Epicardial adipose tissue as a modulator of cardiac arrhythmogenesis

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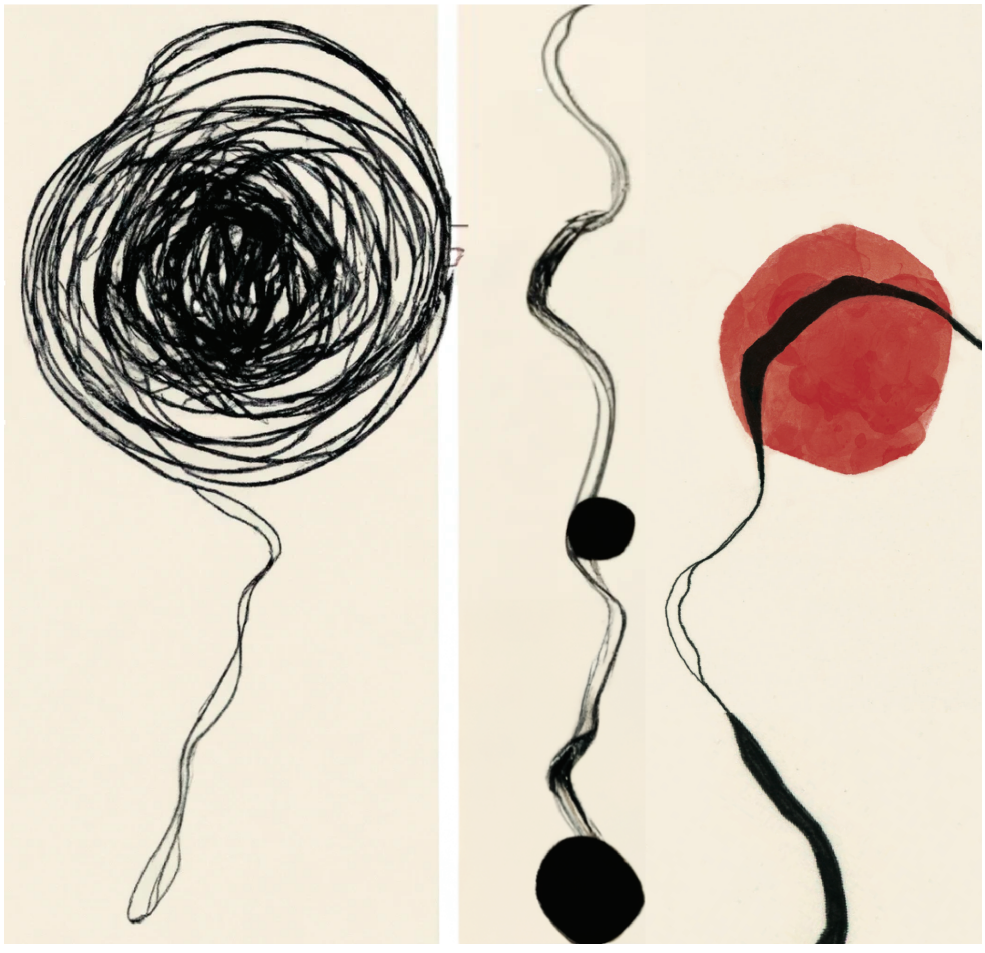

Chapter 2

Modulation of Cardiac Arrhythmogenesis by Epicardial Adipose Tissue

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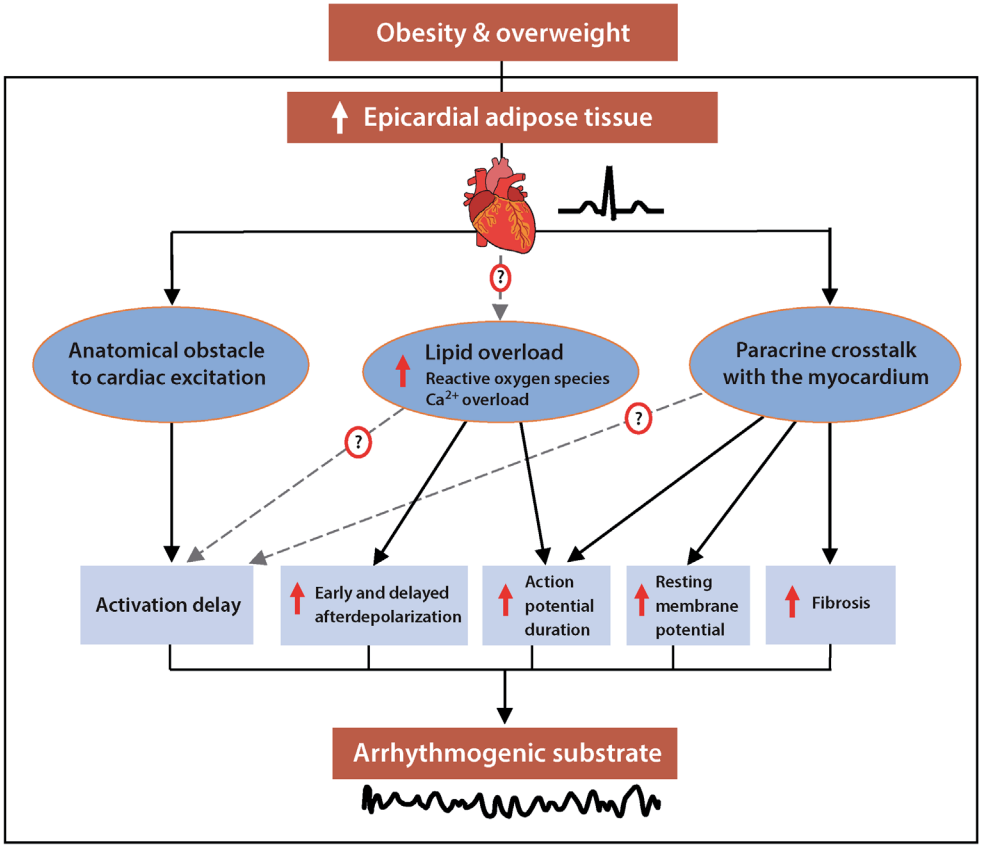
Abstract

Obesity is a significant risk factor for arrhythmic cardiovascular death. Interactions between Epicardial Adipose Tissue (EAT) and myocytes are thought to play a key role in the development of arrhythmias. In this review, we investigate the influence of EAT on arrhythmogenesis. First, we summarize electrocardiographic evidence showing the association between increased EAT volume, atrial and ventricular conduction delay. Second, we detail the structural cross talk between EAT and the heart and its arrhythmogenicity. Adipose tissue infiltration within the myocardium constitutes an anatomical obstacle to cardiac excitation. It causes activation delay and increases the risk of arrhythmias. Intercellular electrical coupling between cardiomyocytes and EAT can further slow conduction and increase the risk of block, favoring reentry and arrhythmias. Finally, EAT secretes multiple substances that influence cardiomyocyte electrophysiology either by modulating ion currents and electrical coupling, or by stimulating fibrosis. Thus, structural and paracrine crosstalk between EAT and cardiomyocytes facilitates arrhythmias.



