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Review

Exploring the role of epicardial adipose-tissue-derived extracellular vesicles in cardiovascular diseases

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SUMMARY

Epicardial adipose tissue (EAT) is a fat depot located between the myocardium and the visceral layer of the epicardium, which, owing to its location, can influence surrounding tissues and can act as a local transducer of systemic inflammation. The mechanisms upon which such influence depends on are however unclear. Given the role EAT undoubtedly has in the scheme of cardiovascular diseases (CVDs), understanding the impact of its cellular components is of upmost importance. Extracellular vesicles (EVs) constitute promising candidates to fill the gap in the knowledge concerning the unexplored mechanisms through which EAT promotes onset and progression of CVDs. Owing to their ability of transporting active biomolecules, EAT-derived EVs have been reported to be actively involved in the pathogenesis of ischemia/reperfusion injury, coronary atherosclerosis, heart failure, and atrial fibrillation. Exploring the precise functions EVs exert in this context may aid in connecting the dots between EAT and CVDs.

INTRODUCTION

Epicardial adipose tissue (EAT) is a distinctive fat depot that interacts anatomically and functionally with the heart, located between the myocardium and the visceral layer of the epicardium. Because it has been proposed as a biomarker of visceral adiposity, its variability reflects the variation in intra-abdominal fat accumulation. EAT is particularly important for cardiac function, given its location (under the pericardium) and because it acts as a metabolically active endocrine organ, exerting cardioprotective functions and providing a source of energy and heat to the heart. Indeed, in physiological conditions, EAT participates in the homeostasis of the cardiovascular system by storing triglycerides that produce energy in the myocardium and provides structural support preventing coronary arteries from twisting.

In pathological conditions (e.g., obesity), the nature of the effects of EAT switch from being protective to detrimental. Indeed, EAT has been observed to contribute to atherogenesis; it has been associated with hemodynamic impairments, an enhanced ventricular interdependence, a reduced exercise capacity, and an increased risk of atrial fibrillation.[1,2] However, precise molecular mechanisms linking EAT to cardiovascular diseases have not yet been described. The underlying mechanisms that have most generally been hypothesized are related to inflammation exacerbation of the innate immune response, oxidative stress, endothelial damage, adipocyte stress, lipid accumulation, and glucotoxicity.[3] Within this context, extracellular vesicles (EVs) are gaining interest in terms of their role in both physiological and pathological conditions.[4] EVs are known to be key players in intercellular communication, given their ability to transport proteins, lipids, and nucleic acids, all of which are reflective of the state of their specific cell of origin.[5] It is well established that EVs are involved in the multiple CVDs, including atherosclerosis,[6,7] heart failure, cardiomyopathies,[8,9] and atrial fibrillation.[10] Given the difficulties in assessing the paracrine contribution of EAT to CVD, the specific signature carried by EVs derived from EAT may provide an insight on unexplored mechanisms through which this particular tissue may, in part, promote the onset and progression of cardiovascular diseases. Levels of circulating EVs are detectable in plasma of healthy subjects and are elevated in patients with cardiovascular risk factors, such as hypertension and dyslipidaemia.[11] Thus, EVs could also be used as biomarkers to monitor the progression of cardiovascular diseases.[12]

This review addresses the role of EVs as key mediators of the inter-organ crosstalk between EAT and the heart (Figure 1). Firstly, the anatomy and physiology of EAT is discussed, followed by a description of the composition and function of EVs. The role of EAT-derived EVs in some of the main CVDs such as coronary artery disease, atrial fibrillation, and heart failure, which are related to EAT regional distribution, is also described (e.g., EAT surrounding the left atrium is not the same as that infiltrating the coronary arteries).

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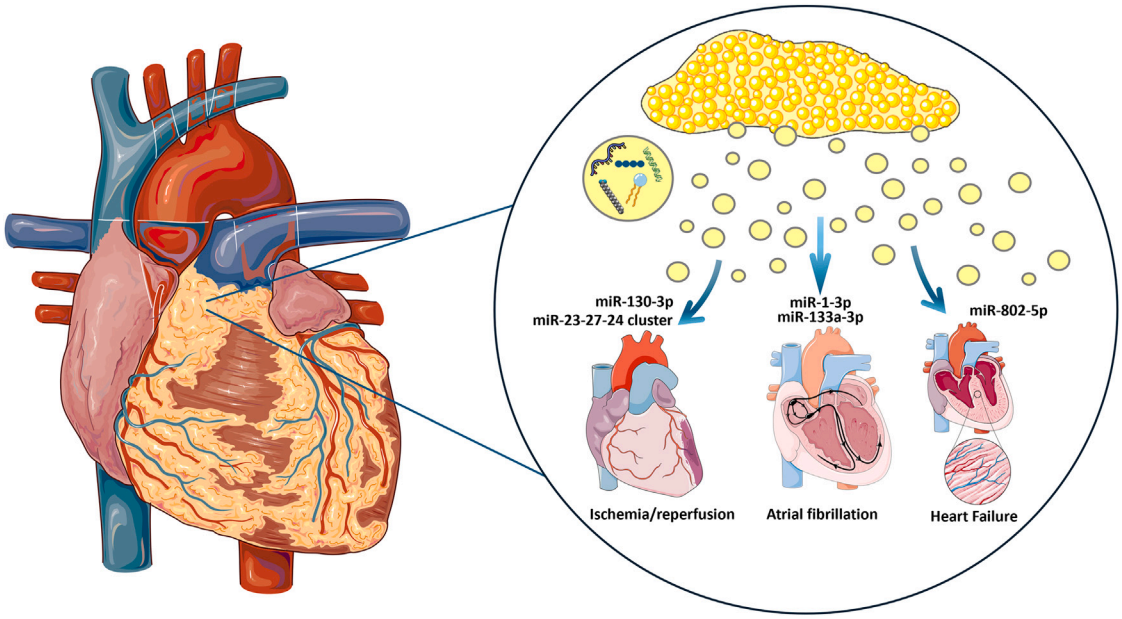

Figure 1. Schematic representation of the involvement of epicardial-adipose-tissue-derived extracellular vesicles in the context of cardiovascular diseases

EAT: ANATOMY AND PHYSIOLOGY

The adipose tissue of the heart can be categorized into two distinct compartments according to their respective anatomical location: epicardial fat and pericardial fat.[13] EAT is the fat depot located between the myocardium and the visceral layer of the epicardium, whereas the pericardial fat is located on the outer surface of the parietal pericardium.[14] EAT accumulates within the interventricular and atrioventricular grooves and is present along the coronary vessels, the right edge of the wall of the right ventricle, on the anterior wall, and around the apex of the heart.[15] According to its specific anatomical distribution, the EAT located directly around or on the coronary artery adventitia is known as "pericoronary EAT"; "myocardial EAT" is the fat depot covering the myocardium, whereas periatrial and periventricular EAT is distributed within and around the atria and ventricles, respectively. Although the externally located pericardial adipose tissue receives its blood supply from branches of the internal thoracic artery,[16] EAT receives its blood supply from branches of the coronary arteries, given their close vicinity. A unique aspect consists in the fact that EAT and the myocardium share the same microcirculation,[17] due to the lack of any kind of barrier or muscle fascia separating them. No other visceral fat depot presents this contiguity with an organ. The proximity between these two anatomical districts enables their interaction through both paracrine and vasocrine pathways. This feature may be explained by the common embryological lineage that EAT in fact shares with the epicardial layer of the myocardium, arising from splanchnopleural mesothelial protrusions so as to form the proepicardial organ.[16] Although not in the remit of the present review, it is worth mentioning that macroscopically, the measurement of EAT thickness can be assessed by echocardiography and computed tomography. Whereas the former consists in a simple, accurate, and readily available method, the latter allows a precise assessment of pericoronary or periatrial epicardial fat volume; however, it is a more expensive and cumbersome technique. For further description, it is suggested to refer to specific reviews.[18] Microscopically, EAT is composed mainly of adipocytes, but also contains nerve cells, inflammatory cells (mainly macrophages and mast cells), stromal cells, vascular cells, and immune cells. There are distinct cell subpopulations of EAT. Larger unilocular adipocytes are primarily involved in the storage of triglycerides for *de novo* lipogenesis to serve as a potential energy source for the epicardium, whereas smaller adipocytes expressing uncoupling protein 1 (UCP-1) found in the periventricular area are associated with thermogenesis.[16] Similarly to visceral adipose tissue, EAT can secrete adipokines. In this way, it may exert a protective role by releasing anti-inflammatory adipokines with cardioprotective effects (i.e., adiponectin).[13]

EAT is a white adipose tissue but also has brown fat-like and beige fat-like features.[19] Human biopsies showed that the adipocytes of EAT are smaller than those in peritoneal and subcutaneous fat and display a higher basal rate of fatty acid metabolism and lipogenesis. Adult EAT maintains brown fat-like and beige fat-like features, which are associated with improved cardiometabolic health. Loss of brown fat-like features is associated with aging and accelerated in chronic diseases.[20,21]

Physiologically, EAT has a protective role. Indeed, it represents an indispensable energy source for the myocardium, providing energy through the beta-oxidation of long chain fatty acids, which are then used by the heart as its main energy source.[13] EAT exhibits a greater ability to take up and release FFAs compared with other fat depots and, given the proximity between EAT and cardiomyocytes, FFAs may directly diffuse from EAT into the myocardial cells, or they can be actively transported through the coronary circulation.[17,22] Adipocyte fatty-acid-binding protein (also known as FABP4), highly expressed in EAT, participates in the intracellular transport of fatty acids from epicardial fat into the

myocardium, while however protecting the myocardium from exposure to high fatty acid levels. Moreover, EAT possesses "beige-like" features, due to the expression of uncoupling protein 1 (UCP1), a specific mediator of thermogenesis in brown adipocytes, suggesting that EAT may protect the heart from hypothermia through heat production.[23] Specifically, this adipose depot could protect the myocardium from, for example, developing fatal ventricular arrhythmias during a drop in core body temperature from cold exposure.[24] EAT thus possesses cardioprotective functions, providing a source of energy and heat to the heart. As its transcriptome, the EAT secretome is unique and very different from that of other fat depots, being enriched in genes encoding cardioprotective adipokines, such as adiponectin, with potential anti-inflammatory and anti-atherogenic properties, and omentin, or involved in the regulation of endothelial function, extracellular matrix remodeling, thrombosis, coagulation, potassium transport, and inflammation.[25,26] Conversely, under pathological circumstances, epicardial fat becomes harmful for the heart, e.g., in the case of obesity, EAT produces inflammatory mediators. Specifically, pericoronary EAT seems to be implicated in atherogenesis, whereas EAT accumulated around the atria is associated with atrial fibrillation and those accumulated over the right or left ventricles show association with ventricle mass.[2,27,28]

Despite the positive correlation observed between the size of adipocytes from different fat depots and body mass index, the explanation of this relationship is still not clear because it did not seem due to an increased hypertrophy of EAT adipocytes. A plausible explanation may lie in the augmented proportion of smaller adipocytes in EAT of patients with obesity when compared with overweight and normal individuals, thus suggesting a hyperplastic remodeling of EAT in this pathological condition.[29] Larger adipocytes usually release increased amounts of proinflammatory mediators such as leptin and resistin and lower amounts of adiponectin.[30] In patients with obesity, a dysregulation of the pro- and anti-inflammatory adipocytokine production leads to a state of chronic low-grade inflammation, which may contribute to the development of atherosclerosis and cardiovascular diseases.[30] This dysregulation is also observed in EAT, which exhibits significantly increased levels of chemokine (monocyte chemoattractant protein-1 [MCP-1]) and inflammatory cytokines (interleukin-1 [IL-1], IL-6, tumor necrosis factor alpha [TNF-α]) compared with subcutaneous fat in patients with CVD.[31] This condition, together with obesity, has also been associated with a decreased production of the anti-inflammatory adipokine adiponectin.[32] The close relationship existing between EAT and inflammation therefore appears clear: the release of proinflammatory adipokines from EAT may contribute to the systemic inflammatory state, and systemic inflammation promotes the accumulation of EAT, contributing to local inflammation.[33] The cellular population of EAT is rich in M1 macrophages and mast cells that infiltrate the coronary artery adventitia.[31,34]

The dysfunction of EAT in obesity could be also related to an eventual onset of insulin resistance and excessive influx of FFA. Generally, insulin resistance increases with body fat, particularly in the visceral compartment. In the case of obesity, EAT adipocyte size is positively correlated with insulin resistance, measured through the HOMA (homeostatic model assessment) index.[30] Activation of the advanced glycation end products/receptor advanced glycation products (AGE/RAGE) system in diabetic EAT contributes to the oxidative stress and endothelial damage associated with diabetic CVD.[35]

Dysfunctional EAT can also be considered an independent risk factor for atrial fibrillation development and recurrence after catheter ablation. The EAT infiltrating the atrium is enriched in genes encoding proteins with potential arrhythmogenic properties that regulate oxidative phosphorylation, muscular contraction, and calcium signaling.[19]

Finally, accumulation of ventricular EAT is associated with myocardial fibrosis, ventricular hypertrophy, and increased cardiac filling pressures, which are features of heart failure with preserved ejection fraction.

