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TOPICAL REVIEW

The role of epicardial adipose tissue in cardiac biology: classic concepts and emerging roles

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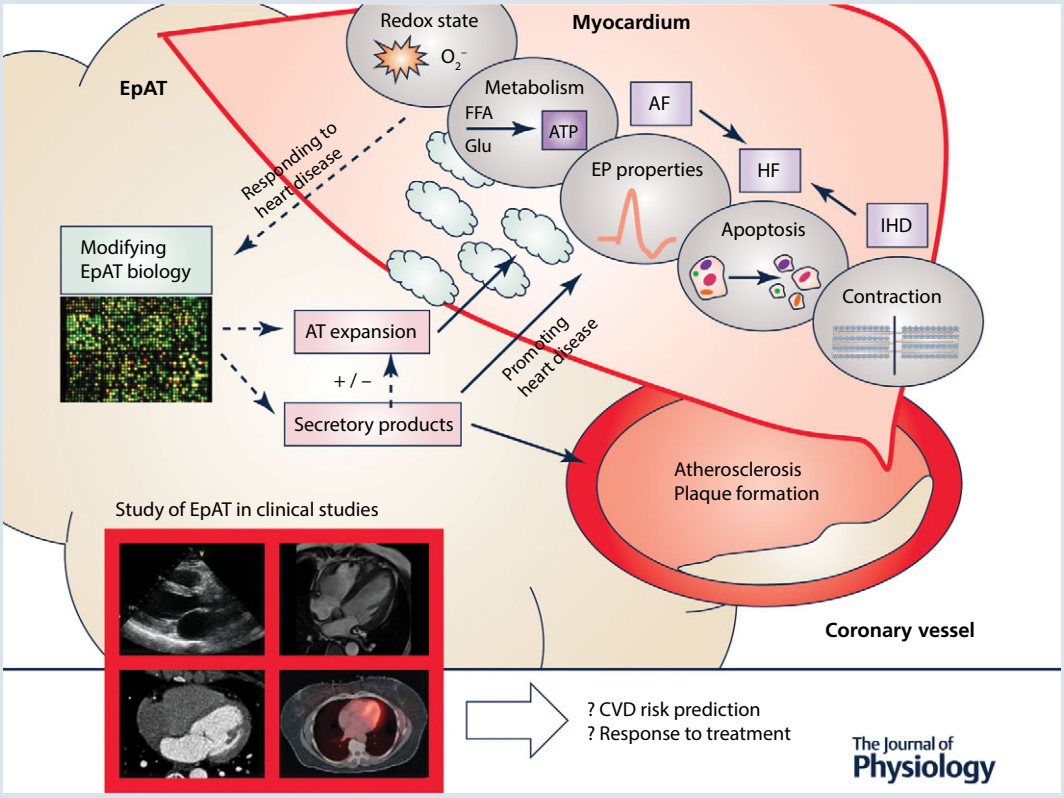

Abstract Classic concepts about the role of epicardial adipose tissue (EpAT) in heart physiology include its role in cardiac metabolism, mechanical protection of coronaries, innervation and possibly cryoprotection of the heart too. Nevertheless, recent evidence has revealed that epicardial adipose tissue regulates multiple aspects of cardiac biology including myocardial redox state, intracellular Ca^{2+} cycling, the electrophysiological and contractile properties of cardiomyocytes,

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cardiac fibrosis as well as coronary atherosclerosis progression. Moreover, it is now understood that the communication between EpAT and the heart is regulated by complex bidirectional pathways, since not only do adipokines regulate cardiac function, but also the heart affects EpAT biology via paracrine 'reverse' signalling. Such complex interactions as well as epicardial fat accumulation as a consequence of cardiac disease and epicardium to adipocyte differentiation should be taken into account by the clinical studies investigating EpAT as a risk marker and its potential as a therapeutic target against cardiovascular disease. Further in-depth exploration of the molecular mechanisms regulating the cross-talk between the heart and EpAT is expected to enhance our understanding regarding the role of the latter in cardiac physiology and relevant disease mechanisms.

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Abstract figure legend Epicardial adipose tissue (EpAT) affects cardiac pathogenesis by direct paracrine effects on cardiomyocyte biology, but also receives signals from the heart and modifies its biology in the presence of cardiac disease. The study of EpAT as a biomarker and/or therapeutic target in cardiovascular disease should take into account these complex interactions and approach alterations in its biology not only as the cause but also as the consequence of cardiac disease. AF, atrial fibrillation; FFA, free fatty acids; HF, heart failure; IHD, ischaemic heart disease.

Abbreviations AF, atrial fibrillation; AT, adipose tissue; CT, computed tomography; IHD, ischaemic heart disease; CVD, cardiovascular disease; EpAT, epicardial adipose tissue; MRI, magnetic resonance imaging; NPR, natriuretic peptide receptor; PET, positron emission tomography; PPARG, peroxisome proliferator activator γ.