Central illustration. Epicardial adipose tissue accumulation participates in creating an arrhythmogenic substrate. In obesity and overweight the increased epicardial adipose tissue (EAT) volume creates an anatomical obstacle to cardiac excitation which delays conduction. EAT accumulation may induce lipid overload and ROS production, leading to prolongation of the action potential and early and delayed afterdepolarizations. Paracrine crosstalk between EAT and myocardium induces fibrosis, prolongs the action potential and depolarize cardiomyocytes. All three pathways increase the risk of cardiac arrhythmias. The dotted lines describe potential mechanism of arrhythmogenicity.

Introduction

The prevalence of obesity has doubled in more than 70 countries since 1980[1]. The Global Burden of Disease study reported that 107.7 million (5%) children and 603.7 million (12%) adults were obese worldwide in 2015[1]. Obesity and overweight (body mass index (BMI)>25 kg/m^2) cause 2.8 to 4 million deaths every year. More than two-thirds of the deaths associated with high BMI are due to cardiovascular disease[1]. A BMI >25 is associated with a higher risk of sudden cardiac death[2] and atrial fibrillation (AF)[3]. Also, a high BMI and obesity are correlated with prolongation of QTc interval and QRS duration[4], which are both independent risk factors for cardiac arrhythmias. Thus, obesity plays an important role in the genesis of life-threatening arrhythmias.

Visceral adipose tissue tends to accumulate in the abdomen around internal organs and around the heart[5]. There is a significant relation between BMI on one hand and the amount of visceral adipose tissue and of fat on the surface of the heart (epicardial adipose tissue, EAT) on the other[6]. Because of its proximity to the heart, EAT has attracted considerable interest regarding its potential pro-arrhythmic effect. EAT volume is positively related to the incidence, duration, and recurrence of AF[7]. Also, EAT on the ventricular free walls correlates with the frequency of occurrence of premature ventricular contractions[8], and the sum of paracardial (i.e. surrounding the parietal pericardium) and epicardial adipose tissues is positively related to the development of ventricular arrhythmias in patients with heart failure[9].

Although a growing body of literature documents the arrhythmogenicity of epicardial adipose tissue, the underlying electrophysiological mechanisms are unknown. In this review, we summarize the literature on the topic in order to integrate the current knowledge on the arrhythmogenicity of EAT into clinical practice.

1. EAT: anatomy and origin

Epicardial Adipose Tissue refers to the visceral adipose tissue located between the myocardium and the epicardium[10]. This adipose tissue depot is enclosed in the pericardial sac, and shares blood supply with the heart. Accordingly, EAT is mostly located along the atrioventricular and interventricular grooves , and extends around the atria as well as along the circumflex and the left anterior descending coronary arteries[11] (Figure 1). It can also be found on the right ventricular free wall and left ventricular apex, covering almost the entire surface of the heart in some cases[11]. The absence of fascia separating EAT from the underlying myocardium allows direct cross-talk between adipocytes and neighboring cardiomyocytes[5].

The epicardium is thought to play a key role in the development and accumulation of EAT. It is the source of resident multipotent adult cardiac progenitor cells that can undergo epithelial-to-mesenchymal transition[12]. The epicardial progenitor derived cells migrate and can give rise, amongst other cell types, to adipocytes[13].

EAT is frequently present in both healthy and diseased individuals. Its volume increases during the first 40 years of life. Thereafter, its size does not depend on age but rather on BMI[14]. The average EAT represents 20% of total heart weight with prominent inter-individual variability[15]. EAT may also accumulate as a result of inflammation, or underlying cardiomyopathy[16].

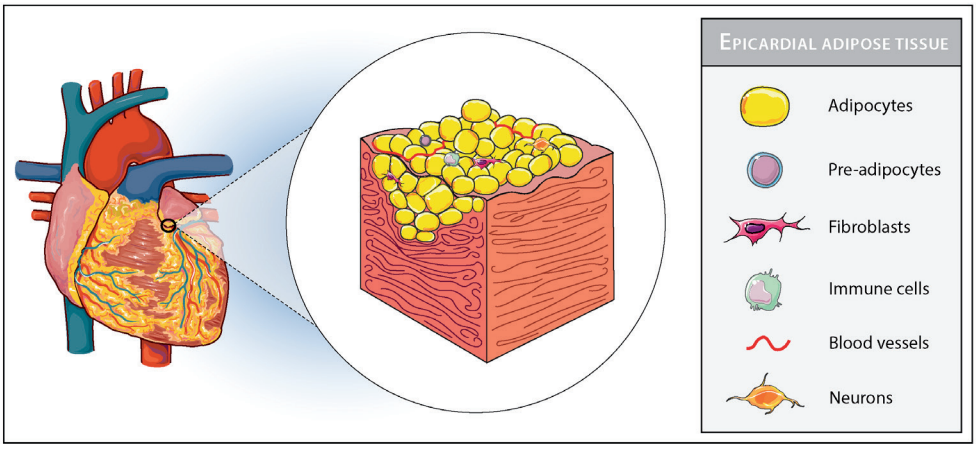


Figure 1. Epicardial adipose tissue anatomy and cellular composition.
Epicardial adipose tissue is a diverse microenvironment composed of adipocytes, pre-adipocytes, fibroblasts, immune cells, nerve cells and blood vessels.

Species differences should be taken into account regarding the amount of EAT. Rodents lack a substantial amount of EAT and are less suitable as animal model for cardiac adiposity[13]. Paracardial Adipose Tissue (PAT) surrounding the parietal pericardium is often used as a substitute for EAT in studies using rodents. However, PAT and EAT are embryologically, anatomically, and biochemically very distinct fat depots[17]. PAT derives from the primitive thoracic mesenchyme and is vascularized by branches of the mammary arteries. EAT on the other hand originates from the splanchnopleuric mesoderm[18] and shares the coronary circulation with the heart. While EAT is directly adjacent to the myocardium, PAT is located outside the pericardium and is considered as thoracic. PAT is therefore not directly in contact with the heart, does not share its circulation, and its paracrine and structural arrhythmogenic effects on the myocardium are likely different from those of EAT.

EAT is a complex microenvironment in which adipocytes, stromovascular cells (pre-adipocytes, fibroblasts, endothelial cells), nerve cells, immune cells, and other types of cells interact[19] (Figure 1). Adrenergic and cholinergic nerves are present in EAT, allowing communication with the cardiac sympathetic and parasympathetic nervous system[20]. Moreover, EAT is a source of biosynthesis of catecholamines, including (nor)epinephrine[21].

2. Role of Epicardial Adipose Tissue in Regulating Cardiac metabolism

EAT is thought to protect the heart from mechanical deformation, to facilitate vessel remodeling, and to provide Free Fatty Acids (FFAs), the main source of energy for the myocardium[22].

Adipose tissue is a highly active metabolic organ which serves as primary storage compartment for fatty acids. EAT present a higher FFAs uptake than subcutaneous adipose tissue[23], and it is more sensitive to changes of the lipid content of the diet[24]. It was hypothesized that FFAs can be sequestered by EAT as a buffering and protective mechanism against lipotoxicity, similar to visceral adipocytes[25] (Figure 2).