EXTRACELLULAR VESICLES: BIOLOGY AND FUNCTION

EVs are a heterogeneous population of small phospholipid bilayer-delimited particles, devoid of a nucleus, released from all cell types in most biological fluids. While once thought to be a means of eliminating cellular waste,[36] EVs are now known to play a pivotal role in various physiological and pathological processes. Owing to their ability to transfer molecular cargo (i.e., proteins, nucleic acids, lipids, even whole and/or fragmented organelles) from one cell to another, it is generally accepted that EVs consist in crucial players in the scheme of intercellular communication in multicellular organisms.[5]

The fundamental heterogeneity of EVs is what renders their classification complicated. So as to avert further contradictory definitions and inaccurate experimental expectations, the International Society of Extracellular Vesicles recommends that EV description be performed using operational terms based on size, according to which EVs may be defined as small ("sEVs," <100 nm or <200 nm) or medium/large ("mEVs"/"lEVs," >200 nm); density range (low, middle, or high); biochemical composition, referring to the presence of specific proteins known as tetraspanins (cluster of differentiation 63 [CD63], CD81) or Annexin A5; and/or state of the conditions of the cell of origin (i.e., "hypoxic EVs," "cardiomyocyte-derived EVs," and so on).[37] Evidence reporting that protein content substantially differs between small and large EVs further supports the feasibility of employing size as a method for EV classification.[38]

EV isolation is achieved according to one and/or more of these characteristics by a multitude of techniques, from various sources. For a summary of techniques most employed for performing EV isolation, refer to Table 1. Recent efforts have also been made at developing novel EV classification methods, such as Kanao's group who proposed to categorize EVs according to their surface glycan structures.[39]

Although not in the remit of the present review, it is worth mentioning that non-vesicular extracellular nanoparticles (NVEPs), particles lacking an outward lipid bilayer also found to be quite abundant within the extracellular space and bodily fluids, have recently acquired appreciation within the context of extracellular nanosized "anuclear" particles.[54] These types of particles include the well-established groups of lipoproteins and nucleosomes but also the recently discovered exomeres[55,56] and supramers.[57,58]

Some of the first evidence of the existence of EVs dates to 1946, following Peter Wolf's demonstrations, in which a phospholipid-rich material present within plasma responsible for its coagulative activity was identified and defined as "platelet dust."[59] Initially these

Table 1. Most commonly employed techniques for extracellular vesicle isolation

Method of isolation	Description of method	Pros and cons
Differential Ultracentrifugation	Based on the difference in particle sedimentation coefficient (dependent on particle size and density). Particles with different sedimentation coefficients pellet at different centrifugation speeds. Sequentially increasing the centrifugal force (1000–3000 g) removes non-EV particles from the sample. The "pre-clearance" procedure is followed by ultracentrifugation (100,000–200,000 g) to ensure the obtainment of an EV-enriched pellet. Factors including rotor type and sample viscosity must be taken into consideration.[40,41]	**Pros:** well-established and accessible method.[40] **Cons:** co-isolation mostly with HDL[42]; can result in EV clumping and/or damage.[43]
Density Gradient Centrifugation	A density gradient is applied to isolate EVs, which distribute throughout a density gradient matrix according to particle sedimentation and flotation index. The EV isolation can depend on particle size and mass density ("top-down gradient") or mass density only ("bottom-up gradient"). Sucrose and iodixanol are the density media most often employed.	**Pros:** EV preparation obtained devoid of protein contaminants.[44] **Cons:** laborious, low throughput, and unable of guaranteeing total depletion of lipoproteins.[45]
Size Exclusion Chromatography (SEC)	SEC is a size- and shape-based method for EV isolation. Samples flow through a stationary phase within a chromatography column with a defined pore size, whereby large particles elute earlier than smaller particles. For optimization of EV yield, this method is used as a first step in combination with other EV isolation methods.[46]	**Pros:** 99% of soluble plasma proteins, along with >95% of HDL, are removed, EV integrity is retained, and their biological activity is not impacted. This method has the advantage of being fast and straight-forward and does not require large starting sample volumes.[47] **Cons:** this method is prone to lipoprotein contamination. The major co-isolated non-EV components are particles above the size cutoff, which mainly include chylomicrons (especially present in plasma from non-fasting subjects), VLDLs, and LDLs.[48]
Ultrafiltration	Based on particle size. The use of membrane filters provides enrichment of EVs based on their size in relation to membrane pore size, enabling separation from soluble components. This method is often employed in combination with other EV isolation techniques, such as SEC.[49]	**Pros:** time efficient; capable of concentrating down large volumes of starting sample; high EV recovery.[50] **Cons:** presence of protein aggregates and lipoproteins as major contaminants in the obtained EV isolate.[51]
Immunoaffinity	This method is based on the interaction between specific EV surface proteins and antibodies, which can be employed to select desired EV populations or trap unwanted EV populations (negative selection or immune-depletion).[49]	**Pros:** high-throughput technique as parallel assays may be performed[40]; all particles that do not bind to the protein of interest are removed.[52] **Cons:** high costs and poor biological activity of isolated EVs.[53]

HDL, high-density lipoprotein; EV, extracellular vesicles; LDL, low-density lipoproteins; SEC, size exclusion chromatography; VLDL, very low-density lipoproteins.

platelet-derived vesicular entities were thought to represent the majority of circulating EVs (70%–90%), whereas studies then demonstrated that these comprise around 30%–50% of total EVs within the circulation.[60] Indeed, circulating EVs can derive from multiple cell types and tissues and are not only involved in countless physiological processes (i.e., maintenance of homeostasis)[61] but also recognized to be well-established contributors to disease onset and progression.[62] The ubiquitous release of EVs from cells into most biological fluids (i.e., blood, saliva, lymph, pericardial fluid, urine), along with the enrichment in biological cargo that reflects the state of their cellular origin, bestows upon EVs the unique property of serving as readily accessible "molecular fingerprints," providing a proxy measurement of tissues and organs. This feature is what renders these nano-sized structures most attractive, as they potentially possess the characteristics of what is defined as a "good biomarker." This potential that EVs yield also owes to the fact that their internal content is protected, being enclosed within a hydrophilic lumen surrounded by a phospholipid bilayer and is therefore shielded from enzymes that would catalyze their degradation.[63] Additionally, the enrichment of molecular cargo within EVs enables the detection of proteins and nucleic acids such as miRNAs

Table 2. Online databases that contain lists of RNA, proteins, lipids, and metabolites that are identified in EVs

Online resource	Description
Vesiclepedia (http://microvesicles.org)	Web-based manually curated collection of EV-associated molecular data, including mRNAs, miRNAs, proteins, lipids, and metabolites from both published and unpublished studies.[79]
EVpedia (http://evpedia.info)	Integrated proteome, transcriptome, and lipidome database of EVs derived from archaea, bacteria, and Eukarya, including human. It allows to perform comparisons of vesicular datasets by ortholog identification, Gene Ontology enrichment analyses, and network analyses of vesicular proteins and provides databases of vesicular mRNAs, miRNAs, and lipids.[80]
exoRBase 2.0 (http://www.exoRBase.org)	exoRBase is a depositary of EVs long RNAs (exLRs) derived from RNA-seq data analyses in four different human body fluids. The exLRs contain messenger RNA (mRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA). The updated website features the integration and visualization of RNA expression profiles, as well as the functional pathway-level changes and the heterogeneity of origins of circulating EVs.[81]
EV-TRACK Platform (http://evtrack.org)	Knowledgebase that records experimental parameters of EV-related studies and implements a bottom-up community consensus approach, encouraging researchers to upload published and unpublished experiments while providing feedback.[82]
exRNA Atlas (http://exrna-atlas.org)	Repository including small RNA sequencing and qPCR-derived exRNA profiles from human and mouse biofluids.[83]

EV, extracellular vesicle; RNA, ribonucleic acid.

that would otherwise be missed,[64] due to the high dynamic range of concentration of these molecules within biological fluids, especially in blood plasma.[65]

The amount and type of biomolecular content loaded within and/or on EVs are influenced by the conditions of the releasing cells, along with the stimuli that modulate their release, whether they be of physiological or pathological nature.[66] Among the broad range of bioactive molecules carried by EVs, RNA molecules, especially microRNAs (miRNAs), are garnering much interest in the emerging field of liquid biopsies.[67,68] Although the debate continues regarding the implication of EV-miRNAs in modulating biological activities at the molecular level,[69] given their low average copy number, it is clear that their concentrations are sufficient to influence cell programming.[70] This is particularly noteworthy also given the well-established association between EV-miRNA expression patterns and certain cardiac diseases.[71]

Concerning EV protein content, whether they reside within the lumen, the membrane structure, or upon their outer surface, EV proteins represent important elements that not only possess relevance for EV subtype classification,[72–74] but also on a functional and clinical level.[7] The use of EV protein profiles, which sheds light on their cellular origin, is becoming increasingly popular to monitor disease onset and progression. By reflecting changes on a cellular level during certain stages of disease, EV proteins further support the plausibility of using these biological nanosized particles in a clinical context.[75]

Although lipids constitute basic structural and functional components of EVs, responsible for maintaining their stability within the extracellular environment, bioactive lipid mediators shuttled by EVs also cover a significantly relevant role in EV biology.[76] Indeed, EVs constitute of membrane lipids organized in a bilayer structure. Studies have reported that cholesterol is the most present of lipids in EV preparations, representing one of the main constituents of both the outer and inner EV plasma membrane. Phosphatidylserine has also been mentioned as a relevant lipid in EV preparations and seems to have a functional role in signaling mechanisms with certain cell types.[77] EVs are also enriched in certain bioactive lipid species enclosed within the vesicular lumen. These biomolecules attract much attention in the context of EV biology not only because of their potential role in biological processes but also given the possibility of using them as biomarkers for various diseases.[78]

Due to the clear relevance of the molecular composition of EVs, an online database called Vesiclepedia containing a list of EV-associated lipids, metabolites, proteins, and RNA molecules has been developed, which users can either browse, query, or download.[79] Other web-based resources in which databases of EV-associated information can be found include EVpedia, exoRBase, EV-TRACK Platform, and exRNA Atlas. For specifics on each database, refer to Table 2.

