Epicardial adipose tissue and atrial fibrillation

Stéphane N. Hatem1,2 and Prashanthan Sanders3

Atrial fibrillation (AF) is the most frequent cardiac arrhythmia in clinical practice. AF is often associated with profound functional and structural alterations of the atrial myocardium that compose its substrate. Recently, a relationship between the thickness of epicardial adipose tissue (EAT) and the incidence and severity of AF has been reported. Adipose tissue is a biologically active organ regulating the metabolism of neighbouring organs. It is also a major source of cytokines. In the heart, EAT is contiguous with the myocardium without fascia boundaries resulting in paracrine effects through the release of adipokines. Indeed, Activin A, which is produced in abundance by EAT during heart failure or diabetes, shows a marked fibrotic effect on the atrial myocardium. The infiltration of adipocytes into the atrial myocardium could also disorganize the depolarization wave front favouring micro re-entry circuits and local conduction block. Finally, EAT contains progenitor cells in abundance and therefore could be a source of myofibroblasts producing extracellular matrix. The study on the role played by adipose tissue in the pathogenesis of AF is just starting and is highly likely to uncover new biomarkers and therapeutic targets for AF.

Keywords
Atrial fibrillation • Cardiac adipose tissue • Cardiac fat depot

This article is part of the Spotlight Issue on: Heterocellular signalling and crosstalk in the heart in ischaemia and heart failure.

1. Epidemiological and co-morbidity factors predisposing to atrial fibrillation

Atrial fibrillation (AF) is the most common sustained heart rhythm disorder affecting humans and is responsible for increasing morbidity and mortality. Consequently, it is now recognized as one of the most common cardiovascular causes of hospitalization and therefore contributes significantly to health-care costs. These observations may, in part, be due to the growing prevalence of AF attributed to an aging population with a prevalence of co-morbidity factors. Several clinical conditions are recognized to predispose the development of AF and include common diseases such as hypertension, heart failure, coronary artery disease, diabetes mellitus, and sleep apnoea.

Structural remodelling, along with its resultant electrophysiological consequences, has emerged as the dominant factor contributing to permissive substrates for AF. The importance of diffuse atrial fibrosis and conduction abnormalities for the development of AF (see below) was initially described in experimental heart failure studies. These findings have now been confirmed in several pre-clinical studies of heart failure, hypertension, and coronary artery disease. Consistent observations have also been reported in several clinical studies evaluating the AF-predisposing substrate caused by heart failure, sinus node disease, aging, hypertension, valvular heart disease, and sleep apnoea. Interestingly, evaluation of those patients with ‘lone AF’ has also demonstrated similar findings, suggesting the presence of disregarded or novel risk factors.

Obesity is increasingly recognized as a novel risk factor that contributes significantly to the burgeoning frequency of AF. Several epidemiological cohorts have observed that obesity independently increased the risk of developing AF. These studies have suggested that each additional unit of body mass index (BMI) was associated with a 4–8% increase in the development of AF. Gami et al. highlight the importance of co-existing risk factors with obesity and report an interaction between obesity and sleep apnoea in the increasing prevalence of AF. While in clinical studies obesity frequently co-exists with other established risk factors for AF such as hypertension, diabetes, and sleep apnoea, a recent experimental animal study of weight gain from our laboratory, devoid of many of these associated conditions, demonstrated diffuse atrial fibrosis and conduction abnormalities. Extension of these findings with sustained obesity in the same model observed greater cardiac ectopic fat and fat infiltration of the local atrial myocardium, potentially a novel substrate specific to obesity.

BMI, the traditional measure of obesity, has been used by the majority of studies identifying associations between weight gain and cardiovascular disease. Concomitant with advances in non-invasive imaging which now permit the characterization of cardiac ectopic fat content, there has been a growing interest in assessing the impact of local fat deposits on cardiovascular disease. Indeed, there are suggestions that
this ectopic fat deposit may be more specific in predicting cardiovascular disease than the traditional measures of obesity.\textsuperscript{33,34} Visceral ectopic fat deposits are thought to exert both local and systemic effects. This review will focus on the emerging interest in epicardial adipose tissue (EAT) and its relationship to AF.