Given the proximity between EAT and cardiomyocytes, the release of FFAs from adipocytes in EAT into the plasma may act as a local and rapidly mobilizable cardiac energy supply[11] (Figure 2). Indeed, mitochondrial oxidation of FFAs represent 60 to 90% of the metabolic substrate of cardiomyocytes that take-up FFAs by protein-mediated transport across the sarcolemma (Figure 2), depending on the concentration of plasma FFAs[22].
Although the heart preferentially uses lipids as metabolic substrate, lipid overload is toxic[26]. If cardiomyocyte FFA uptake overwhelms the oxidative capacity of mitochondria, toxic lipids (such as ceramides) accumulate, leading to mitochondrial dysfunction, endoplasmic reticulum dysfunction, calcium dysregulation and increased reactive oxygen species (ROS) production[27]. The resulting cytosolic calcium-overload and spontaneous release of calcium, plays an important role in arrhythmogenesis based on delayed afterdepolarizations and triggered activity[28]. ROS induce early after-depolarization and facilitate ventricular arrhythmias in rat[29]. ROS also alter electrical coupling between cardiomyocytes potentially resulting in conduction slowing and increase the risk of arrhythmias[29].

FFAs can be stored as myocardial cytosolic lipid droplets (LDs)[27] (Figure 2). LDs thus may prevent lipotoxicity and act as a reservoir for energy and for signaling lipids, and

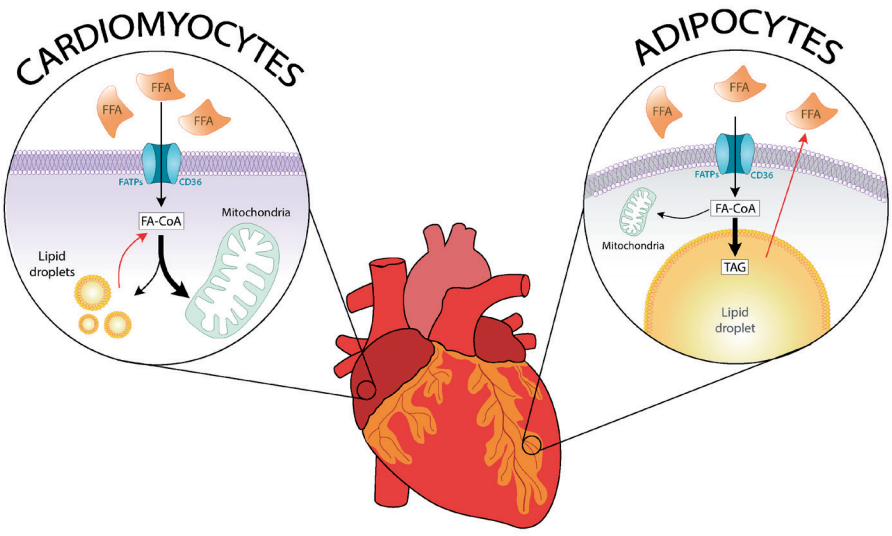


Figure 2. Adipocytes and cardiomyocytes FFAs uptake.
In the setting of excessive lipid concentration in the coronary circulation, Free Fatty Acids (FFAs) in the plasma can be taken-up by adipocytes in Epicardial Adipose Tissue. Once entered in the adipocyte, FFAs are converted into fatty acyl-coenzyme A (FA-CoA), before being stored as triacylglycerol (TAG) in the lipid droplet of the adipocyte. FFAs are also taken-up by cardiomyocytes to be used as mitochondrial substrate. FFAs are esterified into FA-CoA in the cytosol of cardiomyocytes, before being transported into the mitochondria to enter the fatty acid ß-oxidation cycle. Finally, if cardiomyocyte FFAs supply exceeds mitochondrial capacity, FFAs can be stored in lipid droplets to avoid the production of toxic lipids metabolites such as ceramide.

provide lipids for membrane expansion[30]. Cardiac LDs have been identified often at close proximity to mitochondria, suggesting an interplay between the two[31]. The role played by LDs in cardiovascular diseases and arrhythmias is not yet elucidated[32]. While LDs are protective against ischemia-reperfusion injury in adult rat cardiomyocytes[33], defects in their formation may be linked to an increased lipotoxicity, ROS production, and increased risk of arrhythmias[32]. To our knowledge, there are no studies investigating the crosstalk between EAT and LDs deposition in cardiomyocytes. We surmise that EAT accumulation is associated with or caused by increased plasma levels of FFAs. A portion of those FFAs will be used for cardiomyocyte metabolism (Figure 2), while the excess will be encapsulated into LDs and may protect the cell from lipotoxicity. When the LDs capacity of the cell is saturated, or dysfunctional, this may induce excessive ROS production and facilitate arrhythmias (see above). Indeed, lipid storage diseases are associated with lethal arrhythmias[34].

3. EAT and electrocardiographical parameters

Cardiac arrhythmia mechanisms are traditionally divided in abnormal impulse generation or abnormal impulse conduction . Although not all arrhythmia mechanisms can be categorized in this scheme, the dichotomy is used in this review. Abnormalities in impulse generation are related to pacemaking and triggered activity, the latter being dependent on prolonged APD or altered calcium ion (Ca^{2+}) handling. Abnormalities in impulse conduction can lead to anatomically or functionally based reentrant arrhythmias. Spatial heterogeneity in repolarization times provide a basis for unidirectional block and reentry. The electrocardiogram (ECG) contains information on APD, automaticity or conduction slowing and could therefore provide mechanistic information on arrhythmogenesis. We therefore reviewed the association between EAT and ECG parameters.

3.1 Atrial conduction

P-wave

Atrial conduction delay is reflected in the electrocardiogram as a prolonged P-wave. Several studies reported an association between EAT volume and atrial conduction delay (Table 1). In the Framingham Heart Study[35], a positive relation between EAT thickness and P-wave duration was described in healthy subjects after covariates adjustment (Table 1). Similarly, Jhuo et al.[36] described that the amount of EAT positively correlates with P-wave duration and inter-atrial conduction block. EAT thickness also correlates with P-wave duration in morbidly obese patients[37], but also with the left atrial diameter. Thus, in healthy individuals with normal atrial dimensions, the association between EAT and P-wave duration likely reflects slowed atrial conduction, whereas in morbid obese patients, P-wave prolongation at least in part reflects atrial enlargement.

P-wave dispersion is defined as the difference between the longest and the shortest P-wave duration recorded from multiple surface electrocardiographic (ECG) leads. It is a strong marker of anatomical remodeling and heterogeneous propagation of activation in the atria[38]. In healthy persons, P-wave dispersion is associated with EAT thickness[39,40] (Table 1). We hypothesize that the process of accumulating EAT results in anisotropic, heterogeneous propagation of sinus impulses, which may facilitate reentry (Figure 3). The extent of infiltrated adipose tissue in the atrial septum is independently associated with the number of P-wave fragmentations (a marker for heterogeneous conduction) in patients with paroxysmal AF and individuals at risk of AF[41]. Overall, this demonstrates an association between infiltrated adipose tissue in the atrial septum and slowed and discontinuous atrial conduction.

P-R interval

The P-R interval represents the sum of the time needed for conduction through the right atrium, the atrioventricular (AV) node, His bundle, and bundle branches. EAT volume is linearly correlated with a longer PR interval after adjustment for covariates[35,36,42,43] (Table 1). In patients with the highest EAT volume the PR interval was 10ms[43] to 16 ms[42] longer than in those with the smallest EAT volume.