ROLE OF ADIPOCYTE-DERIVED EVs IN ISCHEMIA/REPERFUSION INJURY, CORONARY ATHEROSCLEROSIS, HEART FAILURE, AND ATRIAL FIBRILLATION

Over the last decade, several strategies have been employed to promote cardiac repair, and in this context the role of the epicardium cannot be overlooked. The epicardium is an evolutionarily conserved layer of mesothelium covering the outermost cell layer of the vertebrate heart.

During fetal development, the epicardium serves as a progenitor source, contributing to the production of multipotent cells that subsequently give rise to the cardiac mesenchyme. Indeed, epicardium-derived progenitor cells undergo epithelial-to-mesenchymal transition, invade the underlying myocardium, and differentiate into different cardiac lineages.[84,85] Although the epicardium becomes dormant after birth, functioning as a simple barrier between the myocardium and the pericardial cavity, cardiac injury reactivates the regenerative properties of this tissue. Within this context, epicardium-derived cells play a crucial role; they are a powerful population of cardiac progenitor cells that reinvoke an embryonic gene program and developmental characteristics following cardiac injury.[86]

A possible mechanism through which these cells exert effects on cardiomyocytes may depend on EVs. Epicardial-derived EVs can promote the proliferation of cardiomyocytes in either infarcted mouse hearts or human models of myocardial injury and have been observed to improve contractile function.[87] Another aspect worth considering is the role played by human amniotic fluid stem cells in cardiac repair and multipotent mesenchymal progenitor cells whose secretome was found to send cardioprotective paracrine signals in a preclinical model of rat myocardial ischemia/reperfusion injury.[88] In this context, EVs isolated from human amniotic-fluid-derived stem cells can aid in cardiac repair and trigger cardiac regeneration via paracrine modulation of endogenous mechanisms.[89]

Ischemia/reperfusion injury

Cell-based therapy (e.g., by combining human cardiac stem cells and bone marrow mesenchymal stromal cells) reduces scar size and represses adverse remodeling secondary to myocardial infarction in preclinical models[90] and in clinical trials of patients with ischemic cardiomyopathy.[91] EAT represents a depot of mesenchymal stromal cells possibly promoting cardiac regeneration following ischemic injury,[92] and so its potential role in aiding cardiac regeneration, particularly in response to ischemic insult, is an aspect worth considering. Administration of EVs isolated from adipose-derived mesenchymal stromal cells in rats subjected to ischemia/reperfusion injury significantly reduced size of myocardial infarction, along with serum levels of creatinine kinase-myocardial band, lactate dehydrogenase, and cardiac troponin I. The apoptotic process was attenuated as highlighted by the upregulation of Bcl-2 and the downregulation of Bax and the reduced activity of Caspase 3 in the rat myocardium. The benefit of administering EVs secreted by adipose-derived mesenchymal stromal cells was confirmed in a model of embryonic rat cardiomyocytes (H9c2) exposed to hypoxia-/reoxygenation-induced apoptosis. At the molecular level, the enhanced viability was related to the activation of Wnt/β-catenin pathway, an effect counteracted by the Wnt/β-catenin inhibitor XAV939.[93]

EAT-derived mesenchymal stromal cells are a highly heterogeneous population of cells that are potentially involved in cardiac regeneration. In a study conducted by Thankam et al., EAT-derived mesenchymal stromal cells were exposed to simulated ischemia and reperfusion *in vitro* and subsequently underwent single-cell RNA sequencing, with the aim of identifying the different existing subpopulations in such conditions. Interestingly, 18 unique EAT-derived mesenchymal stromal cell clusters were identified, and the genes upregulated in these clusters were involved in myocardial homeostasis, cell integrity and cell cycle, prevention of fibroblast differentiation, differentiation to myocardial lineage, anti-inflammatory responses, prevention of endoplasmic reticulum-stress, and an improved energy metabolism. These findings not only demonstrate the intrinsic heterogeneity of EAT-derived mesenchymal stromal cells but also suggest that distinct subpopulations display cardioprotective potential under ischemia.[94] The same cellular model was employed to assess the potential impact of ischemic conditions on the cargo of EVs isolated from EAT-derived mesenchymal stromal cells. Indeed, EVs derived from ischemic EATDS were discovered to harbor ribosomal proteins (40SRPS18, 40SRPS30, 60SRPL14, and 40SRPSA), suggesting their potential extracellular function in the case of ischemic stress.[95] Additionally, the potential of mesenchymal stromal cells primed with EVs derived from EAT in cardiac healing was the aim of further study by the same group.[96] After the isolation of EAT-derived mesenchymal stromal cells and cardiac fibroblasts from Yucatan micro swine, EAT-derived mesenchymal stromal cells were challenged with hypoxic medium for 2 h and then EVs were collected. Treatment of cardiac fibroblasts with ischemic EVs caused them to shift toward a cardiomyocyte-like phenotype. The expression of cardiomyocyte-specific transcription factors (e.g., GAT-4, Nkx2.5, IRX4, and TBX5) and of myofibroblast healing factors (e.g., α-SMA) were upregulated in cardiac fibroblasts; conversely, cardiac biomarkers including connexin-43 and troponin-I and fibroblast biomarkers including podoplanin, vimentin, and FSP-1 (fibroblast-specific protein 1) were downregulated. LC-MS/MS analysis suggested that LGALS1 (galectin 1), PRDX2 (peroxiredoxin 2), and CCL2 (monocyte chemoattractant protein-1) contained in the ischemic EVs facilitated survival responses in cardiac fibroblasts following ischemia. These findings therefore imply that in the context of ischemic insult, EVs released from EAT-derived stem cells exhibit the capacity to induce a regenerative phenotype in adult cardiac fibroblasts, which are therefore "reprogrammed" in a manner to mature into cardiomyocytes and become part of the surviving myocardium. Overall, this study sheds light on the healing properties of EVs released from EAT-derived stem cells, which harness great translational potential in the context of regenerative cardiology.[96]

Considering that diabetes mellitus is known to worsen myocardial ischemia/reperfusion injury, it is of interest to highlight how intramyocardial injection of EVs derived from diabetic mice in the nondiabetic heart significantly exacerbated myocardial ischemia/reperfusion injury, as evidenced by a poorer cardiac function recovery, larger infarct size, and greater cardiomyocyte apoptosis. Similarly, intramyocardial or systemic administration of EVs isolated from diabetic adipocytes or high-glucose-/high-lipid-challenged nondiabetic adipocyte significantly aggravated myocardial ischemia/reperfusion. A mechanistic investigation identified that miR-130b-3p was significantly increased in diabetic serum EVs, diabetic adipocyte EVs, and those derived from high-glucose-/high-lipid-challenged nondiabetic adipocyte. AMPKα1/α2, Birc6, and Ucp3 resulted to be the downstream molecular targets mediating the pro-apoptotic effect driven by miR-130b-3p.[97] Treatment with EVs purified from patients with diabetes mellitus significantly exacerbated ischemia-/reperfusion-induced neonatal rat ventricular myocyte cell death, an effect attenuated by a miR-130b-3p inhibitor.[97] Within this context, it is worth mentioning that dysfunctional-adipocyte-derived EVs isolated from patients with obesity/diabetes mellitus promote pathologic organ remodeling, including inflammatory cytokine release, systemic insulin resistance, liver transforming growth factor beta (TGF-β) pathway dysregulation.[98–100]

A further aspect to consider is the presence of brown adipose tissue within cardiac adipose tissue in the healthy state, which undergoes a brown-to-white transition in obesity. Cardiac adipose tissue is phenotypically brown during the early stages of life and despite whitening with age, it retains brown characteristics in adulthood. In order to provide clues regarding the cardiovascular benefits of brown-adipose tissue on the heart, by using a murine model of myocardial ischemia/reperfusion injury, Zhao et al. found that brown-adipose-tissue-derived EVs participated in exercise cardio protection by delivering miR-125b-5p, miR-128-3p, and miR-30d-5p to the heart, which suppressed the activation of the proapoptotic-mitogen-associated protein kinase (MAPK) pathway.[101]

On the other hand, cardiomyocytes may adversely impact adipose function. The heart secretes various cytokines (known as cardiokines) that mediate interorgan and intercellular communication. Cardiokines regulate lipid uptake, β-oxidation, lipolysis, lipid mobilization, and mitochondrial biogenesis in adipocytes.[102] EVs derived from cardiomyocytes can serve as cardiokines regulating the function of other cells, such as bone marrow progenitor cells and adipose-derived mesenchymal stromal cells. By using a mouse model of myocardial ischemia/reperfusion, it was demonstrated that EVs mediated the communication between injured heart and adipose tissue, resulting in adipocyte endocrine dysfunction. When exposed to EVs isolated from ventricular cardiomyocytes from mice subjected to myocardial ischemia/reperfusion, 3T3-L1 adipocytes showed a reduced expression of adiponectin and leptin. In this study, the miR-23-27-24 cluster was the most important molecule transported by EVs that induced endoplasmic reticulum stress in adipocytes. Endoplasmic reticulum is an important cytoplasm organelle responsible for protein assembly and modification. Different pathological stresses lead to an imbalance between the demand for protein folding and the capacity of the endoplasmic reticulum to perform such protein folding, thereby causing endoplasmic reticulum stress. Finally, administration of cardiomyocytes-specific miR-23-27-24 sponges abolished adipocyte miR-23-27-24 elevation in animals that had undergone myocardial ischemia/reperfusion, supporting the cardiomyocyte origin of adipocyte miR-23-27-24.[103]

The main findings of this section have been reported in Table 3.

Coronary atherosclerotic heart disease

It is known that the metabolism of EAT has an important connection to coronary atherosclerotic heart disease. Specifically, in the presence of coronary atherosclerotic heart disease, metabolic disorders of EAT are observed, and it is thought that the metabolic dysfunction of this tissue may be due to abnormal adipogenic differentiation of epicardial adipose stem cells. However, the mechanisms through which these may lead to metabolic disruption have not been fully elucidated. In the study conducted by Yang et al.,[104] it was observed that EAT-derived EVs inhibited the adipogenic differentiation of epicardial adipose stem cells. Additionally, EAT-derived EVs isolated from patients with coronary atherosclerotic heart disease displayed an increased expression of miR-3064-5p. The latter is involved in the regulation of the adipogenic differentiation of epicardial adipose stem cells through interaction with the gene neuronatin.[104] In general, loss of neuronatin increases the expression of UCP1 and the key genes in mitochondrial oxidative phosphorylation in primary subcutaneous white adipocytes, indicative of a "browning" effect.[106] Going back to the study by Yang, EVs modified with the miR-3064-5p inhibitor significantly upregulated the expression of neuronatin protein in EAT stem cells, leading to an improved adipogenic differentiation. Thus, by inhibiting the transcriptional expression of neuronatin, EAT-derived EVs enriched in miR-3064-5p were identified as potential key players in the disruption of normal adipocyte maturation of EAT stem cells, therefore providing a possible explanation behind the link between altered EAT metabolism and coronary atherosclerotic heart disease.[104] A further aspect to be considered in patients with an increased cardiovascular risk is the understanding of how a dysregulated adipose tissue metabolome can influence the vascular redox. By an unbiased metabolomic approach, Akawi et al. characterized the metabolome differentially secreted by thoracic and subcutaneous adipose tissues isolated from obese patients with atherosclerosis and demonstrated how these metabolites were linked to dysregulated vascular redox signaling in patients undergoing coronary bypass surgery. Compared with subcutaneous adipose tissue, the thoracic depots secreted a higher amount of sphingolipid, with C16:0-ceramide and derivates being the most abundant species released within adipocyte-derived EVs.[105] In particular, plasma $Cer_{16:0}$ is associated with an increased risk for major adverse cardiovascular events, including heart failure and myocardial infarction.[107,108]

The main findings of this section have been reported in Table 3.