Introduction

Visceral adipose tissue (AT) has a well-established role in cardiovascular disease (CVD) pathogenesis by determining systemic insulin resistance and releasing active adipokines into the systemic circulation (Antonopoulos *et al.* 2014). However, further to visceral adiposity, research has recently been focused on the role of ectopic fat depots in CVD pathogenesis. In this aspect, epicardial AT (EpAT) is a fat depot with a potentially important role in cardiac biology, given its close anatomical affinity with the heart. Over the last decade imaging studies have treated EpAT as a quantifiable CVD risk marker (Wang *et al.* 2009*a*; Jacobson *et al.* 2011; Nakanishi *et al.* 2012; Dabbah *et al.* 2014; Mahabadi *et al.* 2014; Stojanovska *et al.* 2015; Mazurek *et al.* 2016), while strong translational evidence (Greulich *et al.* 2011, 2012; Blumensatt *et al.* 2013; Burgeiro *et al.* 2016) supports the existence of a cross-talk between EpAT and the myocardium that is involved in cardiac disease pathogenesis.

Epicardial adipose tissue and cardiac physiology: established knowledge

EpAT is located between the visceral pericardium and the heart, in direct contact with the myocardium. Other terms such as pericardial fat or paracardial fat have been wrongly used in the past to refer to EpAT, but they have a distinct meaning and should not be used interchangeably (Fig. 1). Species-specific differences in EpAT are marked (Marchington & Pond, 1990; Chiou *et al.* 1997); whilst in rodents EpAT is almost absent, in humans it can cover up to 80% of the surface of the heart, found even in sub-epicardium, infiltrating human myocardium (Cherian *et al.* 2012). Interestingly, EpAT has a common embryonic origin with the heart from the splachnopleuric mesoderm, and is supplied with blood by the coronary circulation, facts which suggest that EpAT may be important for cardiac physiology (Cherian *et al.* 2012).

Intrathoracic adipose tissue: the visceral adipose tissue of the chest comprising pericardial and epicardial adipose tissue depots as well as any other adipose tissue located inside the chest wall (excluding any subcutaneous adipose tissue).

Epicardial adipose tissue: the adipose tissue adjacent to the epicardium surrounding the heart, located inside the pericardial sac.

Pericardial adipose tissue: the adipose tissue of the thorax, surrounding the heart located outside the pericardial sac (does not include peri-aortic adipose tissue).

Paracardial adipose tissue: a term that has been used to refer to adipose tissue close to the heart. Should be abandoned since it has a less clear meaning.

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Figure 1. Definitions of epicardial, pericardial and paracardial adipose tissue

EpAT consists of white adipocytes, preadipocytes, stroma-vascular cells as well as ganglionic, nerve and immune cells. The exact role of EpAT in cardiac physiology of mammals remains unclear, but evidence suggests that it is multifaceted: its lipogenic capacity suggests that it serves as a local energy store for the heart and protects cardiomyocytes against influx of high free fatty acid levels and lipotoxicity (Marchington & Pond, 1990); its thermogenic capacity implies that it can protect heart against hypothermia (Sacks *et al.* 2009); it serves as the anatomical site for the ganglia innervating myocardium (Chiou *et al.* 1997); and it mechanically protects the heart and coronary arteries. These classic concepts about the role of EpAT in cardiac physiology are summarized in Fig. 2.

Interest in the study of epicardial adiposity by non-invasive imaging in clinical studies is steadily increasing; echocardiography, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography/CT (PET/CT) have all been employed for the imaging of EpAT (Antonopoulos *et al.* 2016*b*). In agreement with basic science findings, clinical imaging studies have strongly associated EpAT with cardiac disease. Recent evidence from clinical studies using a cross-modality approach (CT/MRI/proton and phosphorus MR spectroscopy) suggest that obese, diabetic patients have increased ectopic (including epicardial) adiposity and this is associated with impaired myocardial energetics and myocardial mechanics (Levelt *et al.* 2016). Inflammatory activity of pericoronary EpAT as assessed by PET/CT imaging, despite certain technical limitations, is associated with coronary atherosclerosis (Mazurek *et al.* 2016). Quantification of EpAT volume by CT imaging has been independently associated with obesity and metabolic syndrome (Wang *et al.* 2009*a*), but also with coronary plaque burden, plaque composition and vulnerability (Hassan *et al.* 2016), the development of coronary atherosclerosis in healthy subjects and the risk of future coronary events in ischaemic heart disease (IHD) patients (Kunita *et al.* 2014). EpAT volume is also an independent risk factor for atrial fibrillation (AF) development. This is expected given the strong association of obesity with AF risk, but EpAT volume provides prognostic information for AF development independently of body mass index and classic cardiovascular risk factors (Zhu *et al.* 2015). Periatrial or total EpAT volume also predicts AF recurrence in patients undergoing AF ablation therapy or AF development post-coronary artery bypass grafting (Kocyigit *et al.* 2015).