Observational studies suggest an association between a prolonged P-R interval and an increased incidence in atrial fibrillation, heart failure, and mortality[44]. Indeed, high EAT volumes have been associated with the incidence of AF[7].

Ultimately, these results indicate that a high volume of epicardial adipose tissue is associated with a prolonged atrio-ventricular conduction. Because the PR-interval includes conduction through different structures, it is not clear whether AV-conduction delay in subjects with increased EAT volume is the result of conduction delay in the atria (cf 3.1), AV-node and/or His bundle.

3.2 Ventricular conduction

QRS-complex

Prolonged QRS duration indicates slowing of ventricular conduction or may result from hypertrophy. An increased BMI is associated with a prolonged QRS-complex duration[4].

In a study on 3087 healthy subjects, the authors identified that EAT volume is strongly associated with longer QRS duration after adjusting for several co-variates. Subjects with EAT volume above the 95% upper limit compared to those below the 5% limit had a 6.7ms longer QRS complex[42]. In another study on 287 subjects, a significant correlation was observed between EAT volume and QRS duration[43] (Table 1). Furthermore, the presence of a fragmented QRS-complex (fQRS) was associated with increased EAT in both healthy individuals and hypertensive patients[45,46] (Table 1). In a study on 114 hypertensive patients, EAT thickness was significantly increased in patients with fQRS. In the same study, EAT thickness above 4.5mm predicted the presence of fQRS (sensitivity of 75%, specificity of 58%)[45]. Finally, in a study on 308 healthy subjects, EAT thickness was significantly increased in individuals with fQRS[46]. Heterogeneous anisotropic ventricular conduction is associated with the presence of a fractionated-QRS-complex and may underlie reentrant arrhythmias[48].

Table 1. Association between EAT and ECG parameters. Summary of the studies on the association between EAT extent and ECG parameters: atrial conduction (measured as P-wave duration, amplitude, fragmentation, dispersion and P-R interval) and ventricular depolarization/repolarization (measured as QRS duration and fragmentation (fQRS), corrected QT interval (QTc), QT interval dispersion (QTd), and the interval from the peak to the end of the T wave (TpTe)). (Continued on next pages)

Studies characteristics					P wave				PR
First author, Ref. #	**Population**	**Groups**	**Sample size**	**Fat depot**	**Duration**	**Amplitude**	**Fragmentation**	**Dispersion**	**P-R interval**
Friedman et al.[35]	Healthy subjects	No group within the population	1,946	PCF	Associated (+) with EAT, P<0.002	Associated (+) with EAT, P<0.001			Associated (+) with EAT, P=0.049
Jhuo et al.[36]	Healthy subjects	No group within the population	100	EAT	Correlated (+) with EAT, P<0.05				Correlated (+) with EAT, P<0.001
Fernandes-Cardoso et al.[37]	Morbidly obese patients	Morbidly obese vs normal weight patients	40	EAT	Correlated (+) with EAT, P<0.001				
Murthy et al.[41]	Paroxysmal atrial fibrillation patients	Patients in sinus rhythm with structural heart diseases and paroxysmal AF or with AF risk factor	90	EAT, interatrial fat			Associated (+) with interatrial fat, P=0.001		
Quisi et al.[39]	Healthy subjects	Patients with low EAT: <5.35 mm, and high EAT: ≥5.35 mm	216	EAT	No association			Associated (+) with EAT, P=0.001	No association
Çiçek et al.[40]	Healthy subjects	No group within the population	70	EAT	Associated (+) with EAT, P=0.004			Associated (+) with EAT, P=0.026	

Studies characteristics					P wave				PR
First author, Ref. #	**Population**	**Groups**	**Sample size**	**Fat depot**	**Duration**	**Amplitude**	**Fragmentation**	**Dispersion**	**P-R interval**
Hung et al.[43]	Healthy subjects	No group within the population	287	EAT					Correlated (+) with EAT, P=0.003
Chi et al.[42]	Healthy subjects	No group within the population	3,087	PCF					Associated (+) with PCF, P<0.001
Bekar et al.[45]	Hypertensive patients	Patients with fQRS or without	114	EAT					
Yaman et al.[46]	Healthy subjects	Patients with fQRS or without	308	EAT					
Kaplan et al.[47]	Healthy subjects	Patients whose EAT thickness ≥ 9 mm and control subjects with EAT thickness < 9 mm	90	EAT					

Table 1. Continued

Studies characteristics					QRS				T-wave
First author, Ref. #	**Population**	**Groups**	**Sample size**	**Fat depot**	**Duration**	**Fragmentation**	**QTc**	**QTd**	**TpTe**
Friedman et al.[35]	Healthy subjects	No group within the population	1,946	PCF					
Jhuo et al.[36]	Healthy subjects	No group within the population	100	EAT			No association	No association	
Fernandes-Cardoso et al.[37]	Morbidly obese patients	Morbidly obese vs normal weight patients	40	EAT					
Murthy et al.[41]	Paroxysmal atrial fibrillation patients	Patients in sinus rhythm with structural heart diseases and paroxysmal AF or with AF risk factor	90	EAT, interatrial fat					
Quisi et al.[39]	Healthy subjects	Patients with low EAT: <5.35 mm, and high EAT: >/=5.35 mm	216	EAT	No association		Associated (-) with EAT, P=0.004		
Çiçek et al.[40]	Healthy subjects	No group within the population	70	EAT			No association	No association	
Hung et al.[43]	Healthy subjects	No group within the population	287	EAT	Correlated (+) with EAT, P=0.018		No association		
Chi et al.[42]	Healthy subjects	No group within the population	3,087	PCF	Associated (+) with greater visceral adiposity burden, P<0.001				
Bekar et al.[45]	Hypertensive patients	Patients with fQRS or without	114	EAT		Associated (+) with EAT, P=0.001			

Studies characteristics					QRS				T-wave
First author, Ref. #	**Population**	**Groups**	**Sample size**	**Fat depot**	**Duration**	**Fragmentation**	**QTc**	**QTd**	**TpTe**
Yaman et al.[46]	Healthy subjects	Patients with fQRS or without	308	EAT		Associated (+) with EAT, P<0.001			
Kaplan et al.[47]	Healthy subjects	Patients whose EAT thickness >/= 9 mm and control subjects with EAT thickness < 9 mm	90	EAT				Associated (+) with EAT, P<0.001	Correlated (+) with EAT, P<0.001

3.3 Ventricular Repolarization

QT interval and dispersion, Tpeak to Tend interval

The QT interval (QT) reflects the time needed for depolarization and repolarization of the ventricles and is usually corrected (QTc) for heart rate by the Bazett-formula[49]. QTc prolongation often leads to ventricular arrhythmias such as Torsade de pointes[50]. Research on the relation between EAT amount and QT interval and its dispersion (QTd, the difference between various ECG-leads) is limited. Although a single study has reported a negative association between EAT volume and QTc[39], others do not report a relation between the two parameters[36,39,40,43] (Table 1). Thus, the relation between EAT and ventricular repolarization remains unclear.