Heart failure

The various depots of EAT are known to contain adipose stem cells. Lambert et al. explored the distinct functional properties of the adipose stem cells contained in two different EAT locations in the heart of transplant-eligible patients with advanced heart failure.[92] EAT was isolated from the apex of the heart and the area covering the epicardial arterial sulcus of the left anterior descending artery. EVs released by adipose stem cells potentially have a role in mediating pro-angiogenic effects and spontaneous regeneration of damaged tissue, and it was demonstrated how this effect may vary according to the presence of cardiovascular risk factors such as obesity and diabetes. In this case, the pluripotency self-renewal properties of adipose stem cells are reduced. EVs released from ventricular myocardium adipose stem cells in non-obese and non-diabetic heart failure patients contained a significantly higher amount of tissue factor compared with those of obese and diabetic patients. In addition, non-obese and non-diabetic patients had EVs deriving from myocardium adipose stem cells that carried a higher amount of miRNA126-3p,[92] which is one of the most important miRNAs in angiogenesis.[109] These EVs were able to improve microvascular endothelial cell viability and migration rate. The same effects were not observed in EVs isolated from myocardium adipose stem cells of obese and diabetic patients. This study therefore sheds light on the potential of exploiting adipose-stem-cell-derived EVs within EAT depots in the stimulation of spontaneous regeneration and repair in the damaged myocardium of patients with advanced heart failure.[92] In this regard, it must be acknowledged that EVs released from subcutaneous adipose stem cells of obese subjects have impaired pro-angiogenic potential and possess reduced miR-126 content.[110]

Table 3. Myocardial regeneration upon ischemia/reperfusion injury and coronary atherosclerotic heart disease

Disease	Experimental model	Findings
Myocardial regeneration upon ischemia/reperfusion injury[93]	1) Mesenchymal stromal cells isolated from Sprague-Dawley rats 2) Embryonic rat cardiomyocytes (H9c2) exposed to hypoxia/reoxygenation	• Implantation of EVs isolated from adipose-derived mesenchymal stromal cells reduced the myocardial infarction area in rat hearts subjected to ischemia/reperfusion injury with an attenuation of the apoptotic process • EVs isolated from adipose-derived mesenchymal stromal cells attenuated hypoxia-/reoxygenation-induced apoptosis and promoted cell survival in H9c2 cells
Myocardial regeneration upon ischemia/reperfusion injury[96]	1) EAT-derived stem cells and cardiac fibroblast were isolated from Yucatan micro swine 2) Ischemic-EAT-derived stem cells were obtained by 2-h exposure to ischemic buffer; reperfusion was obtained with complete media overnight	• EVs isolated from ischemic EAT-derived stem cells lead to an increased mRNA expression of cardiomyocyte lineage genes and a decreased expression of fibroblast biomarkers in the treated cardiac fibroblast • EV treatment portended toward the upregulation of cardiomyocyte specific transcription factors and myofibroblast biomarkers and decreased the cardiac and fibroblast biomarkers in ischemic cardiac fibroblasts • Protein mediators contained in ischemic EVs were associated with biological processes involved in cardiac regeneration and inflammatory responses
Myocardial regeneration upon ischemia/reperfusion injury[97]	1) Neonatal rat ventricular myocyte cell 2) C57B6/J mice were fed high fat diet (HFD, 60% fat) for 12 weeks to induce obesity/diabetes 3) Forty patients with type 2 diabetes mellitus and forty healthy control	• Intramyocardial or systemic administration of EVs isolated from diabetic adipocyte or high-glucose-/high-lipid-challenged nondiabetic adipocyte significantly exacerbated myocardial ischemia/reperfusion • miR-130b-3p enrichment in dysfunctional-adipocyte-derived EVs exacerbated myocardial ischemia/reperfusion injury in the diabetic heart
Myocardial regeneration upon ischemia/reperfusion injury[103]	1) Sham-operated control animals or animals subjected to 30 min MI followed by 3-h reperfusion 2) 3T3L1 adipocytes	• Circulating EV in myocardial/reperfusion injury animals may suppress adipocyte endocrine function, adversely regulating systemic metabolism • miR-23-27-24 cluster was the most important molecule transported by EVs that induced endoplasmic reticulum stress in adipocytes
Coronary atherosclerotic heart disease[104]	EAT biopsy samples were collected from the aortic root near the right coronary artery of patients undergoing cardiac surgery	• EAT-derived EVs from patients with coronary atherosclerotic heart disease inhibited adipogenic differentiation of EAT stem cells *in vitro* • miR-3064-5p was highly expressed in EAT-derived EV
Coronary atherosclerotic heart disease[105]	Patients undergoing cardiac surgery (n = 633); paired biopsies of subcutaneous adipose tissue (n = 48) and thoracic adipose tissue from 31 obese and 17 propensity-matched nonobese patients	• Thoracic adipose tissue volume was associated significantly with arterial oxidative stress • C16:0-ceramide and derivatives were the most abundant species released within adipocyte-derived EVs

EAT, epicardial adipose tissue; HFD, high-fat diet; EV, extracellular vesicles; MI, myocardial infarction; n, numerosity.

Cardiac insulin resistance represents a strong predictor for diabetic cardiomyopathy and subsequent heart failure. Wen et al. demonstrated how the adipocytes within epicardial adipose tissue may play a role in the underlying mechanisms of insulin resistance in cardiac cells.[111] Hypertrophic 3T3-L1 adipocytes (obtained by incubating 3T3-L1 with palmitic acid) released EVs enriched in miR-802-5p (a biomarker for type 2 diabetes[112]), which caused insulin resistance in neonatal rat ventricular myocytes. Indeed, the insulin-stimulated phosphorylation of $AktT_{308}$ and insulin-stimulated glucose uptake was suppressed when compared with treatment with control-adipocyte-derived EVs. The direct impact of miR-802-5p on insulin resistance in neonatal rat ventricular myocytes was confirmed by using miR-802-5p mimic and a miR-802-5p inhibitor, which caused the phosphorylation of Akt to decrease and increase, respectively. In search of specific mechanisms, EVs released by hypertrophic 3T3-L1 adipocytes reduced HSP60 protein levels in neonatal rat ventricular myocytes.[111] The link between EV-derived miR-807-5p and HSP60 was demonstrated when the miR-802-5p mimic significantly downregulated HSP60 protein levels in neonatal rat ventricular myocytes. HSP60 is a mitochondrial chaperone protein with anti-inflammatory effects, whose loss determines mitochondrial functionality impairment, a known abnormality at the basis of cardiac insulin resistance and diabetic cardiomyopathy.[113]

Finally, in the context of browning, EAT of individuals with cardiac disease is associated with a brown-to-white *trans*-differentiation with a significant decrease in thermogenic genes and an upregulation of white adipogenesis. This brown-to-white phenotype is associated with a significant increase in the production of oxygen species by EAT with an enrichment of inflammatory biomarkers.[20] In the context of brown

Table 4. Heart failure and atrial fibrillation

Disease	Experimental model	Findings
Heart failure[92]	EAT was obtained from 30 patients undergoing cardiac transplant surgery	• EVs released by ventricular myocardium adipose tissue significantly induced human microvascular endothelial cell migration • EVs released by ventricular myocardium adipose tissue of non-obese and non-diabetic patients contain a significant higher amount of tissue factor than those isolated from obese non-diabetic patients
Heart failure[111]	1) Hypertrophic 3T3-L1 adipocytes 2) Neonatal rat ventricular myocytes	• Hypertrophic adipocyte-derived EVs highly expressed miR-802-5p • EVs released by hypertrophic 3T3-L1 adipocytes reduced HSP60 protein levels in neonatal rat ventricular myocytes
Heart failure[114]	1) Brown-adipocyte-specific β3-adrenergic receptors (ADRB3) knockout mice and littermate control mice 2) Brown adipocytes	• EV-bioactive enzyme inducible NOS from brown adipose tissue promoted angiotensin-II-induced cardiac remodeling • ADRB3 in brown adipose tissue negatively regulated EV-inducible NOS and pathological cardiac remodeling
Atrial fibrillation[10]	EAT specimens were collected from patients with (n = 32) and without AF (n = 30) during elective heart surgery	• EAT-derived EVs from individuals with atrial fibrillation carried a greater amount of IL-1α and IL-6, profibrotic cytokines, and proteins involved in pathways related to fibrosis and hypertrophy
Atrial fibrillation[115]	EAT was isolated from 17 patients with symptomatic, persistent, or long-standing persistent atrial fibrillation who underwent thoracoscopic pulmonary vein isolation	• miR-1-3p and miR-133a-3p were upregulated in EAT-derived EVs compared to subcutaneous adipose tissue • Overexpression of miR-1-3p and miR-133a-3p in neonatal rat ventricular myocytes decreased conduction velocity and increased conduction heterogeneity via reducing the gene expression of *Kcnj2* and *Kcnj12* • *Kcnj2 encodes* Kir2.1, which is a subunit of the potassium channel responsible for the inward rectifier potassium current during cardiac repolarization

ADRB3, β3-adrenergic receptors; AF, atrial fibrillation; EAT, epicardial adipose tissue; EV, extracellular vesicles; IL, interleukin; n, numerosity; NOS, nitric oxide synthase.

adipose tissue-to-heart crosstalk, Lin et al. demonstrated that a specific knockout of β-adrenoreceptor 3 in brown adipose tissue leads to an increased release of inducible nitric-oxide-synthase-containing EVs, which directly aggravate cardiac remodeling. Furthermore, EVs isolated from brown adipocytes treated with a β-adrenoreceptor 3 antagonist accelerated angiotensin-II-driven cardiac remodeling, whereas EVs isolated from adipocytes treated with a β-adrenoreceptor 3 agonist attenuated the pathological phenotype.[114]

The main findings of this section have been reported in Table 4.

Atrial fibrillation

In the context of CVD, atrial fibrillation is a condition that can form blood clots in the chambers of the heart and lead to stroke, heart failure, and other cardiovascular complications. Patients with excess EAT are at increased risk of developing cardiac arrhythmias as EAT favors the depolarization of the membrane of cardiomyocytes, slowing down conduction and facilitating re-entrance arrhythmias. Shaihov-Teper et al.'s work represents a pillar study in this field, as it was demonstrated that EVs derived from EAT of patients with atrial fibrillation carried a distinctive proinflammatory, profibrotic, and proarrhythmic signature.[10] Specifically, patients with chronic atrial fibrillation released an increased amount of EVs compared with both patients with paroxysmal atrial fibrillation or without atrial fibrillation. EAT-derived EVs from individuals with atrial fibrillation carried a greater amount of interleukin (IL)-1α and IL-6, profibrotic cytokines (osteopontin), and proteins involved in pathways related to fibrosis and hypertrophy (TGF-β). This evidence was further confirmed by an increased expression of miRNA involved in profibrotic events (e.g., miR-146b, miR-133a, and miRNA-29a, involved in collagen synthesis and atrial remodeling). Adipose tissue has been recently recognized as one of the most important sources of circulating-EV-containing miRNAs that can regulate gene expression even in remote tissues.[116] When EVs isolated from EAT of individuals with atrial fibrillation were incubated with pluripotent-stem-cell-derived cardiomyocytes, they induced a sustained reentry.[10]