A summary of the studies exploring the value of EpAT as a biomarker for CVD risk is provided in Table 1. These findings could also help explain the 'obesity paradox', i.e. the association of obesity with better clinical outcome in patients with established CVD (Antonopoulos *et al.* 2016*b*), which has been based solely on the use of body mass index to define obesity. Clinical studies using CT-based volumetric measurements of fat have shown that increased epicardial fat volume is a strong predictor of the risk of IHD and AF, over and above systemic adiposity indices, contradicting thus the notion of an 'obesity paradox'. It should be noted though that any findings related to the volumetric analysis of EpAT in humans may be biased, since in clinical studies EpAT volume measurements are rarely adjusted for body or heart size, and pathology studies of autoptic human hearts suggest a constant fat/muscle mass ratio irrespectively of the presence of cardiac disease (Antonopoulos *et al.* 2016*b*); for example in a large cohort the association between EpAT volume and AF was lost when adjusted for LA size (Mahabadi *et al.* 2014). Even so, imaging of

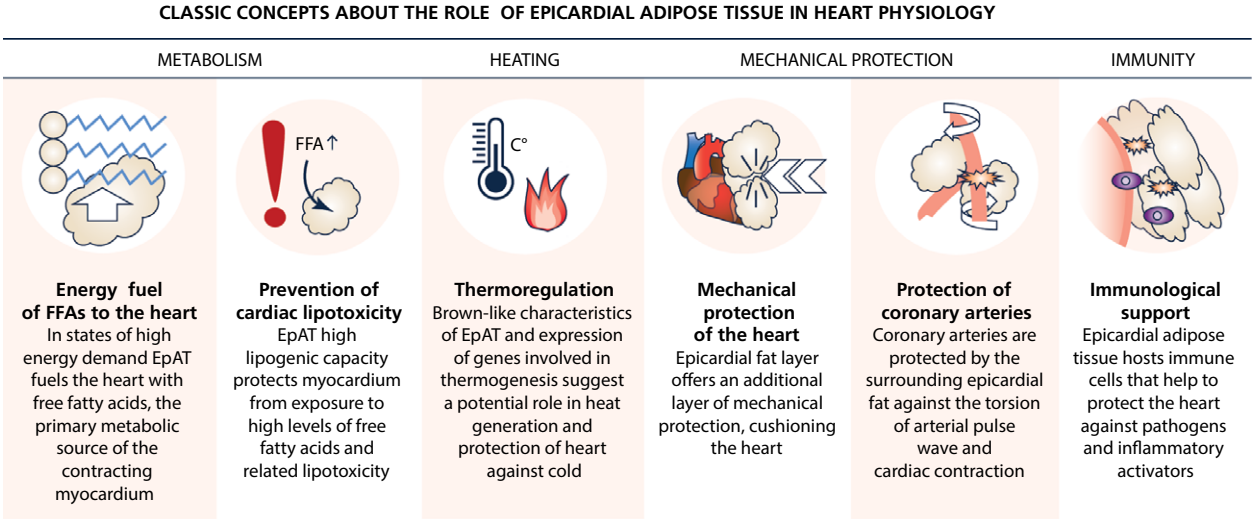

Figure 2. The classic concepts about the role of epicardial adipose tissue in heart physiology

Table 1. Important clinical studies on epicardial/pericardial adipose tissue volume as a risk marker for cardiovascular disease

Study	Study population	FU period	Endpoint	Conclusion
Coronary artery disease				
Mahabadi *et al*. (2013)	4093 healthy subjects	8 years	Coronary events	With each doubling of EpAT increased risk for coronary events HR = 1.54 (95% CI: 1.09–2.19)
Cheng *et al*. (2010)	2571 patients with no CAD	4 years	MACEs	Doubling of PAT volume associated with increased MACE risk OR = 1.74 (95% CI: 1.03–2.95)
Forouzandeh *et al*. (2013)	760 patients with acute chest pain	3.3 years*	MACEs	EpAT volume independently associated with future MACEs
Kunita *et al*. (2014)	722 CAD patients	3.7 years	Coronary events	Increased EpAT volume is a risk factor for coronary events
Nakanishi *et al*. (2014)	517 non-obese CAD patients	> 1 year	ACS	EpAT volume is a strong predictor of future acute coronary syndromes
Ding *et al*. (2009)	998 cases-controls		CAD	For every SD increase in PAT volume, increased HR = 1.33 (95% CI: 1.15–1.54) for CAD
Tamarappoo *et al*. (2010)	1,777 CAD patients	6 months	SPECT–ischaemia	PAT volume is an independent predictor of ischemia at 6 months
Atrial fibrillation				
Mahabadi *et al*. (2014)	3467 healthy subjects	5 years	AF	Left atrial size –but not EpAT volume – is independently associated with AF
Zhu *et al*. (2015)	Meta-analysis of 10 case–control studies		AF	EpAT volume may be associated with an increased risk of AF.
Nakanishi *et al*. (2012)	279 subjects undergoing MDCT	3.3 years	New AF	Periatrial EpAT volume predicts the development of new-onset AF in subjects undergoing MDCT
Yorgun *et al*. (2015)	618 patients (in AF and SR)	n/a	AF	Periatrial and epicardial adipose tissue thickness is independently associated with AF
Kocyigit *et al*. (2015)	249 AF patients post-ablation	29 months	Late AF recurrence	EpAT thickness was an independent predictor for late AF recurrence.