2. Pathophysiology of AF: the biology of the substrate

The occurrence and persistence of AF require the combined action of a trigger, a substrate, and activation of the autonomous nervous system.\textsuperscript{35,36} The trigger consists of focal spontaneous electrical activity localized to preferential sites, mainly the pulmonary vein, where repetitive activity with a very short cycle length has been recorded.\textsuperscript{37} The substrate refers to the various alterations of the structural and functional properties of the atrial myocardium, which result in the shortening of atrial refractoriness, formation of re-entry circuits, and local conduction block patterns.\textsuperscript{35,36} Alteration in the electrical properties of atrial myocytes is an important determinant of AF pathogenesis.\textsuperscript{35,36,38–41} In addition, a large body of work indicates that structural remodelling of the myocardium is also central to the generation of AF substrate. As already mentioned, the role of fibrosis in the formation of local conduction blocks and disorganization of the conduction wave is well established.\textsuperscript{42–44} Increased fibrosis has been observed around trabeculae and in the interstitial spaces of the atrial myocardium, in both animal models and human biopsies.\textsuperscript{45,46} One mechanism by which fibrosis can alter the conduction of the depolarization wave is by favouring the delocalization of connexin gap junction channels.\textsuperscript{47} In addition to the interstitial fibrosis seen during AF and in dilated atria, myocytes tend to enlarge; their contractile apparatus is replaced by an accumulation of glycogen granules. These myocytes with extensive myocyte lysis return to a dedifferentiated state with structural and phenotypic characteristics typical of those observed during the development of ontogenic cardiac muscle.\textsuperscript{45,48}

A large research effort has been dedicated to deciphering the biological mechanisms underlying the AF substrate, the goal being to identify new biomarkers and therapeutic targets for the arrhythmia. Several mechanisms have been already identified. For instance, activation of the renin–angiotensin–aldosterone system, platelet-derived growth factor, connective tissue growth factor, and local inflammatory responses secondary to endothelial dysfunction and thrombi formation are all major actors in the accumulation of fibrosis.\textsuperscript{49–53} Less well understood are those pathogenic factors involved in the occurrence of myofibrocyte myocytes. Abnormal cell–cell contacts could be implicated, as supported by the observation that fibroblast–myocyte contact favours the dedifferentiation of human atrial myocytes.\textsuperscript{54,55}

Within this context, the discovery of a relationship between the abundance of atrial fatty deposits, and the risk and severity of AF, has opened new research perspectives on the biology of the arrhythmogenic substrate. Indeed, cardiac fatty deposits can impact the neighbouring myocardium through different processes.

3. Anatomy and physiology of cardiac fat tissue

Cardiac, or pericardial, adipose tissue comprises the paracardial fat located outside the visceral pericardium and the EAT situated between the visceral pericardium and the epicardium. There are also intra-myocardial fat deposits that are dedicated to triglyceride storage in the myocardium. Paracardial and epicardial fat are embryologically different, but both evolve from brown adipose tissue as intra-abdominal visceral fat.\textsuperscript{56} Some white adipose tissue can be detected in the atrioventricular and inter-ventricular grooves of the adult heart. While paracardial fat is separated from the myocardium by visceral pericardium, there is no distinct barrier between the EAT and the adjacent myocardium, supporting the possibility of cross talk between the two tissues. Indeed, paracrine processes have been described to underlie the biological interactions between EAT and its neighbouring myocardium. In addition, a vasocrine pathway is also possible via the vasavasorum.\textsuperscript{57} Figure 1 shows an example of dense fatty tissue infiltrating the atrial and ventricular myocardium. Well-organized adipocytes with pericellular fibrosis are in close proximity to myocytes, supporting the feasibility of an EAT secretome paracrine effect on neighbouring myocardium.\textsuperscript{57} Finally, cytokines secreted by EAT can accumulate in the pericardial fluid and could also contribute to local effects of the EAT secretome on the heart.\textsuperscript{58}

4. Experimental and clinical evidence for a relationship between epicardial fat and AF

The association between pericardial fat and AF has now been described by several groups. Noteworthy, though the distinction between epicardial and paracardial zones is clear on gross histological examination, it is far from evident when imaging regions adjacent to the atria.\textsuperscript{59} Noteworthy, the term ‘pericardial fat’ has been used interchangeably in studies evaluating the relationship with AF, with some studies defining epicardial fat as pericardial fat (i.e. inside the pericardial sac),\textsuperscript{31,60} while others defining pericardial fat as the sum of both epicardial and paracardial adipose tissues.\textsuperscript{33}