Changes in T-wave shape or duration reflect heterogeneity of ventricular repolarization[51]. A longer time interval between the peak and the end of the T wave (T_{peak}–T_{end} (TpTe)) mirrors repolarization heterogeneity and predicts sudden cardiac death and mortality in the general population[52]. In the only observational study available on the relation between EAT and TpTe, this interval was increased in subjects with higher EAT[47]. Furthermore, in one study on 90 healthy individuals high EAT volume was associated to an increased QT-dispersion[47], which points to increased repolarization heterogeneity.

These combined changes facilitate reentrant arrhythmias by causing unidirectional block following a premature beat. This suggests an association between EAT, repolarization heterogeneity, and the risk of ventricular arrhythmias and sudden cardiac death.

In the next section we will systematically study the available literature on the direct and indirect electrophysiological consequences of the proximity of epicardial adipose tissue to cardiomyocytes, and will identify the key players of this adipo-cardiac cross-talk.

4. Electrophysiological cross-talk between EAT and myocardium.

4.1. Structural cross talk and arrhythmogenesis

Adipose tissue infiltration and fibrosis

Two types of myocardial fat infiltration have been observed in autopsy cases and cardiac biopsies[5]. Adipocytes are either organized in thin and compact cords originating from the epicardium and infiltrating between cardiac bundles, or can be surrounded by dense fibrotic areas resembling a scarring process (fibro-fatty infiltration)[5]. It has been suggested that adipose tissue infiltrates can remodel in response to inflammatory triggers, resulting in fibro-fatty infiltration[53,54]. Atrial fibro-fatty replacement is more extensively observed in persistent than in paroxysmal AF patients[54,55], emphasizing its importance in arrhythmogenicity. Haemers et al.[54] showed that AF induces an increased accumulation of fibro-fatty infiltration in sheep left atria in comparison to control. Although the proportion of EAT infiltrates was not different between AF sheep and controls, the degree of fibrosis of the adipose tissue infiltrates was significantly increased in AF, suggesting that AF induced fibrosis of atrial fatty infiltrates. De Coster et al.[56] used computer modeling to investigate the effect of fat infiltration and/or fibrosis on arrhythmogenesis. They found that fibrosis is more arrhythmogenic than adipose tissue in terms of percentage of non-conductive tissue necessary to induce an arrhythmia.

Adipocyte infiltration into the myocardium can lead to nonuniform anisotropic propagation of an activation wavefront similar to what can be observed in chronic myocardial infarction[57]. The adipose tissue separates cardiomyocytes from each other and creates an anatomical barrier, thus forcing the electrical impulse to follow a 'zig-zag' path (Figures 3A-B). This discontinuous conduction results in electrogram fractionation[58] (Figure 3C) and may lead to conduction delay and reentry (Central Illustration). In a sheep model of myocardial infarction, intramyocardial adiposity was associated with myocardial discontinuity, decreased conduction velocity, reduced electrogram amplitude and increased risk of arrhythmias[59]. In the same study, the authors observed lateralization of connexin 43 (Cx43) in myocytes adjacent to fibro-fatty infiltrations. Finally, in patients with coronary artery disease, local slower conduction and electrogram fractionation (measured by epicardial mapping), increased fibrosis, and lateralization of connexin 40 have been shown at sites with a local high EAT volume[60].

Gap junctions

Cardiomyocytes form a syncytium through intercellular gap junctions. These allow the exchange of current, ions and matter between cells. Gap junctions play a critical role

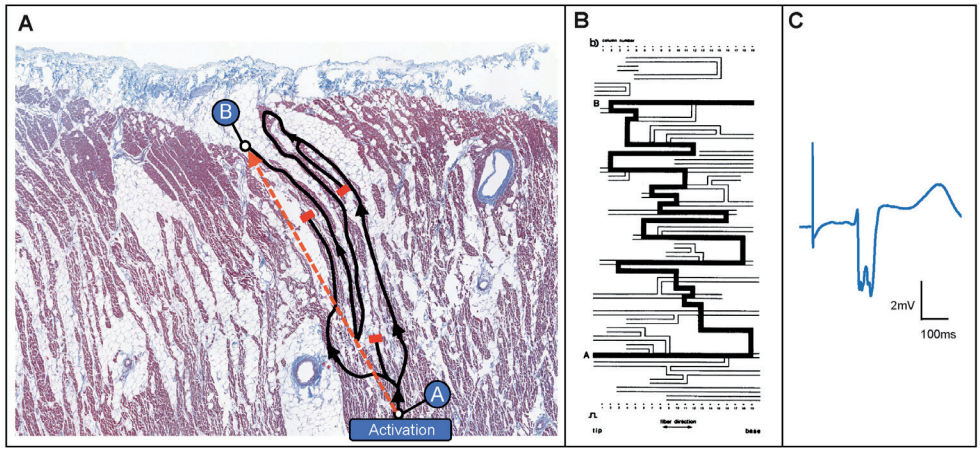


Figure 3. Direct effect of adipose tissue on arrhythmogenesis

(A) Masson's trichrome staining from Vigmond et al.[103] showing adipose tissue infiltration in the myocardium. Black arrows indicate a 'zigzag' path of the activation wave from point 'A' to 'B'. The activation wave has to follow a longer path, thus prolonging activation time. The orange arrow indicates the fastest activation path without anatomical obstacle, similar to the observations by de Bakker et al.[57] (B) in the setting of arrhythmogenesis in myocardial infarction. We surmise that adipose tissue can provide the substrate for tortuous activation pathways and activation delay that can lead to reentry. In this schematic drawing (B) the length of the route from A to B was 18 times longer than the shortest distance between A and B. (C) Fractionated electrograms from Vigmond et al.[103] recorded from this tissue (A). Note activation delay and fractionation resulting from discontinuous conduction induced by adipose tissue infiltration in the myocardium.

in cardiac conduction. Adipocytes also express gap junctional channels proteins such as Cx43, which are highly expressed in cardiomyocytes[61]. Indeed, adipocytes from white adipose tissue of mice are functionally coupled to each other through Cx43[61]. Whether adipocytes in EAT can electrically couple to cardiomyocytes is unknown. Gap junctions have been described between human adipose-derived mesenchymal stem cells and rat neonatal cardiomyocytes[62]. Other cell types in EAT such as fibroblasts, myofibroblasts, and macrophages may also form gap junctions with cardiomyocytes[63-65]. In atrial and ventricular cardiomyocytes, the resting membrane potential (RMP) is around -90mV, whereas white adipocytes, the main cellular constituent of the EAT, present a membrane potential of approximately -30mV[66]. Fibroblasts and macrophages have a membrane potential of between -10 and -30mV[63,67]. The non-myocytes do not generate an action potential. If we assume that EAT-adipocytes express gap junctions and that they electrically couple with cardiomyocytes (Figure 4), a change of the resting potential of the coupled cells may result. If the relatively depolarized non-myocardial cells in EAT are electronically coupled with cardiomyocytes, they will allow the direct exchange of ions and current between the cells. This

would lead to a relative depolarization of cardiomyocytes and a hyperpolarization of the adipocyte (Figure 4). A slight depolarization of cardiomyocytes may increase cardiac excitability by decreasing the potential difference between the RMP and the excitation threshold ("superexcitability") (Figure 4). Further depolarization of the resting membrane, however, will partially inactivate fast sodium channels, thereby decreasing the action potential upstroke and slowing conduction velocity. Finally, an extreme depolarization will lead to complete inactivation of the fast sodium channels. Propagation may then solely depend on the L-type calcium-current (very slow).
No proof of cardiomyocyte-adipocyte electrical coupling has been shown. However, electrical coupling between cardiomyocyte and fibroblasts has been demonstrated in rabbit hearts[68]. In computer modeling study, fibroblast-cardiomyocyte coupling induces changes in action potential shapes (a reduction of the plateau level of the action potential and shortening of the APD)[69] (Figure 4). Furthermore, cardiomyocytes co-cultured in direct contact with myofibroblasts elicit spontaneous electrical activity because of the diastolic depolarization (abnormal automaticity)[70]. We surmise therefore that electrotonic coupling between adipocytes and cardiomyocytes can exert electrophysiological effects comparable to that between fibrocytes, myofibroblasts, macrophages on the one hand, and cardiomyocytes on the other (Figure 4).