The analysis of EAT and subcutaneous adipose tissue isolated from 17 patients with symptomatic, persistent, or long-standing persistent atrial fibrillation who had undergone thoracoscopic pulmonary vein isolation showed that the EV concentration was higher in the EAT secretome than in the subcutaneous adipose tissue secretome. Among 824 identified miRNAs carried by EVs, miR-1-3p and miR-133a-3p were upregulated in EAT-derived EVs compared with subcutaneous adipose tissue. Due to the limited amount of RNA isolated from EVs and the limited amount of EVs in the secretome, the authors performed the validation in tissue. All candidate miRNAs identified by sequencing in EAT-secretome-derived EVs were expressed in EAT. In order to understand the contribution of miR-1-3p and miR-133a-3p in EAT-secretome-induced arrhythmogenicity, the overexpression of these two miRNAs in neonatal rat ventricular myocytes decreased conduction velocity and increased conduction heterogeneity via reducing the gene expression of *Kcnj2* and *Kcnj12*.[115] *Kcnj2* encodes Kir2.1, which is a subunit of the potassium channel responsible for the inward rectifier potassium current during cardiac repolarization. These findings could provide further insight on a previous observation reporting that the secretome of EAT isolated from patients with atrial fibrillation slowed conduction,

depolarized the resting potential, altered electrical cell-cell coupling, and facilitated re-entrant arrhythmias in neonatal rat ventricular myocytes.[117]

The main findings of this section have been reported in Table 4.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

EAT is an ectopic fat depot that can functionally modulate the heart in a direct manner. EAT is a highly metabolically active tissue and secretes bioactive molecules that are transported to the adjacent myocardium through vasocrine and/or paracrine pathways. Given the lack of anatomical barriers separating the two tissues, the EAT secretome directly reaches the myocardium and coronary lumen.[118] Considering that strategies to regenerate cardiac tissue postinjury are limited and that heart transplantation remains the only "cure" for a failing heart, it becomes of interest to consider the regenerative properties of EVs from certain cell sources, which can stimulate functional recovery of injured hearts (e.g., EVs derived from EAT can carry mediators to the ischemic myocardium favoring a pro-healing phenotype). Conversely, the dysregulation of EV signaling in injured tissues tends to contribute to maladaptive responses, including myofibroblast activation, cardiomyocyte hypertrophy, vascular destruction, or adipose endocrine dysfunction. Based on this knowledge, recent studies have tried to deliver exogenous EVs therapeutically to the injured heart, with the aim of improving cardiac function. Although EVs deliver functional cargo with decreased immune clearance when administered systemically to rodents, more evaluation in clinically relevant systems and direct, quantitative comparison with liposome-based alternatives are required to comprehensively assess the risk-benefit ratio. Indeed, the immunogenicity and biocompatibility of each individual EV formulation must be evaluated, as in the case of liposomal carriers. In addition to organ distribution studies and careful selection of EV labeling methods, studies evaluating repeated dosing under various regimes are required to allow further clinical investigations.[119] Within this context, to overcome some limitations of EVs such as inefficient cell targeting, on demand delivery, and low yield, EV can be engineered by modifying the donor cells (e.g., stress induction or 3D cell culture) or by a direct modification of the EVs (e.g., a direct loading or membrane surface strategies).[120] Efforts are already being made in deciphering the mechanisms underlying targeted EV uptake, through which the delivery of functional EV-encapsulated biomolecules to recipient cells occurs[74]; superiorly robust EV detection methods will also contribute to the establishment of EV usage in clinical contexts.[121]

While short half-life and bioactivity in the circulation, dose selection, dosage strategy, route of administration, pharmacokinetics, or pharmacodynamics represent first steps toward fostering the understanding of EVs,[122] the development of detailed mechanistic understanding of EVs in the heart also represents a significantly complicated roadblock to harnessing the clinical potential of cardiac EVs.[123]

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Hatem, S.N., and Sanders, P. (2014). Epicardial adipose tissue and atrial fibrillation. Cardiovasc. Res. *102*, 205–213. https://doi.org/10.1093/cvr/cvu045.
2. Goldman, S.A., Requena-Ibanez, J.A., Devesa, A., Santos-Gallego, C.G., Badimon, J.J., and Fuster, V. (2023). Uncovering the Role of Epicardial Adipose Tissue in Heart Failure With Preserved Ejection Fraction. JACC (J. Am. Coll. Cardiol.): Advances *2*, 100657.
3. Hearon, C.M., Jr., Reddy, S., Dias, K.A., Shankar, A., MacNamara, J., Levine, B., and Sarma, S. (2023). Characterizing regional and global effects of epicardial adipose tissue on cardiac systolic and diastolic function. Obesity *31*, 1884–1893. https://doi.org/10.1002/oby.23782.
4. Shetty, A.K., and Upadhya, R. (2021). Extracellular Vesicles in Health and Disease. Aging Dis. *12*, 1358–1362. https://doi.org/10.14336/AD.2021.0827.
5. van Niel, G., Carter, D.R.F., Clayton, A., Lambert, D.W., Raposo, G., and Vader, P. (2022). Challenges and directions in studying cell-cell communication by extracellular vesicles. Nat. Rev. Mol. Cell Biol. *23*, 369–382. https://doi.org/10.1038/s41580-022-00460-3.
6. Macchi, C., Greco, M.F., Favero, C., Dioni, L., Cantone, L., Hoxha, M., Vigna, L., Solazzo, G., Corsini, A., Banach, M., et al. (2021). Associations Among PCSK9 Levels, Atherosclerosis-Derived Extracellular Vesicles, and Their miRNA Content in Adults With Obesity. Front. Cardiovasc. Med. *8*, 785250. https://doi.org/10.3389/fcvm.2021.785250.
7. Greco, M.F., Rizzuto, A.S., Zarà, M., Cafora, M., Favero, C., Solazzo, G., Giusti, I., Adorni, M.P., Zimetti, F., Dolo, V., et al. (2022). PCSK9 Confers Inflammatory Properties to Extracellular Vesicles Released by Vascular Smooth Muscle Cells. Int. J. Mol. Sci. *23*, 13065. https://doi.org/10.3390/ijms232113065.
8. Vilella-Figuerola, A., Padró, T., Roig, E., Mirabet, S., and Badimon, L. (2022). New factors in heart failure pathophysiology: Immunity cells release of extracellular

vesicles. Front. Cardiovasc. Med. *9*, 939625. https://doi.org/10.3389/fcvm.2022.939625.
9. Rizzuto, A.S., Faggiano, A., Macchi, C., Carugo, S., Perrino, C., and Ruscica, M. (2024). Extracellular vesicles in cardiomyopathies: A narrative review. Heliyon *10*, e23765. https://doi.org/10.1016/j.heliyon.2023.e23765.
10. Shaihov-Teper, O., Ram, E., Ballan, N., Brzezinski, R.Y., Naftali-Shani, N., Masoud, R., Ziv, T., Lewis, N., Schary, Y., Levin-Kotler, L.P., et al. (2021). Extracellular Vesicles From Epicardial Fat Facilitate Atrial Fibrillation. Circulation *143*, 2475–2493. https://doi.org/10.1161/CIRCULATIONAHA.120.052009.
11. Badimon, L., Padro, T., Arderiu, G., Vilahur, G., Borrell-Pages, M., and Suades, R. (2022). Extracellular vesicles in atherothrombosis: From biomarkers and precision medicine to therapeutic targets. Immunol. Rev. *312*, 6–19. https://doi.org/10.1111/imr.13127.
12. Davidson, S.M., Boulanger, C.M., Aikawa, E., Badimon, L., Barile, L., Binder, C.J., Brisson, A., Buzas, E., Emanueli, C., Jansen, F., et al. (2023). Methods for the identification and characterization of extracellular vesicles in cardiovascular studies: from exosomes to microvesicles. Cardiovasc. Res. *119*, 45–63. https://doi.org/10.1093/cvr/cvac031.
13. Braescu, L., Gaspar, M., Buriman, D., Aburel, O.M., Merce, A.P., Bratosin, F., Aleksandrovich, K.S., Alambaram, S., and Mornos, C. (2022). The Role and Implications of Epicardial Fat in Coronary Atherosclerotic Disease. J. Clin. Med. *11*, 4718. https://doi.org/10.3390/jcm11164718.
14. Iacobellis, G. (2022). Epicardial adipose tissue in contemporary cardiology. Nat. Rev. Cardiol. *19*, 593–606. https://doi.org/10.1038/s41569-022-00679-9.
15. Cheładze, P., Martuszewski, A., Poręba, R., and Gać, P. (2022). The Importance of the Assessment of Epicardial Adipose Tissue in Scientific Research. J. Clin. Med. *11*, 5621. https://doi.org/10.3390/jcm11195621.
16. Ayton, S.L., Gulsin, G.S., McCann, G.P., and Moss, A.J. (2022). Epicardial adipose tissue in obesity-related cardiac dysfunction. Heart *108*, 339–344. https://doi.org/10.1136/heartjnl-2020-318242.
17. Ernault, A.C., Meijborg, V.M.F., and Coronel, R. (2021). Modulation of Cardiac Arrhythmogenesis by Epicardial Adipose Tissue: JACC State-of-the-Art Review. J. Am. Coll. Cardiol. *78*, 1730–1745. https://doi.org/10.1016/j.jacc.2021.08.037.
18. Demircelik, M.B., Yilmaz, O.C., Gurel, O.M., Selcoki, Y., Atar, I.A., Bozkurt, A., Akin, K., and Eryonucu, B. (2014). Epicardial adipose tissue and pericoronary fat thickness measured with 64-multidetector computed tomography: potential predictors of the severity of coronary artery disease. Clinics *69*, 388–392. https://doi.org/10.6061/clinics/2014(06)04.
19. Iacobellis, G. (2023). Epicardial fat links obesity to cardiovascular diseases. Prog. Cardiovasc. Dis. *78*, 27–33. https://doi.org/10.1016/j.pcad.2023.04.006.
20. Aldiss, P., Davies, G., Woods, R., Budge, H., Sacks, H.S., and Symonds, M.E. (2017). 'Browning' the cardiac and peri-vascular adipose tissues to modulate cardiovascular risk. Int. J. Cardiol. *228*, 265–274. https://doi.org/10.1016/j.ijcard.2016.11.074.
21. Doukbi, E., Soghomonian, A., Sengenès, C., Ahmed, S., Ancel, P., Dutour, A., and Gaborit, B. (2022). Browning Epicardial Adipose Tissue: Friend or Foe? Cells *11*, 991. https://doi.org/10.3390/cells11060991.
22. van Woerden, G., van Veldhuisen, D.J., Westenbrink, B.D., de Boer, R.A., Rienstra, M., and Gorter, T.M. (2022). Connecting epicardial adipose tissue and heart failure with preserved ejection fraction: mechanisms, management and modern perspectives. Eur. J. Heart Fail. *24*, 2238–2250. https://doi.org/10.1002/ejhf.2741.
23. Santos, D., and Carvalho, E. (2022). Adipose-related microRNAs as modulators of the cardiovascular system: the role of epicardial adipose tissue. J. Physiol. *600*, 1171–1187. https://doi.org/10.1113/JP280917.
24. Sacks, H.S., Fain, J.N., Holman, B., Cheema, P., Chary, A., Parks, F., Karas, J., Optican, R., Bahouth, S.W., Garrett, E., et al. (2009). Uncoupling protein-1 and related messenger ribonucleic acids in human epicardial and other adipose tissues: epicardial fat functioning as brown fat. J. Clin. Endocrinol. Metab. *94*, 3611–3615. https://doi.org/10.1210/jc.2009-0571.
25. McAninch, E.A., Fonseca, T.L., Poggioli, R., Panos, A.L., Salerno, T.A., Deng, Y., Li, Y., Bianco, A.C., and Iacobellis, G. (2015). Epicardial adipose tissue has a unique transcriptome modified in severe coronary artery disease. Obesity *23*, 1267–1278. https://doi.org/10.1002/oby.21059.
26. Gaborit, B., Venteclef, N., Ancel, P., Pelloux, V., Gariboldi, V., Leprince, P., Amour, J., Hatem, S.N., Jouve, E., Dutour, A., and Clément, K. (2015). Human epicardial adipose tissue has a specific transcriptomic signature depending on its anatomical peri-atrial, peri-ventricular, or peri-coronary location. Cardiovasc. Res. *108*, 62–73. https://doi.org/10.1093/cvr/cvv208.
27. Wang, T.D., Lee, W.J., Shih, F.Y., Huang, C.H., Chen, W.J., Lee, Y.T., Shih, T.T.F., and Chen, M.F. (2010). Association of epicardial adipose tissue with coronary atherosclerosis is region-specific and independent of conventional risk factors and intra-abdominal adiposity. Atherosclerosis *213*, 279–287. https://doi.org/10.1016/j.atherosclerosis.2010.07.055.
28. Nalliah, C.J., Bell, J.R., Raaijmakers, A.J.A., Waddell, H.M., Wells, S.P., Bernasochi, G.B., Montgomery, M.K., Binny, S., Watts, T., Joshi, S.B., et al. (2020). Epicardial Adipose Tissue Accumulation Confers Atrial Conduction Abnormality. J. Am. Coll. Cardiol. *76*, 1197–1211. https://doi.org/10.1016/j.jacc.2020.07.017.
29. Aitken-Buck, H.M., Moharram, M., Babakr, A.A., Reijers, R., Van Hout, I., Fomison-Nurse, I.C., Sugunesegran, R., Bhagwat, K., Davis, P.J., Bunton, R.W., et al. (2019). Relationship between epicardial adipose tissue thickness and epicardial adipocyte size with increasing body mass index. Adipocyte *8*, 412–420. https://doi.org/10.1080/21623945.2019.1701387.
30. Bambace, C., Telesca, M., Zoico, E., Sepe, A., Olioso, D., Rossi, A., Corzato, F., Di Francesco, V., Mazzucco, A., Santini, F., and Zamboni, M. (2011). Adiponectin gene expression and adipocyte diameter: a comparison between epicardial and subcutaneous adipose tissue in men. Cardiovasc. Pathol. *20*, e153–e156. https://doi.org/10.1016/j.carpath.2010.07.005.
31. Mazurek, T., Zhang, L., Zalewski, A., Mannion, J.D., Diehl, J.T., Arafat, H., Sarov-Blat, L., O'Brien, S., Keiper, E.A., Johnson, A.G., et al. (2003). Human epicardial adipose tissue is a source of inflammatory mediators. Circulation *108*, 2460–2466. https://doi.org/10.1161/01.CIR.0000099542.57313.C5.
32. Fitzgibbons, T.P., and Czech, M.P. (2014). Epicardial and perivascular adipose tissues and their influence on cardiovascular disease: basic mechanisms and clinical associations. J. Am. Heart Assoc. *3*, e000582. https://doi.org/10.1161/JAHA.113.000582.
33. Packer, M. (2018). The epicardial adipose inflammatory triad: coronary atherosclerosis, atrial fibrillation, and heart failure with a preserved ejection fraction. Eur. J. Heart Fail. *20*, 1567–1569. https://doi.org/10.1002/ejhf.1294.
34. Vianello, E., Dozio, E., Arnaboldi, F., Marazzi, M.G., Martinelli, C., Lamont, J., Tacchini, L., Sigrüner, A., Schmitz, G., and Corsi Romanelli, M.M. (2016). Epicardial adipocyte hypertrophy: Association with M1-polarization and toll-like receptor pathways in coronary artery disease patients. Nutr. Metab. Cardiovasc. Dis. *26*, 246–253. https://doi.org/10.1016/j.numecd.2015.12.005.
35. Dozio, E., Vianello, E., Briganti, S., Lamont, J., Tacchini, L., Schmitz, G., and Corsi Romanelli, M.M. (2016). Expression of the Receptor for Advanced Glycation End Products in Epicardial Fat: Link with Tissue Thickness and Local Insulin Resistance in Coronary Artery Disease. J. Diabetes Res. *2016*, 2327341. https://doi.org/10.1155/2016/2327341.
36. Bazzan, E., Tinè, M., Casara, A., Biondini, D., Semenzato, U., Cocconcelli, E., Balestro, E., Damin, M., Radu, C.M., Turato, G., et al. (2021). Critical Review of the Evolution of Extracellular Vesicles' Knowledge: From 1946 to Today. Int. J. Mol. Sci. *22*, 6417. https://doi.org/10.3390/ijms22126417.
37. Welsh, J.A., Goberdhan, D.C.I., O'Driscoll, L., Buzas, E.I., Blenkiron, C., Bussolati, B., Cai, H., Di Vizio, D., Driedonks, T.A.P., Erdbrügger, U., et al. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J. Extracell. Vesicles *13*, e12404. https://doi.org/10.1002/jev2.12404.
38. Lischnig, A., Bergqvist, M., Ochiya, T., and Lässer, C. (2022). Quantitative Proteomics Identifies Proteins Enriched in Large and Small Extracellular Vesicles. Mol. Cell. Proteomics *21*, 100273. https://doi.org/10.1016/j.mcpro.2022.100273.
39. Kanao, E., Wada, S., Nishida, H., Kubo, T., Tanigawa, T., Imami, K., Shimoda, A., Umezaki, K., Sasaki, Y., Akiyoshi, K., et al. (2022). Classification of Extracellular Vesicles Based on Surface Glycan Structures by Spongy-like Separation Media. Anal. Chem. *94*, 18025–18033. https://doi.org/10.1021/acs.analchem.2c04391.
40. Coumans, F.A.W., Brisson, A.R., Buzas, E.I., Dignat-George, F., Drees, E.E.E., El-Andaloussi, S., Emanueli, C., Gasecka, A., Hendrix, A., Hill, A.F., et al. (2017). Methodological Guidelines to Study Extracellular Vesicles. Circ. Res. *120*, 1632–1648. https://doi.org/10.1161/CIRCRESAHA.117.309417.
41. Livshits, M.A., Khomyakova, E., Evtushenko, E.G., Lazarev, V.N., Kulemin, N.A., Semina, S.E., Generozov, E.V., and Govorun, V.M. (2015). Isolation of exosomes by differential centrifugation: Theoretical analysis of a