To date, the largest study stems from the Framingham Heart study. In this study involving 2317 participants of the Framingham Heart Study Offspring and Third Generation Cohorts, the investigators characterized pericardial fat (adipose tissue within the pericardial sac) with computed tomography (CT) and observed that pericardial fat volume predicted AF risk independent of other measures of adiposity with an odds ratio (OR) of 1.28 [95% confidence interval (CI) 1.05–1.60, \(P = 0.03\)] for every standard deviation increase in pericardial fat volume.\textsuperscript{31} This association was maintained after adjusting for other AF risk factors. Indeed, total pericardial fat, but not intra-thoracic or visceral fat, was associated with AF. Batal et al.\textsuperscript{61} evaluated 169 consecutive patients who had CT angiograms for either coronary artery disease or AF, and demonstrated that the CT-derived posterior left atria (LA) fat thickness (comprise between the oesophagus, the main pulmonary artery, and descending thoracic aorta) was associated with AF burden, independent of LA area and BMI. They showed that a 1 cm increase in this fat deposit thickness was associated with an OR of 6.06 (95% CI 1.9–19.25, \(P = 0.002\)). This has been further confirmed by Al Chekakie et al.\textsuperscript{60}, who demonstrate that pericardial fat volume (CT, adipose tissue within the pericardial sac) was associated with AF after adjusting for BMI, other traditional risk factors, and LA enlargement. Finally, Wong et al.\textsuperscript{33} studied pericardial fat (adipose tissue within the pericardial sac) via cardiac magnetic resonance in 130 patients, and reported that atrial pericardial fat volumes are associated with the prevalence and severity of AF, which persisted after adjusting for body weight. After adjusting for risk factors and body weight, both peri-atrial fat (OR of 5.33, 95% CI:
1.25–22.66; \( P = 0.02 \) and peri-ventricular fat (OR of 11.97, 95% CI: 1.69–84.88; \( P = 0.01 \)) were predictive of AF. Peri-atrial fat volumes, but not BMI or body surface area, were predictive of this association with AF. Recently, Shin et al.\(^62\) also reported that CT-measured epicardial fat volumes (adipose tissue within the pericardial sac) and peri-atrial fat thickness were significantly associated with the prevalence and persistence of AF. These studies have suggested that EAT volume may be more predictive of the presence and severity of AF than the traditional measures of obesity. They provide the foundation for the intriguing postulate that the direct impact of obesity on the atrial substrate may be mediated via EAT.

Some investigators have reported an association of pericardial fat with long-term clinical outcomes following AF ablation. Wong et al.\(^33\) reported that pericardial fat volume was associated with AF recurrence after radiofrequency ablation in a cohort of 110 patients with paroxysmal and persistent AF. Tsao et al.\(^63\) reported similar results with an association of CT-measured epicardial fat volumes predicting worse outcomes after radiofrequency ablation in a cohort of 68 patients with paroxysmal and persistent AF. Nagashima et al.\(^64\) likewise reported higher epicardial fat volumes (adipose tissue within the pericardial sac) in patients with AF recurrence after radiofrequency ablation. In another study, the same authors report an association of dominant frequency sites with epicardial fat locations. They also reported greater epicardial fat volumes and higher levels of serum inflammatory markers in patients with persistent AF when compared with paroxysmal AF.\(^65\) These studies suggest that EAT is an important determinant of the AF substrate, which can then predict the outcomes of rhythm control strategies.

### 5. Paracrine effects of EAT on the atrial myocardium

Visceral adipose tissue is a complex organ characterized by intense biological activity.\(^56,66\) For instance, EAT is actively involved in lipid and energy homeostasis. Compared with other visceral fat depots, this fatty tissue is characterized by a greater capacity to metabolize and secrete free fatty acids, whereas the glucose utilization is lower.\(^56,67\) This high lipolytic activity suggests that EAT is a source of free fatty acids for myocardial energetic metabolism, and could...
induced atherogenic changes in monocytes and endothelial cells.\textsuperscript{84} whereas conditioned media from EAT obtained from the same patients also reported in EAT from patients with a coronary artery disease, of anti-inflammatory and anti-atherogenic adiponectins has been contributing to myocardium insulin resistance. Reduced expression increase both lipolysis and the release of non-esterified fatty acids, derived TNF-\(\alpha\) could inhibit insulin receptor signalling and could also increase both lipolysis and the release of non-esterified fatty acids, contributing to myocardium insulin resistance. Reduced expression of anti-inflammatory and anti-atherogenic adiponectins has been also reported in EAT from patients with a coronary artery disease, whereas conditioned media from EAT obtained from the same patients induced atherogenic changes in monocytes and endothelial cells.\textsuperscript{84}

In addition to its role in energetic and lipid metabolism, adipose tissue produces a myriad of bioactive molecules, such as inflammatory mediators and adipocytokines, that have been shown to mediate the effect of visceral fat on neighbouring tissues.\textsuperscript{70} The biological activity of EAT has biological activity on neighbouring cardiovascular tissues.