In summary, adipocyte infiltrations in the myocardium can slow conduction and facilitate the development of arrhythmias. Further research is needed to understand whether intercellular coupling between EAT cells and cardiomyocytes can exacerbate arrhythmogenicity.

4.2. Paracrine cross talk and arrhythmogenesis

Cells communicate with each other through the release of signaling molecules[71]. The term 'EAT secretome' refers to all molecules secreted by epicardial adipose tissue[72]. It includes a variety of soluble factors (growth factors, cytokines, bioactive lipids) and extracellular vesicles (EVs). The latter carry various cargo (proteins, lipids and signaling molecules) that may be transferred between cells and therefore play a wide and significant role in intercellular communication[73] (Figure 5). EVs also contain coding RNAs (messenger RNAs) and noncoding RNAs (long noncoding RNAs, microRNAs, circular RNAs)[74] that can be internalized by recipient cells and lead to major functional consequences. Recent work from Shaihov-Teper et al.[75] identified that epicardial fat-derived EVs from patients with AF contain proinflammatory and profibrotic cytokines, as well as profibrotic miRNA, which, when incubated with human-induced pluripotent stem cells-based cardiac cell sheet for 7 days shortened the APD_{80} and induced sustained rotors.

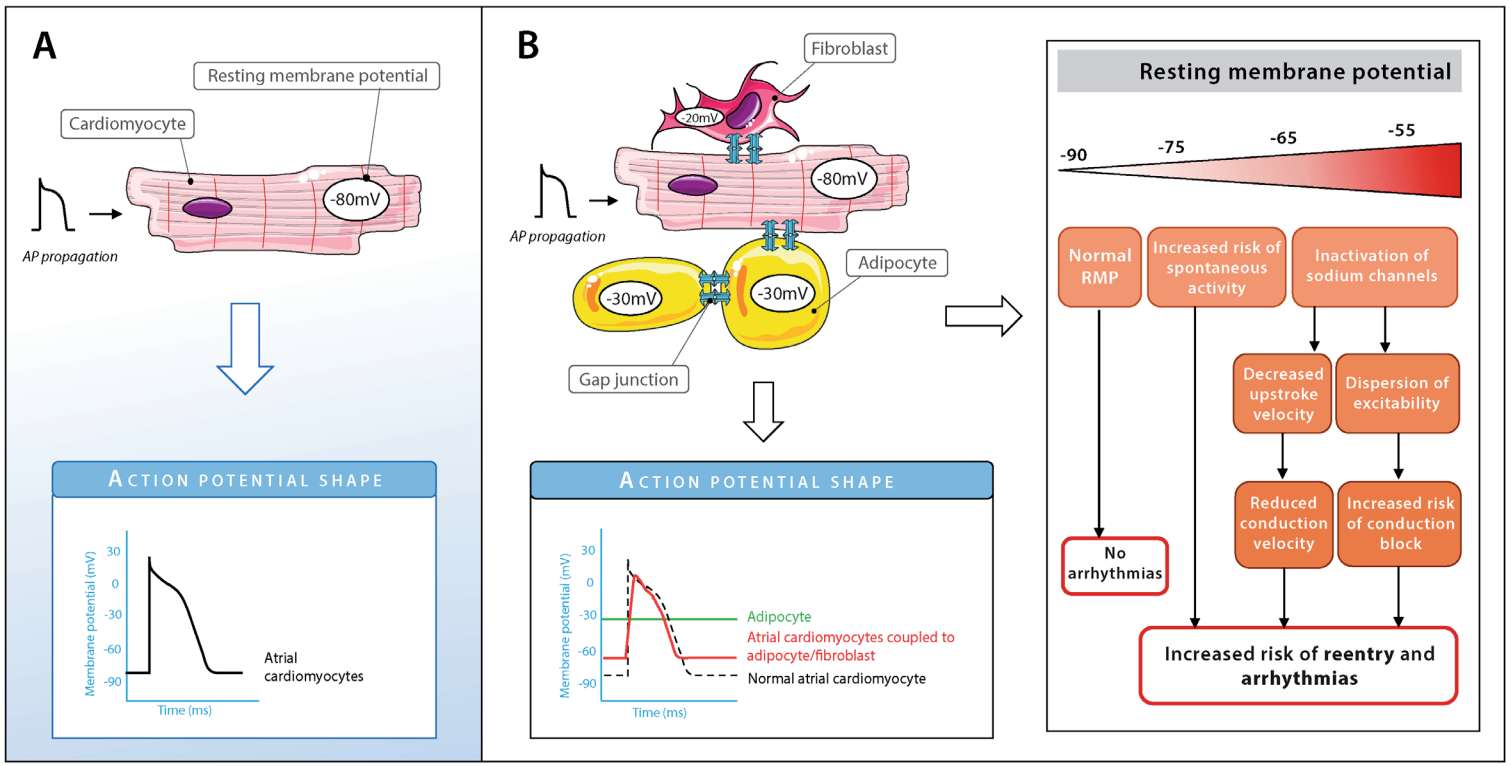


Figure 4. Hypothetical direct effect of adipose tissue on arrhythmogenesis: intercellular coupling.

(**A**) Atrial cardiomyocytes exhibit a Resting Membrane Potential (RMP) of approximately -80 mV when coupled to other cardiomyocytes. The RMP plays a critical role in activation since it sets the excitability level of cardiomyocyte. (**B**) Cardiomyocyte-fibroblast and cardiomyocyte-adipocyte coupling can hypothetically lead to the depolarization of cardiomyocyte with various consequences. A slight depolarization between -75 and -65 mV would decrease the potential difference between the RMP and the activation threshold, thus increasing excitability. Larger depolarization between -65 and -55 mV would lead to the inactivation of most sodium channels, thus decreasing excitability and inducing dispersion of excitability. The latter will lead to a reduced conduction velocity and an increased risk of conduction block.

