies thereby are needed to determine the role of LA epicardial fat and its cause-effect relationship with AF, and to determine its clinical relevance in targeting LA adiposity as a therapeutic goal.

### Study limitations

First, we did not investigate the epicardial fat surrounding the RA, because all patients had AF triggers originating from PVs. We analyzed the epicardial fat surrounding the left atrium, and focused on the relationship between the LA substrate and epicardial fat. Second, pericardial fat is divided into two layers. Because of the CT scan resolution limit, it is difficult to differentiate the epicardial fat from paracardial adipose tissue. However, we used the same definition to compare the fat derived from the CT scan to minimize any bias. Third, two out of 9 persistent AF patients had procedural termination during the ablation procedure. However, because of the small patient number, we did not evaluate the relationship between AF termination and the DF or epicardial fat. It is worth an additional study in the future. Fourth, the sample size was limited and there might have been a type II error in the comparison of the total epicardial fat between the paroxysmal and persistent AF groups. That is a universal limitation because it is not easy to achieve sinus rhythm in persistent AF patients unless electrical cardioversion is performed immediately before CT scanning.

### CONCLUSIONS

This is the first human study that highlighted a significant and consistent association between the regional epicardial fat and the LA DF, and its potential role not only in the dynamics of AF initiation, but also in its maintenance by virtue of a significant neuromodulation of the ICANS and atrial substrate modification. Radiofrequency ablation targeting the LA DF (AF nest) may affect the long-term outcome. Therefore, modifying the LA adiposity may constitute an important therapeutic approach for the management of AF.

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## CONFLICT OF INTERESTS DISCLOSURES

All authors have no conflicts of interest to declare.

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