*Median value. ACS, acute coronary syndrome; CAD, coronary artery disease; CI, confidence interval; EAT, epicardial adipose tissue; HR, hazard ratio; MACE, major adverse cardiac event; MDCT, multiple-row detector computed tomography; MESA, Multi-Ethnic Study of Atherosclerosis; n/a, not applicable; OR, odds ratio; PAT, pericardial adipose tissue; SPECT, single photon-emission computed tomography.

EpAT in large clinical cohorts suggests that epicardial adiposity (total/periatrial EpAT, pericoronary EpAT or intramyocardial fat content) may be a more specific and sensitive marker to assess the obesity burden to the heart and its impact on cardiac disease development (Antonopoulos *et al.* 2016*b*).

Recent translational evidence on the role of epicardial adipose tissue in cardiac physiology

The growing interest in the links between cardiac physiology and EpAT has led to the detailed investigation of their communication. EpAT is considered to be a type

of visceral AT; the latter is an active endocrine organ directly involved in cardiovascular physiology by secretion of active adipokines into the circulation (Antonopoulos *et al.* 2014), but whether EpAT can participate in such a role has been debated, given its negligible mass compared to other fat depots. EpAT contains smaller adipocytes and lower insulin-induced glucose uptake compared to subcutaneous AT and expresses low levels of fat-mobilizing genes, having therefore lower lipid storage and lipolytic capacity compared to other fat depots (Burgeiro *et al.* 2016). The metabolic activity of EpAT is further altered in the presence of heart failure, and even though the physiological significance of the decreased lipid storage and lipolytic capacity of EpAT is unknown, it has been suggested that it could represent a protective mechanism against cardiac lipotoxicity (Burgeiro *et al.* 2016). Evidence also suggests that EpAT expresses brown AT-signature genes, e.g. uncoupling protein 1 (Sacks *et al.* 2009), and its transcriptome significantly differs from that of subcutaneous or visceral fat, being even site-specific for the EpAT around the coronaries, atria or ventricles (Gaborit *et al.* 2015).

Studies have now confirmed the important role of EpAT as a modulator of cardiac disease-related mechanisms (Greulich *et al.* 2011, 2012; Antonopoulos *et al.* 2016*a*). Secretory products of EpAT affect cardiomyocytes function either via paracrine mechanisms (i.e. passive diffusion through interstitial space and cellular membranes) or in a vasocrine manner (via coronary vasa vasorum) or secretion into the coronary circulation (Cherian *et al.* 2012). Adipokines secreted by EpAT can exert protective effects on the myocardium; e.g. EpAT-released orosomucoid inhibits caspase-3-mediated apoptosis of cardiomyocytes (Lage *et al.* 2015) and adiponectin binding to its receptors (AdipoR1/2 and T-cadherin) exerts beneficial metabolic anti-oxidant effects on cardiomyocytes (Wang *et al.* 2009*b*). Adiponectin reduces O_2^- generation from myocardial NADPH-oxidase (Antonopoulos *et al.* 2016*a*) and improves nitric oxide bioavailability and endothelial function in the coronary vasculature (Margaritis *et al.* 2013; Antonopoulos *et al.* 2015). Our recent studies have highlighted the importance of Akt and AMP-kinase intracellular pathways in mediating the effects of adiponectin on the vasculature and human myocardium, respectively (Margaritis *et al.* 2013; Antonopoulos *et al.* 2014, 2015, 2016*a*).

Next to their beneficial effects, EpAT-released adipokines can also activate monocytes, directly favouring atherogenesis, via their effects on coronary endothelial and vascular smooth muscle cells (Karastergiou *et al.* 2010). It has been suggested that pericoronary EpAT could even serve as a local storage and supply site for human oxidized LDL to coronary intima, i.e. the hallmark of coronary plaque formation (Uchida *et al.* 2016). Nevertheless a direct link between pericoronary adipose tissue and atherosclerosis cannot be easily established. For example myocardial bridges, i.e. coronary artery segments not covered by EpAT, are protected against atherosclerosis development (Ishikawa *et al.* 1997), but total absence of EpAT such as in congenital lipodystrophy does not prevent coronary atherosclerosis development (Chandalia *et al.* 1995). Other EpAT-secreted products (such as retinol binding protein-4 or activin A) can negatively affect cardiac metabolism (Blumensatt *et al.* 2013); for example activin A secreted from human EpAT induces the expression of miR-143 in human cardiomyocytes and negatively affects Akt signalling and insulin-mediated glucose uptake, possibly by regulation of the availability of oxysterol-binding protein-related protein 8 in cardiomyocytes (Blumensatt *et al.* 2013). Interestingly diabetes mellitus is associated with increased infiltration of EpAT by $CD14^+$ monocytes (Greulich *et al.* 2012), suggesting increased tissue inflammation. This could explain the respective adverse changes in EpAT secretome in animals challenged with high-fat diet (Greulich *et al.* 2011) or diabetes development (Greulich *et al.* 2012; Blumensatt *et al.* 2013) (e.g. increased activin A, reduced omentin 1 release), which lead to changes in phosphorylation of Akt (at Ser143) or SMAD2 in cardiomyocytes (Greulich *et al.* 2012; Blumensatt *et al.* 2013). Such effects of adipokines on cardiomyocytes' intracellular signalling negatively affect the activity of sarco/endoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} cycling, and promote the contractile dysfunction of cardiomyocytes in diabetic patients (Greulich *et al.* 2011, 2012). Obviously the net effect of EpAT-derived mediators on myocardial signalling depends on the biology of the EpAT, as well as the degree of infiltration by immune cells further to the biology of the adipocytes.