commonly used protocol. Sci. Rep. *5*, 17319. https://doi.org/10.1038/srep17319.
42. Simonsen, J.B. (2017). What Are We Looking At? Extracellular Vesicles, Lipoproteins, or Both? Circ. Res. *121*, 920–922. https://doi.org/10.1161/CIRCRESAHA.117.311767.
43. Yuana, Y., Böing, A.N., Grootemaat, A.E., van der Pol, E., Hau, C.M., Cizmar, P., Buhr, E., Sturk, A., and Nieuwland, R. (2015). Handling and storage of human body fluids for analysis of extracellular vesicles. J. Extracell. Vesicles *4*, 29260. https://doi.org/10.3402/jev.v4.29260.
44. Paolini, L., Zendrini, A., Di Noto, G., Busatto, S., Lottini, E., Radeghieri, A., Dossi, A., Caneschi, A., Ricotta, D., and Bergese, P. (2016). Residual matrix from different separation techniques impacts exosome biological activity. Sci. Rep. *6*, 23550. https://doi.org/10.1038/srep23550.
45. Gupta, D., Zickler, A.M., and El Andaloussi, S. (2021). Dosing extracellular vesicles. Adv. Drug Deliv. Rev. *178*, 113961. https://doi.org/10.1016/j.addr.2021.113961.
46. Monguió-Tortajada, M., Gálvez-Montón, C., Bayes-Genis, A., Roura, S., and Borràs, F.E. (2019). Extracellular vesicle isolation methods: rising impact of size-exclusion chromatography. Cell. Mol. Life Sci. *76*, 2369–2382. https://doi.org/10.1007/s00018-019-03071-y.
47. Nordin, J.Z., Lee, Y., Vader, P., Mäger, I., Johansson, H.J., Heusermann, W., Wiklander, O.P.B., Hällbrink, M., Seow, Y., Bultema, J.J., et al. (2015). Ultrafiltration with size-exclusion liquid chromatography for high yield isolation of extracellular vesicles preserving intact biophysical and functional properties. Nanomedicine. *11*, 879–883. https://doi.org/10.1016/j.nano.2015.01.003.
48. Sódar, B.W., Kittel, Á., Pálóczi, K., Vukman, K.V., Osteikoetxea, X., Szabó-Taylor, K., Németh, A., Sperlágh, B., Baranyai, T., Giricz, Z., et al. (2016). Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection. Sci. Rep. *6*, 24316. https://doi.org/10.1038/srep24316.
49. Liangsupree, T., Multia, E., and Riekkola, M.L. (2021). Modern isolation and separation techniques for extracellular vesicles. J. Chromatogr. A *1636*, 461773. https://doi.org/10.1016/j.chroma.2020.461773.
50. Lobb, R.J., Becker, M., Wen, S.W., Wong, C.S.F., Wiegmans, A.P., Leimgruber, A., and Möller, A. (2015). Optimized exosome isolation protocol for cell culture supernatant and human plasma. J. Extracell. Vesicles *4*, 27031. https://doi.org/10.3402/jev.v4.27031.
51. Ramirez, M.I., Amorim, M.G., Gadelha, C., Milic, I., Welsh, J.A., Freitas, V.M., Nawaz, M., Akbar, N., Couch, Y., Makin, L., et al. (2018). Technical challenges of working with extracellular vesicles. Nanoscale *10*, 881–906. https://doi.org/10.1039/c7nr08360b.
52. Gandham, S., Su, X., Wood, J., Nocera, A.L., Alli, S.C., Milane, L., Zimmerman, A., Amiji, M., and Ivanov, A.R. (2020). Technologies and Standardization in Research on Extracellular Vesicles. Trends Biotechnol. *38*, 1066–1098. https://doi.org/10.1016/j.tibtech.2020.05.012.
53. Mork, M., Handberg, A., Pedersen, S., Jorgensen, M.M., Baek, R., Nielsen, M.K., and Kristensen, S.R. (2017). Prospects and limitations of antibody-mediated clearing of lipoproteins from blood plasma prior to nanoparticle tracking analysis of extracellular vesicles. J. Extracell. Vesicles *6*, 1308779. https://doi.org/10.1080/20013078.2017.1308779.
54. Jeppesen, D.K., Zhang, Q., Franklin, J.L., and Coffey, R.J. (2023). Extracellular vesicles and nanoparticles: emerging complexities. Trends Cell Biol. *33*, 667–681. https://doi.org/10.1016/j.tcb.2023.01.002.
55. Zhang, H., and Lyden, D. (2019). Asymmetric-flow field-flow fractionation technology for exomere and small extracellular vesicle separation and characterization. Nat. Protoc. *14*, 1027–1053. https://doi.org/10.1038/s41596-019-0126-x.
56. Zhang, Q., Higginbotham, J.N., Jeppesen, D.K., Yang, Y.P., Li, W., McKinley, E.T., Graves-Deal, R., Ping, J., Britain, C.M., Dorsett, K.A., et al. (2019). Transfer of Functional Cargo in Exomeres. Cell Rep. *27*, 940–954.e6. https://doi.org/10.1016/j.celrep.2019.01.009.
57. Zhang, Q., Jeppesen, D.K., Higginbotham, J.N., Graves-Deal, R., Trinh, V.Q., Ramirez, M.A., Sohn, Y., Neininger, A.C., Taneja, N., McKinley, E.T., et al. (2021). Supermeres are functional extracellular nanoparticles replete with disease biomarkers and therapeutic targets. Nat. Cell Biol. *23*, 1240–1254. https://doi.org/10.1038/s41556-021-00805-8.
58. Jeppesen, D.K., Zhang, Q., Franklin, J.L., and Coffey, R.J. (2022). Are Supermeres a Distinct Nanoparticle? J. Extracell. Biol. *1*, e44. https://doi.org/10.1002/jex2.44.
59. Chargaff, E., and West, R. (1946). The biological significance of the thromboplastic protein of blood. J. Biol. Chem. *166*, 189–197.
60. Berckmans, R.J., Lacroix, R., Hau, C.M., Sturk, A., and Nieuwland, R. (2019). Extracellular vesicles and coagulation in blood from healthy humans revisited. J. Extracell. Vesicles *8*, 1688936. https://doi.org/10.1080/20013078.2019.1688936.
61. Li, Y., He, X., Li, Q., Lai, H., Zhang, H., Hu, Z., Li, Y., and Huang, S. (2020). EV-origin: Enumerating the tissue-cellular origin of circulating extracellular vesicles using exLR profile. Comput. Struct. Biotechnol. J. *18*, 2851–2859. https://doi.org/10.1016/j.csbj.2020.10.002.
62. Yates, A.G., Pink, R.C., Erdbrügger, U., Siljander, P.R.M., Dellar, E.R., Pantazi, P., Akbar, N., Cooke, W.R., Vatish, M., Dias-Neto, E., et al. (2022). In sickness and in health: The functional role of extracellular vesicles in physiology and pathology *in vivo*: Part II: Pathology: Part II: Pathology. J. Extracell. Vesicles *11*, e12190. https://doi.org/10.1002/jev2.12190.
63. Beetler, D.J., Di Florio, D.N., Bruno, K.A., Ikezu, T., March, K.L., Cooper, L.T., Jr., Wolfram, J., and Fairweather, D. (2023). Extracellular vesicles as personalized medicine. Mol. Aspects Med. *91*, 101155. https://doi.org/10.1016/j.mam.2022.101155.
64. Kverneland, A.H., Østergaard, O., Emdal, K.B., Svane, I.M., and Olsen, J.V. (2023). Differential ultracentrifugation enables deep plasma proteomics through enrichment of extracellular vesicles. Proteomics *23*, e2200039. https://doi.org/10.1002/pmic.202200039.
65. Newman, S.S., Wilson, B.D., Mamerow, D., Wollant, B.C., Nyein, H., Rosenberg-Hasson, Y., Maecker, H.T., Eisenstein, M., and Soh, H.T. (2023). Extending the dynamic range of biomarker quantification through molecular equalization. Nat. Commun. *14*, 4192. https://doi.org/10.1038/s41467-023-39772-z.
66. Rädler, J., Gupta, D., Zickler, A., and Andaloussi, S.E. (2023). Exploiting the biogenesis of extracellular vesicles for bioengineering and therapeutic cargo loading. Mol. Ther. *31*, 1231–1250. https://doi.org/10.1016/j.ymthe.2023.02.013.
67. Mori, M.A., Ludwig, R.G., Garcia-Martin, R., Brandão, B.B., and Kahn, C.R. (2019). Extracellular miRNAs: From Biomarkers to Mediators of Physiology and Disease. Cell Metab. *30*, 656–673. https://doi.org/10.1016/j.cmet.2019.07.011.
68. Xu, D., Di, K., Fan, B., Wu, J., Gu, X., Sun, Y., Khan, A., Li, P., and Li, Z. (2022). MicroRNAs in extracellular vesicles: Sorting mechanisms, diagnostic value, isolation, and detection technology. Front. Bioeng. Biotechnol. *10*, 948959. https://doi.org/10.3389/fbioe.2022.948959.
69. Albanese, M., Chen, Y.F.A., Hüls, C., Gärtner, K., Tagawa, T., Mejias-Perez, E., Keppler, O.T., Göbel, C., Zeidler, R., Shein, M., et al. (2021). MicroRNAs are minor constituents of extracellular vesicles that are rarely delivered to target cells. PLoS Genet. *17*, e1009951. https://doi.org/10.1371/journal.pgen.1009951.
70. Raposo, G., and Stahl, P.D. (2024). Extracellular vesicles, genetic programmers. Nat. Cell Biol. *26*, 22–23. https://doi.org/10.1038/s41556-023-01277-8.
71. Hosen, M.R., Goody, P.R., Zietzer, A., Xiang, X., Niepmann, S.T., Sedaghat, A., Tiyerili, V., Chennupati, R., Moore, J.B., 4th, Boon, R.A., et al. (2022). Circulating MicroRNA-122-5p Is Associated With a Lack of Improvement in Left Ventricular Function After Transcatheter Aortic Valve Replacement and Regulates Viability of Cardiomyocytes Through Extracellular Vesicles. Circulation *146*, 1836–1854. https://doi.org/10.1161/CIRCULATIONAHA.122.060258.
72. Botha, J., Handberg, A., and Simonsen, J.B. (2022). Lipid-based strategies used to identify extracellular vesicles in flow cytometry can be confounded by lipoproteins: Evaluations of annexin V, lactadherin, and detergent lysis. J. Extracell. Vesicles *11*, e12200. https://doi.org/10.1002/jev2.12200.
73. Karimi, N., Dalirfardouei, R., Dias, T., Lötvall, J., and Lässer, C. (2022). Tetraspanins distinguish separate extracellular vesicle subpopulations in human serum and plasma - Contributions of platelet extracellular vesicles in plasma samples. J. Extracell. Vesicles *11*, e12213. https://doi.org/10.1002/jev2.12213.
74. O'Brien, K., Ughetto, S., Mahjoum, S., Nair, A.V., and Breakefield, X.O. (2022). Uptake, functionality, and re-release of extracellular vesicle-encapsulated cargo. Cell Rep. *39*, 110651. https://doi.org/10.1016/j.celrep.2022.110651.
75. Vagner, T., Chin, A., Mariscal, J., Bannykh, S., Engman, D.M., and Di Vizio, D. (2019). Protein Composition Reflects Extracellular Vesicle Heterogeneity. Proteomics *19*, e1800167. https://doi.org/10.1002/pmic.201800167.
76. Broad, K., Walker, S.A., Davidovich, I., Witwer, K., Talmon, Y., and Wolfram, J. (2023). Unraveling multilayered extracellular