Several EAT-expressed adipokines are known to be involved in the formation of the AF substrate. This includes inflammatory cytokines, growth factor, or matrix metalloproteinases (MMPs).\textsuperscript{50,94} Therefore, one explanation for the relationship between EAT abundance and the severity of the arrhythmia could be that EAT-secreted adipokines contribute to structural remodelling of the atrial myocardium, such as fibrosis. This hypothesis was tested directly using an ex vivo model of rat atrial organoculture, which permitted the study of the effects of human adipose tissue secretomes on the myocardium independently of co-morbidity factors. For this study, EAT was obtained from the interventricular and atrio-ventricular grooves of patients undergoing cardiac surgery for coronary bypass or aortic valve replacement. Conditioned medium from human EAT, but not SAT, induced marked fibrosis of the atrial myocardium as illustrated in Figure 2.\textsuperscript{57} In addition, the EAT-conditioned media favoured the transformation of fibroblasts into myofibroblasts, which then produce extracellular matrix components.

Clearly, EAT can have a biological impact on neighbouring cardiovascular tissues.

6. Adipokines involved in the formation of the AF substrate

Among the cytokines found in abundance in the EAT secretome, activin A and MMPs are excellent candidates for causing the fibrotic effect of EAT secretome on the atrial myocardium (Table 1).
Activin A is a member of the TGF-β superfamily. First recognized as an inducer of follicle-stimulating hormone release, activin A is a multifunctional cytokine expressed in various tissue types. A pro-fibrotic effect of activin A has already been described for liver fibrosis. The supplementation of culture media with recombinant human activin A reproduced the atrial myocardial fibrosis observed with EAT secretome. Moreover, anti-activin A antibody neutralized the pro-fibrotic effects induced by the EAT secretome. Both EAT-conditioned medium and activin A induced the expression of TGF-β1 and -β2 in the atria, which could indirectly contribute to the pro-fibrotic effect of activin A. Activin A-induced cardiac effects other than fibrosis have also been described. For instance, this cytokine has anti-hypertrophic and -apoptotic properties on the myocardium when it is exposed to ischaemia/reperfusion and pressure overload injuries. Activin A causes a negative inotropic effect on isolated guinea pig cardiac myocytes, suggesting a direct effect of this cytokine on the excitation–contraction coupling process.

MMPs are key regulators of extracellular matrix homeostasis, including the various collagen fibres and basement membrane components. During AF, it has been demonstrated that up-regulated activity of several MMPs, notably MMP2 and 7, contributes to the accumulation of interstitial fibrosis. MMP8, which is abundantly expressed in EAT, is known to be involved in the formation of atherosclerosis plaques, whereas little is known of its role during myocardial fibrosis.

The observation that EAT secretes adipokines that can induce fibrosis of the atrial myocardium raises the following question: under which clinical circumstances would the biological activity of cardiac fat tissue contribute to AF substrate formation? Is this effect only related to EAT abundance? Or are there specific clinical conditions associated with increased EAT biological activity? Venteclaf et al. found that the level of both activin A and MMP8 is enhanced in patients with heart failure. Moreover, Greulich et al. report that activin A is more abundantly expressed in the EAT of obese patients with Type 2 diabetes than in the other study patients. Heart failure and diabetes are well-established risk factors for AF, highlighting the role of EAT biological activity in these epidemiologic relationships. Of note, it has been reported that higher EAT thickness could be associated with higher biological activity (inflammatory cytokines).

Inflammation is an important determinant of the pathogenesis of AF. For instance, pericarditis, myocarditis, or cardiac surgery, all conditions that are characterized by some degree of myocardial inflammation, are associated with a high risk of AF. In addition, the C-reactive protein, a marker of systemic inflammation, is greater than two-fold higher in patients with none post-operative AF; a higher level was found in the subgroup of patients with chronic compared with those with paroxysmal AF. IL-6, -8, 1b, or TNF-α are other circulating inflammatory factors independently associated with the incidence and prevalence of AF. All these inflammatory factors are produced and secreted by
EAT in abundance, notably during ischaemic cardiopathy, obesity, or diabetes (Table 1). In EAT, not only adipocytes, but monocytes also can be a source of inflammatory cytokines when they are attracted by MCP-1 secreted by expanded adipocytes,83 as described in obese mice.105,106

Epicardial fat is characterized by a higher oxidative stress activity when compared with SAT.107 For instance, in patients suffering ischaemic cardiopathy, EAT contains more reactive oxygen species (ROS) whereas the activity of catalase is reduced compared to SAP; catalase is an antioxidant enzyme that protect cells against ROSH3O2.107 In addition to be produced by the myocardium,108 epicardial fat tissue might be an important source of ROS, for instance, in the context of ischaemic disease or post-operative AF. Clearly, more translational clinical studies using human EAT explants are needed to establish the precise scheme of the relationships between the biological activity of EAT and the remodelling of the myocardial tissue.