Next to the effects on cardiomyocyte metabolism and contractility, EpAT also affects cardiac electrophysiology (Burke *et al.* 1998; Lin *et al.* 2012; Verheule *et al.* 2013; Venteclef *et al.* 2015). EpAT-derived adipokines affect myocardial NADPH oxidase activity, which, as we have previously demonstrated, is a critical determinant for the development of AF in experimental models (Reilly *et al.* 2011) or post-operatively in patients undergoing cardiac surgery (Reilly *et al.* 2011; Antoniades *et al.* 2012). Fibro-fatty infiltrates of EpAT into subepicardium can disrupt the electro-mechanical properties of the myocardium, triggering arrhythmias (Burke *et al.* 1998; Verheule *et al.* 2013). In experimental obesity models, biatrial electrophysiological, electroanatomical and structural remodelling is caused as a consequence of EpAT expansion into atrial tissue and related profibrotic transforming growth factor (TGF) β signalling (Mahajan *et al.* 2015). AF *per se* induces upregulation of adipocyte-specific genes in atrial tissue (Chilukoti *et al.* 2015), favouring intra-atrial fat accumulation, and perpetuating the vicious cycle of fibro-fatty infiltration

in atrial myocardium and AF development. In addition to the direct infiltration of myocardium by fatty tissue, EpAT products can alter the electrophysiological properties of atrial myocytes. Medium from cultured AT negatively affects the action potential duration, L-type Na^+ currents and isoproterenol-triggered beats favouring arrhythmiogenesis (Lin *et al.* 2012). Evidence suggests that the EpAT secretome is rich in adipokines with pro-fibrotic effects, such as thrombospondin 2, vascular endothelial growth factor, activin A, TGF-β1 and matrix metalloproteinase isoforms, in significantly higher levels compared to other fat depots (Venteclef *et al.* 2015). The Hatem group (Venteclef *et al.* 2015) has eloquently shown that EpAT-secreted adipokines induce extensive fibrosis of rat atria in organo-culture models and use of an activin A neutralizing antibody reversed these effects, suggesting that activin A has a pivotal role among secreted adipokines with pro-fibrotic effects and may be even therapeutically targeted (Venteclef *et al.* 2015).

Overall, accumulating experimental evidence suggests a role of EpAT-secreted products in aspects of cardiac biology and the regulation of mechanisms of coronary atherosclerosis (Karastergiou *et al.* 2010; Uchida *et al.* 2016), ischaemic heart failure (Greulich *et al.* 2011, 2012; Antonopoulos *et al.* 2016*a*) and AF (Burke *et al.* 1998; Lin *et al.* 2012; Verheule *et al.* 2013; Venteclef *et al.* 2015) (summarized in Fig. 3).

Evidence for cross-talk between the heart and epicardial adipose tissue. While the causal role of EpAT biology in obesity-related cardiac disease has been widely explored leading to the notion of 'outside-to-inside' signalling, the possibility of a reverse signalling has only recently been investigated (termed 'inside-to-outside' signalling). Studies have supported that the EpAT gene expression profile is shifted towards a pro-inflammatory phenotype in the presence of coronary atherosclerosis (Shimabukuro *et al.* 2013) and heart failure (Burgeiro *et al.* 2016).