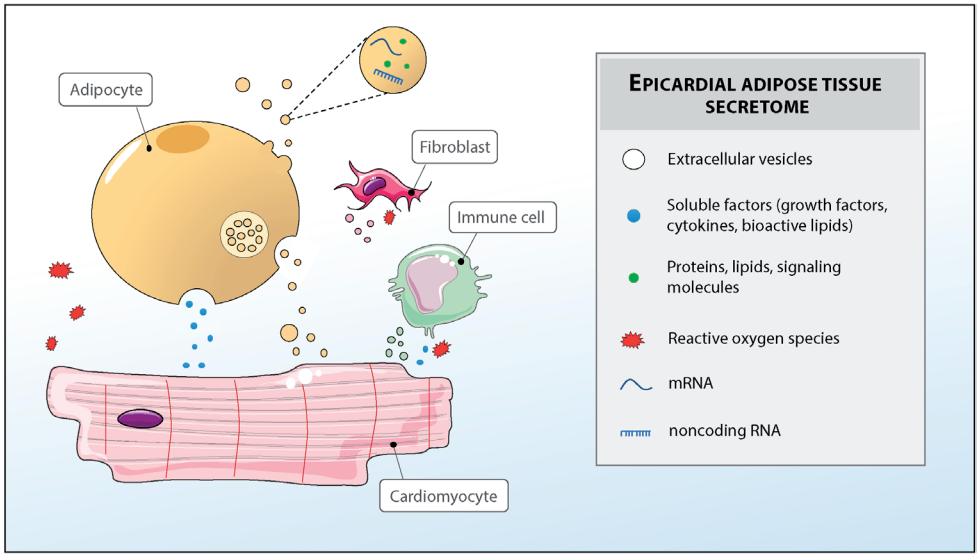


Figure 5 Epicardial adipose tissue secretome.
Epicardial adipose tissue secretes a variety of soluble factors ('secretome', growth factors, cytokines, bioactive lipids) and extracellular vesicles (EVs) carrying various cargo: proteins, lipids, signaling molecules, which may be transferred between cells, therefore playing an important role in intercellular communication. EVs also contain messenger RNAs (mRNAs) and noncoding RNAs (long noncoding RNAs, microRNAs, circular RNAs).

4.2.1 EAT secretome directly modulates cardiomyocytes electrophysiology

Incubation of rabbit atrial cardiomyocytes with isolated EAT adipocytes induces significant changes in action potential characteristics: a longer action potential duration at 90% of repolarization (APD_{90}) and a less negative resting membrane potential[76]. The longer APD can be explained by the changes in several ion currents: a larger late sodium and/or L-type calcium current, combined with a smaller inward rectifier potassium current than control atrial cardiomyocytes, even in the presence of increased transient outward current. In the same way, an 18-hour exposure of H9c2 cells (derived from rat cardiac tissue) to rat EAT secretome induces a significant decrease in the delayed rectifier potassium outward current[77]. Finally, a 24h incubation of sheep epicardial adipose tissue fragments with human induced pluripotent stem cell-derived cardiomyocytes (hIPS-CM) increases the duration of extracellular field potentials, a surrogate measure of action potential duration[60].

Together, these results suggest that EAT secretome exerts a paracrine effect on the myocardium, inducing a prolongation of the APD, thus potentially increasing the

APD heterogeneity in the myocardium (Central illustration) and facilitating arrhythmias.

The net effect of the cellular changes on tissue-level electrophysiology remains to be explored. Using data on the effect of adipocytes on electrophysiological properties[76], De Coster et al.[78] performed an *in-silico* study to project those remodeling effects in a two dimensional monolayer, as well as in a model of human atria. They showed that secretome-induced electrophysiological remodeling of cardiomyocytes created more complexity in the spiral wave dynamics during an pacing induced-arrhythmia in a tissue monolayer model as well as in a human atria model.

The secretome components responsible for the electrophysiological changes observed in cardiomyocytes after EAT secretome incubation are not yet identified. Adipose tissue is recognized as an endocrine organ secreting a high number of cytokines, growth factors and hormones, known as "adipokines".

4.2.2 Adipokines involved in electrical remodeling

Adipokines are produced by adipocytes and by the stromal-vascular fraction of EAT which is composed of endothelial cells, adipocyte progenitors, immune cells, fibroblasts and stromal cell. About 90% of the adipokines released by adipose tissue are secreted by non-adipocytes[79]. Several adipokines are known to electrically remodel cardiomyocytes and stimulate fibrosis. Here, we summarize the relative contribution of adipokines found in EAT[80,81] on electrophysiological changes pertinent to arrhythmogenesis. Of the numerous adipokines identified in EAT, some show potentially pro-arrhythmic effects on in vitro cardiomyocytes through alteration of potassium and calcium currents, and by altering gap junctions.

Transient outward K^+ current (I_{to})

The transient outward K^+ current (I_{to}) plays a critical role in action potential morphology: it is responsible for the early repolarization of cardiomyocyte (phase 1 of the AP). Exposure of cardiomyocytes to adipokines such as Tumor Necrosis Factor alpha (TNF-α) and Interleukin-1β (IL-1β) induces significant changes in I_{to}.

TNF-α, a cytokine involved in systemic inflammation, regulates several key cellular functions including cell proliferation, survival, and apoptosis. TNF-α reduces I_{to} in rat ventricular myocytes[82]. In the same way, IL-1β, a pro-inflammatory cytokine which is part of the Interleukin-1 family of 11 cytokines, is known to alter cardiomyocyte electrophysiology by prolonging the action potential through a decrease in I_{to} in rat hearts[83]. Finally, in hIPS-CM, IL-1β exposure induces a prolonged field potential du-

ration in comparison to untreated hIPS-CM[83]. The duration of the local electrograms (field potential) is used as a surrogate measure of local APD.

These results indicate that TNF-α and IL-1β exposure induce electrical remodeling of cardiomyocyte through a reduced repolarizing K^+ current (I_{to}), resulting in APD prolongation. Since EAT secretome has been shown to increase the APD (cf 4.2.1), it suggests that TNF-α and IL-1β secreted by EAT play an role in this remodeling (Figure 6).

Calcium handling proteins and ion channel modulation

There is evidence that adipokines can modulate calcium dynamics in cardiomyocytes.

Fatty acid-binding protein-4 (FABP4) is involved in transportation of lipids to specific cellular compartments. FABP4 also reduces intracellular systolic peak Ca^{2+} in rat cardiomyocytes[84].

Interleukin-6 (IL-6), a cytokine secreted by several immune cells in EAT, directly modulates L-type calcium channels (an increase or decrease depending on dose and exposure duration) and downregulate SERCA2 activity and expression[85]. This has potential consequences for APD and for Ca^{2+}-induced triggered activity.

IL-1β increases spontaneous diastolic sarcoplasmic reticulum Ca^{2+} release in cardiomyocytes[83] and decrease the expression of Ca^{2+}-handling-proteins[86]. These changes can facilitate reentry by modulating refractoriness and can increase the risk for triggered activity.

Adipokines and gap junction remodeling

Several inflammatory adipokines downregulate the expression of connexin genes (Figure 6). This has consequences for intercellular communication through gap junction in the heart and can result in conduction slowing and increased risk of reentry.

IL-6 reduces Cx40 and Cx43 expression in HL-1 mouse atrial myocytes[87]. High levels of IL-1β are also involved in downregulation and degradation of cardiac Cx43 in mouse models of post-myocardial infarction[88]. Finally, TNF-α exposure reduces Cx40 expression[89] and slows conduction velocity in guinea pig hearts[90].