vesicles: Speculation on cause. J. Extracell. Vesicles *12*, e12309. https://doi.org/10.1002/jev2.12309.

77. Skotland, T., Sagini, K., Sandvig, K., and Llorente, A. (2020). An emerging focus on lipids in extracellular vesicles. Adv. Drug Deliv. Rev. *159*, 308–321. https://doi.org/10.1016/j.addr.2020.03.002.
78. Roy, S., Hochberg, F.H., and Jones, P.S. (2018). Extracellular vesicles: the growth as diagnostics and therapeutics; a survey. J. Extracell. Vesicles *7*, 1438720. https://doi.org/10.1080/20013078.2018.1438720.
79. Chitti, S.V., Gummadi, S., Kang, T., Shahi, S., Marzan, A.L., Nedeva, C., Sanwlani, R., Bramich, K., Stewart, S., Petrovska, M., et al. (2024). Vesiclepedia 2024: an extracellular vesicles and extracellular particles repository. Nucleic Acids Res. *52*, D1694–D1698. https://doi.org/10.1093/nar/gkad1007.
80. Kim, D.K., Kang, B., Kim, O.Y., Choi, D.S., Lee, J., Kim, S.R., Go, G., Yoon, Y.J., Kim, J.H., Jang, S.C., et al. (2013). EVpedia: an integrated database of high-throughput data for systemic analyses of extracellular vesicles. J. Extracell. Vesicles *2*. https://doi.org/10.3402/jev.v2i0.20384.
81. Lai, H., Li, Y., Zhang, H., Hu, J., Liao, J., Su, Y., Li, Q., Chen, B., Li, C., Wang, Z., et al. (2022). exoRBase 2.0: an atlas of mRNA, lncRNA and circRNA in extracellular vesicles from human biofluids. Nucleic Acids Res. *50*, D118–D128. https://doi.org/10.1093/nar/gkab1085.
82. EV-TRACK Consortium, Van Deun, J., Mestdagh, P., Agostinis, P., Akay, Ö., Anand, S., Anckaert, J., Martinez, Z.A., Baetens, T., Beghein, E., et al. (2017). EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. Nat. Methods *14*, 228–232. https://doi.org/10.1038/nmeth.4185.
83. Murillo, O.D., Thistlethwaite, W., Rozowsky, J., Subramanian, S.L., Lucero, R., Shah, N., Jackson, A.R., Srinivasan, S., Chung, A., Laurent, C.D., et al. (2019). exRNA Atlas Analysis Reveals Distinct Extracellular RNA Cargo Types and Their Carriers Present across Human Biofluids. Cell *177*, 463–477.e15. https://doi.org/10.1016/j.cell.2019.02.018.
84. Singh, M.K., and Epstein, J.A. (2012). Epicardium-derived cardiac mesenchymal stem cells: expanding the outer limit of heart repair. Circ. Res. *110*, 904–906. https://doi.org/10.1161/RES.0b013e31825332a3.
85. Quijada, P., Trembley, M.A., and Small, E.M. (2020). The Role of the Epicardium During Heart Development and Repair. Circ. Res. *126*, 377–394. https://doi.org/10.1161/CIRCRESAHA.119.315857.
86. Smart, N., Bollini, S., Dubé, K.N., Vieira, J.M., Zhou, B., Davidson, S., Yellon, D., Riegler, J., Price, A.N., Lythgoe, M.F., et al. (2011). De novo cardiomyocytes from within the activated adult heart after injury. Nature *474*, 640–644. https://doi.org/10.1038/nature10188.
87. Del Campo, C.V., Liaw, N.Y., Gunadasa-Rohling, M., Matthaei, M., Braga, L., Kennedy, T., Salinas, G., Voigt, N., Giacca, M., Zimmermann, W.H., and Riley, P.R. (2022). Regenerative potential of epicardium-derived extracellular vesicles mediated by conserved miRNA transfer. Cardiovasc. Res. *118*, 597–611. https://doi.org/10.1093/cvr/cvab054.
88. Bollini, S., Cheung, K.K., Riegler, J., Dong, X., Smart, N., Ghionzoli, M., Loukogeorgakis, S.P., Maghsoudlou, P., Dubé, K.N., Riley, P.R., et al. (2011). Amniotic fluid stem cells are cardioprotective following acute myocardial infarction. Stem Cells Dev. *20*, 1985–1994. https://doi.org/10.1089/scd.2010.0424.
89. Balbi, C., Lodder, K., Costa, A., Moimas, S., Moccia, F., van Herwaarden, T., Rosti, V., Campagnoli, F., Palmeri, A., De Biasio, P., et al. (2019). Reactivating endogenous mechanisms of cardiac regeneration via paracrine boosting using the human amniotic fluid stem cell secretome. Int. J. Cardiol. *287*, 87–95. https://doi.org/10.1016/j.ijcard.2019.04.011.
90. McCall, F.C., Telukuntla, K.S., Karantalis, V., Suncion, V.Y., Heldman, A.W., Mushtaq, M., Williams, A.R., and Hare, J.M. (2012). Myocardial infarction and intramyocardial injection models in swine. Nat. Protoc. *7*, 1479–1496. https://doi.org/10.1038/nprot.2012.075.
91. Williams, A.R., Hatzistergos, K.E., Addicott, B., McCall, F., Carvalho, D., Suncion, V., Morales, A.R., Da Silva, J., Sussman, M.A., Heldman, A.W., and Hare, J.M. (2013). Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation *127*, 213–223. https://doi.org/10.1161/CIRCULATIONAHA.112.131110.
92. Lambert, C., Arderiu, G., Bejar, M.T., Crespo, J., Baldellou, M., Juan-Babot, O., and Badimon, L. (2019). Stem cells from human cardiac adipose tissue depots show different gene expression and functional capacities. Stem Cell Res. Ther. *10*, 361. https://doi.org/10.1186/s13287-019-1460-1.
93. Cui, X., He, Z., Liang, Z., Chen, Z., Wang, H., and Zhang, J. (2017). Exosomes From Adipose-derived Mesenchymal Stem Cells Protect the Myocardium Against Ischemia/Reperfusion Injury Through Wnt/beta-Catenin Signaling Pathway. J. Cardiovasc. Pharmacol. *70*, 225–231. https://doi.org/10.1097/FJC.0000000000000507.
94. Thankam, F.G., and Agrawal, D.K. (2022). Single Cell Genomics Identifies Unique Cardioprotective Phenotype of Stem Cells derived from Epicardial Adipose Tissue under Ischemia. Stem Cell Rev. Rep. *18*, 294–335. https://doi.org/10.1007/s12015-021-10273-0.
95. Thankam, F.G., Huynh, J., Fang, W., Chen, Y., and Agrawal, D.K. (2022). Exosomal-ribosomal proteins-driven heterogeneity of epicardial adipose tissue derived stem cells under ischemia for cardiac regeneration. J. Tissue Eng. Regen. Med. *16*, 396–408. https://doi.org/10.1002/term.3289.
96. Thankam, F.G., Sedighim, S., Kuan, R., and Agrawal, D.K. (2023). Ischemia challenged epicardial adipose tissue stem cells-derived extracellular vesicles alter the gene expression of cardiac fibroblasts to cardiomyocyte like phenotype. Transl. Res. *254*, 54–67. https://doi.org/10.1016/j.trsl.2022.10.004.
97. Gan, L., Xie, D., Liu, J., Bond Lau, W., Christopher, T.A., Lopez, B., Zhang, L., Gao, E., Koch, W., Ma, X.L., and Wang, Y. (2020). Small Extracellular Microvesicles Mediated Pathological Communications Between Dysfunctional Adipocytes and Cardiomyocytes as a Novel Mechanism Exacerbating Ischemia/Reperfusion Injury in Diabetic Mice. Circulation *141*, 968–983. https://doi.org/10.1161/CIRCULATIONAHA.119.042640.
98. Kranendonk, M.E.G., Visseren, F.L.J., van Balkom, B.W.M., Nolte-'t Hoen, E.N.M., van Herwaarden, J.A., de Jager, W., Schipper, H.S., Brenkman, A.B., Verhaar, M.C., Wauben, M.H.M., and Kalkhoven, E. (2014). Human adipocyte extracellular vesicles in reciprocal signaling between adipocytes and macrophages. Obesity *22*, 1296–1308. https://doi.org/10.1002/oby.20679.
99. Xie, Z., Wang, X., Liu, X., Du, H., Sun, C., Shao, X., Tian, J., Gu, X., Wang, H., Tian, J., and Yu, B. (2018). Adipose-Derived Exosomes Exert Proatherogenic Effects by Regulating Macrophage Foam Cell Formation and Polarization. J. Am. Heart Assoc. *7*, e007442. https://doi.org/10.1161/JAHA.117.007442.
100. Deng, Z.B., Poliakov, A., Hardy, R.W., Clements, R., Liu, C., Liu, Y., Wang, J., Xiang, X., Zhang, S., Zhuang, X., et al. (2009). Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance. Diabetes *58*, 2498–2505. https://doi.org/10.2337/db09-0216.
101. Zhao, H., Chen, X., Hu, G., Li, C., Guo, L., Zhang, L., Sun, F., Xia, Y., Yan, W., Cui, Z., et al. (2022). Small Extracellular Vesicles From Brown Adipose Tissue Mediate Exercise Cardioprotection. Circ. Res. *130*, 1490–1506. https://doi.org/10.1161/CIRCRESAHA.121.320458.
102. Masurkar, N., Bouvet, M., Logeart, D., Jouve, C., Dramé, F., Claude, O., Roux, M., Delacroix, C., Bergerot, D., Mercadier, J.J., et al. (2023). Novel Cardiokine GDF3 Predicts Adverse Fibrotic Remodeling After Myocardial Infarction. Circulation *147*, 498–511. https://doi.org/10.1161/CIRCULATIONAHA.121.056272.
103. Gan, L., Liu, D., Xie, D., Bond Lau, W., Liu, J., Christopher, T.A., Lopez, B., Liu, L., Hu, H., Yao, P., et al. (2022). Ischemic Heart-Derived Small Extracellular Vesicles Impair Adipocyte Function. Circ. Res. *130*, 48–66. https://doi.org/10.1161/CIRCRESAHA.121.320157.
104. Yang, W., Tu, H., Tang, K., Huang, H., Ou, S., and Wu, J. (2021). MiR-3064 in Epicardial Adipose-Derived Exosomes Targets Neuronatin to Regulate Adipogenic Differentiation of Epicardial Adipose Stem Cells. Front. Cardiovasc. Med. *8*, 709079. https://doi.org/10.3389/fcvm.2021.709079.
105. Akawi, N., Checa, A., Antonopoulos, A.S., Akoumianakis, I., Daskalaki, E., Kotanidis, C.P., Kondo, H., Lee, K., Yesilyurt, D., Badi, I., et al. (2021). Fat-Secreted Ceramides Regulate Vascular Redox State and Influence Outcomes in Patients With Cardiovascular Disease. J. Am. Coll. Cardiol. *77*, 2494–2513. https://doi.org/10.1016/j.jacc.2021.03.314.
106. Gburcik, V., Cleasby, M.E., and Timmons, J.A. (2013). Loss of neuronatin promotes "browning" of primary mouse adipocytes while reducing Glut1-mediated glucose disposal. Am. J. Physiol. Endocrinol. Metab. *304*, E885–E894. https://doi.org/10.1152/ajpendo.00463.2012.
107. Laaksonen, R., Ekroos, K., Sysi-Aho, M., Hilvo, M., Vihervaara, T., Kauhanen, D., Suoniemi, M., Hurme, R., März, W., Scharnagl, H., et al. (2016). Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease