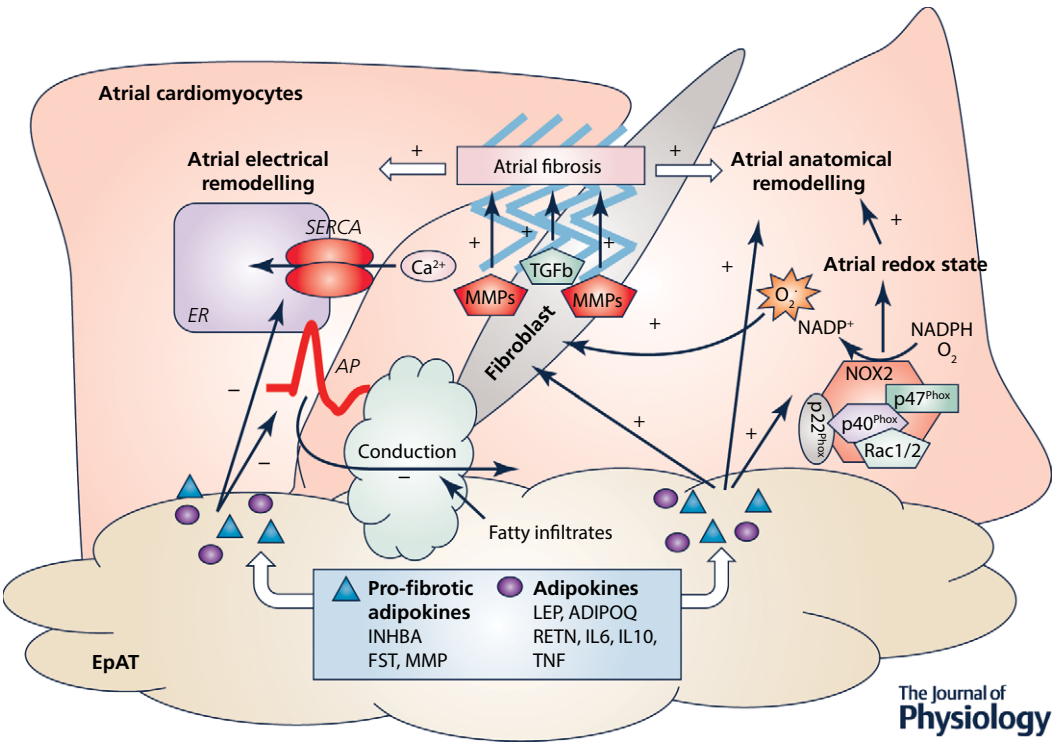

Figure 3. Adipokines and atrial fibrillation development
Adipokines have an impact on atrial electrophysiological properties, action potential (AP) duration and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity of atrial cardiomyocytes, affecting thus arrhythmiogeneicity. Fibrofatty infiltrates into subepicardium also affect *per se* the electrical conduction properties of atrium. Adipokines can modulate NADPH oxidase activity (mainly NOX2) and myocardial redox state in human atria, which is causally involved in atrial fibrillation development. Through the direct effects of adipokines on extracellular matrix (e.g. matrix metalloproteinases) or via their indirect effects on activation of fibroblasts and modulation of myocardial redox state, and promote of atrial fibrosis. The latter is centrally involved in atrial anatomical and electrical remodelling, which disrupts the electrical conduction properties of atrial tissue and favours atrial fibrillation development. ADIPOQ, adiponectin; FST, follistatin; IL, interleukin; INHBA, activin A; LEP, leptin; MMP, matrix metallopeptidases; RETN, resistin; TGFb, transforming growth factor β; TNF, tumour necrosis factor α; list of adipokines is indicative; +, stimulate/induce; –, decrease/impair.