Several adipokines involved in gap junction remodeling are also important regulators of cardiac fibrosis (Figure 6).

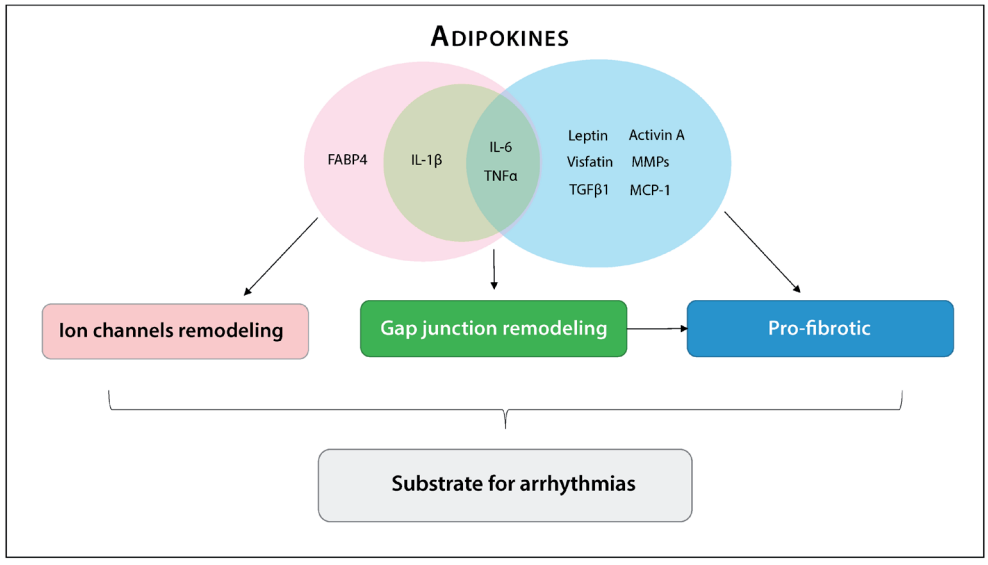


Figure 6. Adipokines secreted by EAT.
Adipokines secreted by epicardial adipose tissue induce ion channel remodeling, gap junction remodeling and are pro-fibrotic.

4.2.3 Pro-fibrotic adipokines

Adipokines have raised much interest over the last decade especially because of their pro-fibrotic effect[72]. Fibrosis is characterized by an excessive extracellular matrix (ECM) production which alters myocardial architecture and impairs conduction[91]. It plays an important role as a potential substrate for arrhythmias, as it is involved in the maintenance of AF and post-myocardial ventricular tachycardias[91]. The following adipokines (Table 2) are either involved in fibroblast proliferation, collagen synthesis and/or myofibroblast activation in vitro, which are well-known mechanisms involved in fibrosis.

Activin-A is part of the TGF-β (transforming growth factor-β) superfamily ligand. Venteclef et al.[72] showed that Activin-A is highly present in the EAT secretome. Treatment of rat atria (placed in organ culture) with Activin-A for 1 week induced increased collagen 1 deposition in comparison to controls (Table 2). This pro-fibrotic effect was blocked with an antibody neutralizing Activin-A.

Visfatin, also called pre-B cell colony-enhancing factor or nicotinamide phosphoribosyltransferase, is secreted by adipocytes and macrophages that have infiltrated into adipose tissue in response to inflammation . Visfatin promotes cardiac fibroblasts proliferation and increased collagen synthesis in rat[92] (Table 2).

Table 2: Pro-fibrotic adipokines secreted by EAT

Summary of the adipokines secreted by Epicardial Adipose Tissue participating in fibrosis through enhancement of fibroblasts proliferation, collagen synthesis and/or myofibroblast activation

Adipokines	Fibroblasts proliferation	Collagen synthesis	Myofibroblast activation
Activin A		↑ (Venteclef[72])	
Visfatin	↑ (YU XY[92])	↑ (YU XY[92])	
TGF-ß1	↑ (Clark[93])	↑ (Seeland[94], Rosenkrank[95])	↑ (Wipff[96])
Leptin		↑ (Zibadi [97])	
Monocyte chemoattractant protein-1 (MCP-1)	↑ (Gharaee-Kermani[98])	↑ (Gharaee-Kermani[98])	↑ (Gharaee-Kermani[98])
Interleukin-6		↑ (Meléndez[99])	

TGF-β_1 is a pro-fibrotic growth factor present in the EAT secretome[100] and plays a major role in cardiac remodeling: it induces the phenotype switch of fibroblasts into myofibroblasts[96] (Table 2), thus promoting ECM production, fibroblast proliferation[93] and fibrosis. Indeed, cardiac overexpression of TGF-β_1 in mice results in interstitial fibrosis[94,95].

Leptin is secreted by EAT adipocytes[80] and is associated with local, increased interstitial fibrosis in rats hearts. Leptin stimulates collagen I production in cardiac myofibroblasts, thus inducing fibrosis (Table 2). Several other studies showed a similar pro-fibrotic effect of leptin[97,101].

Monocyte chemoattractant protein-1 (MCP-1), also known as chemokine ligand 2 (CCL2), regulates cell adhesion and chemotaxis. MCP-1 is secreted by adipocytes and inflammatory cells in EAT[81]. MCP-1 enhances myofibroblast activation, fibroblast proliferation and collagen expression in isolated rat lungs fibroblasts[98] (Table 2).

The contribution of the **interleukin family** to cardiac remodeling is still unknown. IL-6 infusion in rats induces an increased collagen volume fraction[99] (Table 2).

The Matrix metalloproteinases (MMPs) family is composed of proteases that maintain ECM homeostasis by degradation of its components. Amongst others, MMP1, MMP2, MMP8, and MMP9 are secreted at high levels by EAT[72]. TNF-α, which is also secreted by EAT, induces excessive secretion of MMPs[102]. MMPs are likely to participate in ECM remodeling in the heart.

Conclusion and clinical relevance

In this review, we show that epicardial adipose tissue accumulation is closely associated with atrial and ventricular arrhythmias and with electrocardiographic signs associated with arrhythmogenesis. We have identified several key mechanisms elucidating the modulation of arrhythmogenesis by EAT (Central illustration).

Epicardial adipose tissue alters cardiac electrophysiology by creating an anatomical obstacle which delays activation. The heterogeneity of conduction is brought about by the disparate distribution of fat over the atrial myocardium, and by myocardial cellular uncoupling. The resulting heterogeneous conduction slowing facilitates reentrant mechanisms.

Both adipocytes and cardiomyocytes express Cx43, that mediate intercellular exchange of matter and current. Further research is needed to understand whether electrotonic interaction between EAT cells and cardiomyocytes facilitate arrhythmias.

Furthermore, adipokines secreted by EAT induce electrical remodeling and promote fibrosis, thus playing a key role in modulating the substrate for arrhythmias. We hypothesize that paracrine crosstalk and electrotonic coupling between EAT and myocardium leads to resting membrane depolarization of adjacent cardiomyocytes. It remains elusive whether EAT accumulation leads to lipotoxicity which may also contribute to arrhythmogenesis.

Therefore, the various components by which Epicardial Adipose Tissue may alter local electrophysiology will provide an inroad for improved risk assessment and prevention of cardiac arrhythmias.

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