and acute coronary syndromes beyond LDL-cholesterol. Eur. Heart J. *37*, 1967–1976. https://doi.org/10.1093/eurheartj/ehw148.

108. Javaheri, A., Allegood, J.C., Cowart, L.A., and Chirinos, J.A. (2020). Circulating Ceramide 16:0 in Heart Failure With Preserved Ejection Fraction. J. Am. Coll. Cardiol. *75*, 2273–2275. https://doi.org/10.1016/j.jacc.2020.02.062.
109. Wang, S., Aurora, A.B., Johnson, B.A., Qi, X., McAnally, J., Hill, J.A., Richardson, J.A., Bassel-Duby, R., and Olson, E.N. (2008). The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell *15*, 261–271. https://doi.org/10.1016/j.devcel.2008.07.002.
110. Togliatto, G., Dentelli, P., Gili, M., Gallo, S., Deregibus, C., Biglieri, E., Iavello, A., Santini, E., Rossi, C., Solini, A., et al. (2016). Obesity reduces the pro-angiogenic potential of adipose tissue stem cell-derived extracellular vesicles (EVs) by impairing miR-126 content: impact on clinical applications. Int. J. Obes. *40*, 102–111. https://doi.org/10.1038/ijo.2015.123.
111. Wen, Z., Li, J., Fu, Y., Zheng, Y., Ma, M., and Wang, C. (2020). Hypertrophic Adipocyte-Derived Exosomal miR-802-5p Contributes to Insulin Resistance in Cardiac Myocytes Through Targeting HSP60. Obesity *28*, 1932–1940. https://doi.org/10.1002/oby.22932.
112. Higuchi, C., Nakatsuka, A., Eguchi, J., Teshigawara, S., Kanzaki, M., Katayama, A., Yamaguchi, S., Takahashi, N., Murakami, K., Ogawa, D., et al. (2015). Identification of circulating miR-101, miR-375 and miR-802 as biomarkers for type 2 diabetes. Metabolism *64*, 489–497. https://doi.org/10.1016/j.metabol.2014.12.003.
113. Hauffe, R., Rath, M., Schell, M., Ritter, K., Kappert, K., Deubel, S., Ott, C., Jähnert, M., Jonas, W., Schürmann, A., and Kleinridders, A. (2021). HSP60 reduction protects against diet-induced obesity by modulating energy metabolism in adipose tissue. Mol. Metab. *53*, 101276. https://doi.org/10.1016/j.molmet.2021.101276.
114. Lin, J.R., Ding, L.L.Q., Xu, L., Huang, J., Zhang, Z.B., Chen, X.H., Cheng, Y.W., Ruan, C.C., and Gao, P.J. (2022). Brown Adipocyte ADRB3 Mediates Cardioprotection via Suppressing Exosomal iNOS. Circ. Res. *131*, 133–147. https://doi.org/10.1161/CIRCRESAHA.121.320470.
115. Ernault, A.C., de Winter, R., Fabrizi, B., Bracht, J.W.P., Hau, C., van Amersfoorth, S.C.M., Meulendijks, E.R., Tijsen, A.J., Cócera Ortega, L., van der Made, I., et al. (2023). MicroRNAs in extracellular vesicles released from epicardial adipose tissue promote arrhythmogenic conduction slowing. Heart Rhythm O2 *4*, 805–814. https://doi.org/10.1016/j.hroo.2023.10.007.
116. Thomou, T., Mori, M.A., Dreyfuss, J.M., Konishi, M., Sakaguchi, M., Wolfrum, C., Rao, T.N., Winnay, J.N., Garcia-Martin, R., Grinspoon, S.K., et al. (2017). Adipose-derived circulating miRNAs regulate gene expression in other tissues. Nature *542*, 450–455. https://doi.org/10.1038/nature21365.
117. Ernault, A.C., Verkerk, A.O., Bayer, J.D., Aras, K., Montañés-Agudo, P., Mohan, R.A., Veldkamp, M., Rivaud, M.R., de Winter, R., Kawasaki, M., et al. (2022). Secretome of atrial epicardial adipose tissue facilitates reentrant arrhythmias by myocardial remodeling. Heart Rhythm *19*, 1461–1470. https://doi.org/10.1016/j.hrthm.2022.05.011.
118. Rossi, V.A., Gruebler, M., Monzo, L., Galluzzo, A., and Beltrami, M. (2023). The Different Pathways of Epicardial Adipose Tissue across the Heart Failure Phenotypes: From Pathophysiology to Therapeutic Target. Int. J. Mol. Sci. *24*, 6838. https://doi.org/10.3390/ijms24076838.
119. Herrmann, I.K., Wood, M.J.A., and Fuhrmann, G. (2021). Extracellular vesicles as a next-generation drug delivery platform. Nat. Nanotechnol. *16*, 748–759. https://doi.org/10.1038/s41565-021-00931-2.
120. Cheng, W., Xu, C., Su, Y., Shen, Y., Yang, Q., Zhao, Y., Zhao, Y., and Liu, Y. (2023). Engineered Extracellular Vesicles: A potential treatment for regeneration. iScience *26*, 108282. https://doi.org/10.1016/j.isci.2023.108282.
121. Leggio, L., Paternò, G., Vivarelli, S., Bonasera, A., Pignataro, B., Iraci, N., and Arrabito, G. (2023). Label-free approaches for extracellular vesicle detection. iScience *26*, 108105. https://doi.org/10.1016/j.isci.2023.108105.
122. Li, G., Chen, T., Dahlman, J., Eniola-Adefeso, L., Ghiran, I.C., Kurre, P., Lam, W.A., Lang, J.K., Marbán, E., Martín, P., et al. (2023). Current challenges and future directions for engineering extracellular vesicles for heart, lung, blood and sleep diseases. J. Extracell. Vesicles *12*, e12305. https://doi.org/10.1002/jev2.12305.
123. Wagner, K.T., Nash, T.R., Liu, B., Vunjak-Novakovic, G., and Radisic, M. (2021). Extracellular Vesicles in Cardiac Regeneration: Potential Applications for Tissues-on-a-Chip. Trends Biotechnol. *39*, 755–773. https://doi.org/10.1016/j.tibtech.2020.08.005.