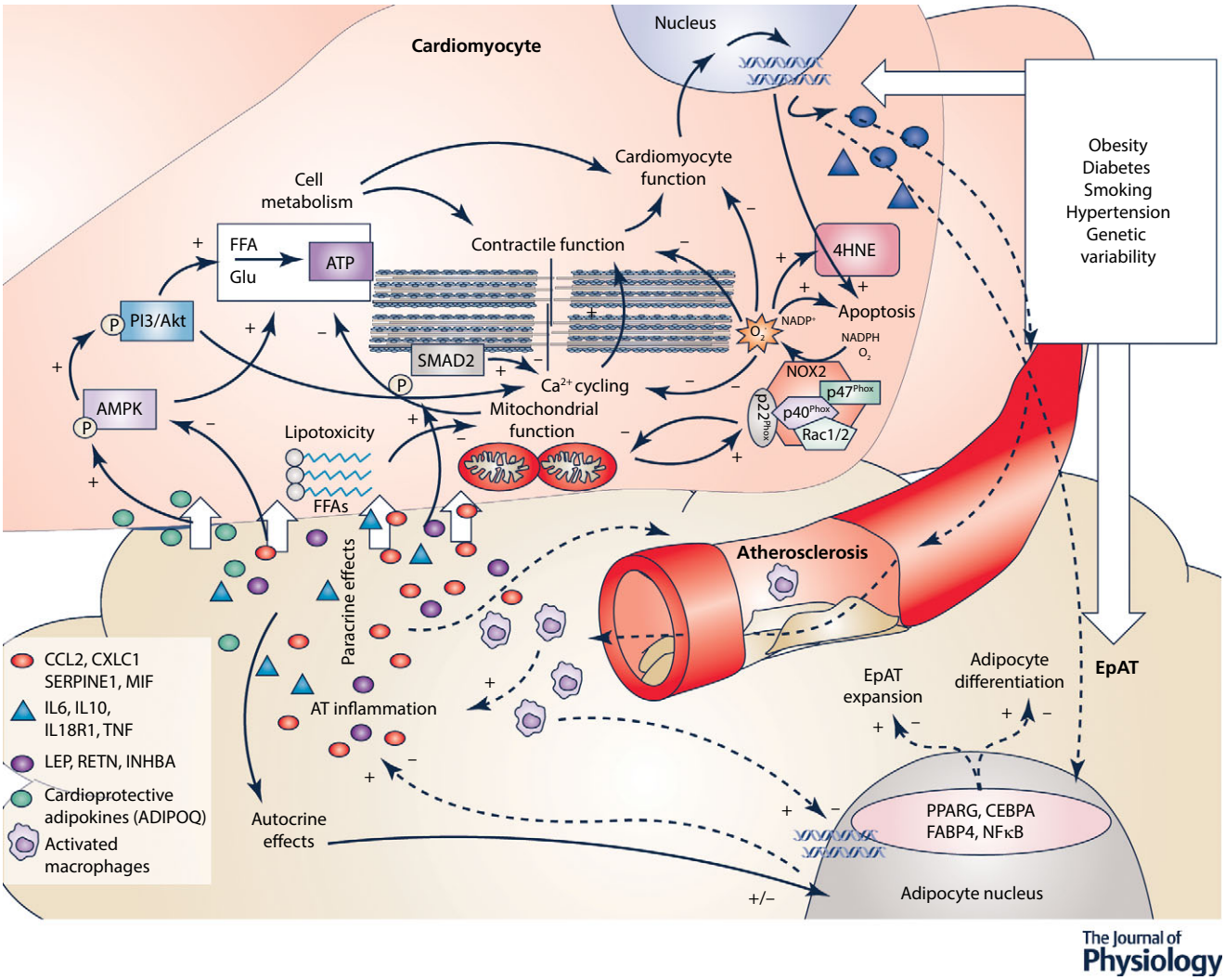

Figure 4. Communication between the cardiomyocytes and epicardial adipose tissue
Epicardial adipose tissue (EpAT) and the cardiomyocyte transcriptomic profile are altered in the presence of cardiovascular risk factors or by genetic variability. Nevertheless, further to any systemic effects, a local cross-talk takes place between cardiomyocytes and EpAT which determines aspects of myocardial biology, cardiac function and coronary atherosclerosis progression. Secreted adipokines (e.g. adiponectin or leptin) differentially affect AMP-activated kinase (AMPK) and phosphoinositide 3-kinase (PI3K)/Akt signalling in cardiomyocytes, which are centrally involved in cardiomyocyte metabolism and substrate utilization. Free fatty acid (FFA)-related lipotoxicity results in mitochondrial dysfunction, impaired oxidative metabolism and increased oxidative stress. NADPH oxidase activity is also enhanced by mitochondrial dysfunction, and alterations in AMPK signalling induced by EpAT-secreted adipokines. Increased phsopshorylation of SMAD2 (e.g. by activin A) and/or reduced PI3K/Akt signalling by pro-inflammatory adipokines negatively affect Ca^{2+} cycling and cardiomyocyte contractility. Increased cardiomyocyte oxidative stress has also direct effects on redox-sensitive proteins of the contractile apparatus and cell apoptosis. Cardiomyocyte stress due to impaired substrate utilization, contractile dysfunction and increased oxidative burden leads to respective changes in cardiomyocyte transcriptome. Products of increased myocardial oxidative stress, such as 4-hydroxynonenal (4HNE, an end product of lipid oxidation) and possibly others among the cardiomyocyte secretome may signal back to EpAT and affect key aspects of its biology, such as the differentiation of adipocytes, adipose tissue expansion and its infiltration by inflammatory cells as well as the regulation of transcriptional factors and relevant gene expression profile, which is shifted towards a pro-inflammatory phenotype. The concept of a bidirectional signalling between the heart and EpAT is represented with continuous ('outside-to-inside' signalling) and dashed arrows ('inside-to-outside' signalling) respectively. ADIPOQ, adiponectin; CEBPA, CCAAT/enhancer-binding protein α; CXCL1, C-X-C motif chemokine ligand 1; FABP4, fatty acid binding protein-4; IL, interleukin; CCL2, C-C motif chemokine ligand; INHBA, activin A; LEP, leptin; MIF, macrophage migration inhibitory factor; NFκB, nuclear factor κB; PPARG, peroxisome proliferator activator receptor γ; RETN, resistin; SERPINE1, serpin family E member 1; SMAD2, mothers against decapentaplegic homolog 2; TNF, tumour necrosis factor α; list of adipokines is indicative. +, stimulate/induce; –, decrease/impair; short white arrows represent diffusion of adipokines.