7. Potential mechanisms underlying the relationships between EAT and AF

An important mechanism of AF is related to the degree of disorganization and loss of homogeneity of the atrial myocardium, which alter the harmonious propagation of the depolarizing wave, favouring the formation of microcircuits and breakthrough of electrical impulses.36,44 Simultaneous endo-epicardial mapping of atrial electrical activity has shown that breakthrough and micro re-entry circuits increase with increasing AF substrate complexity. Lacking epicardial layer continuity due to endo-mysial fibrosis appears to be one of the major determinants of the complexity of fibrillatory conduction pathways.109 EAT accumulation is often associated with fatty infiltration from the epicardial layer, which advances deep into the myocardium (see Figure 1). This may contribute to myocardial functional disorganization and the formation of local arrhythmogenic substrate.30

More speculative is the potential role of EAT in the cellular component of the AF substrate, including the proliferation of myofibroblasts and the number of dedifferentiated and dystrophic myocytes.45,48 The origin of this cellular remodelling is not known. It may be the result of regenerative processes with a corresponding accumulation of precursor cells and their differentiation into various cell lineages. This hypothesis is supported by the finding that cardiac progenitor cells are abundant in the human atrial myocardium.110 In addition, there is an increased density of haematopoietic progenitor cells in the blood of patients with persistent AF.111 In this vein, it is important to note that adipose tissue contains abundant stem cells located in the stroma fraction.112,113 These stem cells are capable of differentiating not only into adipocytes, but also into cardiomyocytes114 or myofibroblasts.115 Therefore, cardiac fatty tissue may constitute a source of precursor cells that can differentiate into myofibroblasts, contributing to the structural remodelling of the atrial myocardium.

8. Conclusions and perspectives

The discovery that the abundance of cardiac fatty tissue is associated with both the severity and risk of developing AF has created new avenues of research on the pathogenesis of this arrhythmia. This adipose tissue is likely to be a major player in the formation of the AF substrate given its important biological activity. It could be the missing link between AF and the clinical conditions associated with changes in the biological activity of cardiac adipose tissue, such as obesity, diabetes, or heart failure. This research field should lead to the identification of new AF treatments, such as targeting specific adipokines. In addition, the improvement of the ability to visualize the abundance, distribution, and biological status of cardiac fatty tissue will accelerate the development of new tools to improve the identification of the AF substrate in clinical practice. More studies which use experimental models reproducing the relationships between the accumulation of adipose tissue and AF are warranted. However, small rodents such as mouse or rat have little or no EAT; therefore, these experimental studies must be conducted in large animal species that characterize by a distinct EAT such as rabbit, pigs, sheep, or monkey.

Conflict of interest: P.S. reports having served on the advisory board of Biosense-Webster, Medtronic, St Jude Medical, Sanofi-Aventis, and Merck, Sharpe and Dohme. P.S. also received lecture and/or consulting fees from Biosense-Webster, Medtronic, St Jude Medical, Boston Scientific, Merck, Sharpe and Dohme, Biotronik, and Sanofi-Aventis. P.S. received research funding from Medtronic, St Jude Medical, Boston Scientific, Biotronik, and Sorin. S.N.H. reports having served on the advisory board of Sanofi-Aventis, Servier laboratory, and Pierre Fabre industry.

Funding

This work is supported by the Fondation Leducq ‘Structural Alterations in the Myocardium and the Substrate for Cardiac Fibrillation’ (S.N.H.) and the European Union (EUTRAF-261057; S.N.H.). This work was also supported by the French National Agency through the National program ‘Investissements d’avenir’ with the reference ANR-10-IAHU-05 (S.N.H.). This work is also supported by the National Heart Foundation of Australia and New Zealand (P.S.). P.S. has received a Practitioner Fellowship from the National Health and Medical Research Council of Australia.

References

Cardiac fat tissue

211