It is now understood that a two-way interaction between adipocytes and cardiomyocytes occurs, with the latter affecting adipocytes' gene expression in a paracrine manner (Anan *et al.* 2011). Proteomic analyses of EpAT demonstrate increased expression of redox-related proteins compared to subcutaneous fat, suggesting that EpAT has been adaptively evolved to cope with high – local – oxidative stress burden, possibly due to signals received from the adjacent myocardium (Salgado-Somoza *et al.* 2010). Recent evidence from our group (Antonopoulos *et al.* 2016*a*) supports a cross-talk between the myocardial redox state and peroxisome proliferator activator γ (PPARG)–adiponectin axis in EpAT. Under conditions of increased myocardial oxidative stress, stress signals (e.g. 4-hydroxynonenal and possibly others too) released from cardiomyocytes affect PPARG/adiponectin expression in EpAT as a means to locally regulate and lower myocardial oxidative stress by inhibition of NADPH oxidase activity (Antonopoulos *et al.* 2016*a*). Besides, not just locally, but also at a systemic level, natriuretic peptide binding to their highly expressed receptors in adipose tissue elicits lipolytic effects. For example, natriuretic peptide receptor type 1 (NPR1) and type 2 (NPR2) signalling stimulates the guanylyl cyclase–cyclic GMP–protein kinase G (PKG) pathway in adipocytes, which increases the expression of hormone-sensitive lipase (HSL) and lipolysis globally in adipocytes (Antonopoulos *et al.* 2014). To the contrary, signalling via NPR3, whose expression is up-regulated in the presence of obesity or diabetes, leads to natriuretic peptide internalization and degradation in lysosomes counteracting the beneficial systemic metabolic effects of natriuretic peptides. The potency of the lipolytic effects of natriuretic peptides on human adipose tissue seems to be highest for type A and lowest for type C natriuretic peptide. Deficiency of natriuretic peptides is causally involved in systemic insulin resistance and diabetes development. Our recent studies on human adipose tissue (Antonopoulos *et al.* 2014) also suggest that B-natriuretic peptide is a driver of adiponectin release globally in all human adipose tissue depots, over-riding any local effects of endogenous adipose tissue inflammation in patients with IHD.

In the cross-talk between the human heart and EpAT, of particular interest is the adipogenic capacity of epicardial cells. This transformation is apparent in murine models of myocardial injury, where mesothelial lineage cells differentiate to adipocytes following myocardial infarction (Zangi *et al.* 2017). Indeed epicardial and subepicardial layers host epicardial progenitor derived cells (EPDCs) which can be engaged in adipocyte transformation via pro-adipogenic factors modulating the epithelial-to-mesenchymal transition process (Suffee *et al.* 2016). Preliminary evidence supports that human atrial myocytes can be the source of such pro-adipogenic factors and regulate this process of EPDC differentiation to mature adipocytes and epicardial fat accumulation (Suffee *et al.* 2016). Such observations suggest that EpAT may be actually derived from the adipogenic transformation of epicardium (Yamaguchi *et al.* 2015). Indeed intramyocardial fat and fibrous tissue infiltrates that are found in AF or cardiomyopathies (such as in arrhythmogenic right ventricular cardiomyopathy) could be the result of this adipogenic process, and thus epicardial adiposity could be the consequence (rather than the cause) of advanced cardiac disease (Yamaguchi *et al.* 2015).

Therefore modulation of pathways pivotally involved in adipocyte differentiation, adipogenesis or lipolysis by paracrine 'inside-to-outside' signalling from cardiomyocytes suggests that EpAT expansion and remodelling may be at least partly regulated by cardiac disease-related mechanisms. It is now understood that EpAT biology is regulated by both systemic factors (e.g. insulin resistance, obesity) and local stimuli from the heart (Antonopoulos *et al.* 2016*a*) or the coronaries (Margaritis *et al.* 2013; Antonopoulos *et al.* 2015), which can further strengthen or even outweigh any systemic effects. Moreover, the triggered 'rescue responses' of EpAT in the presence of cardiac disease, such as the upregulation of adiponectin expression, could be potential therapeutic targets against CVD (Woodward *et al.* 2016). The cross-talk between the heart and EpAT is summarized in Fig. 4.

Unresolved issues and future perspectives

Despite the well-acknowledged roles of EpAT in cardiac physiology, several issues remain less-well clarified. For example it is still not unknown which factors regulate EpAT secretome and the equilibrium between the beneficial and deleterious EpAT-derived adipokines. Systemic factors such as obesity and insulin resistance are strong determinants of EpAT secretome profile, but the latter is also influenced by local signals derived from the heart. In addition, it is currently debatable whether a pro-inflammatory phenotype of EpAT is the result or the cause of coronary atherosclerosis and heart disease, given the two-way interactions between EpAT and the heart. Finally, while the quantification of EpAT volume as a biomarker in cardiac disease has been extensively explored, there are not currently any available imaging modalities to reliably assess EpAT inflammatory status. The complex interactions between the heart and EpAT should be taken into account by the clinical studies investigating the value of EpAT as a risk marker and its potential as a therapeutic target in cardiac disease.

Conclusions

Firm evidence supports that EpAT has a role in cardiac metabolism, mechanical protection of coronaries and thermogenesis, as well as in the regulation of

myocardial redox state, Ca^{2+} currents, electrophysiological and contractile properties of cardiomyocytes, cardiac fibrosis and coronary atherosclerosis progression. Epicardial cells transformation to adipocytes and the paracrine effects of epicardial adipocytes on human cardiomyocyte and fibroblast biology generate a nexus of complex bidirectional actions, which is centrally involved in cardiac disease pathogenesis. In the light of this knowledge, EpAT 'dysfunction' should not be considered only as the cause but also as the consequence of cardiac disease since EpAT receives paracrine 'reverse' signalling from the adjacent myocardium. Further in-depth exploration of the molecular mechanisms regulating the cross-talk between the heart and EpAT is expected to enhance our understanding regarding the role of the latter in cardiac physiology and related disease mechanisms.

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Additional information

Competing interests

None.

Author contributions

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