Role of adipocyte-derived extracellular vesicles in vascular inflammation

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Abstract

Extracellular vesicles (EVs) are nanometre-sized vesicles released from most cells, including adipocytes. Relatively little is known about adipocyte-derived EVs (ADEVs) in comparison to other EV subtypes, though interest in ADEVs as potential paracrine and endocrine communicators of adipose tissue in obesity is building. Current evidence indicates that ADEVs contribute to the development of adipose tissue dysfunction; a key feature of obese adipose tissue that it is associated with obesity-related comorbidities including cardiovascular disease (CVD). This review summarises our current knowledge of ADEVs in the development of adipose tissue dysfunction and the potential of ADEVs to disrupt redox signalling and exert vascular effects that may exacerbate CVD in obesity.

Introduction

In contrast to the wealth of data available regarding the roles of extracellular vesicles (EVs) originating from most cell types in health and disease, our knowledge of the functional characteristics of adipocyte-derived EVs (ADEVs) is less well established. However, the field has recently begun to gain traction and adipocytes are now recognised as important sources of EVs, particularly in obesity, metabolic syndrome, cardiovascular disease (CVD), and certain cancers. This bears a resemblance to the historic consideration of adipose tissue as simply a latent store of excess energy as opposed to the endocrine organ we know it as today. This lag in interest may also be due in part to the relative inaccessibility of adipocytes and adipose tissue as a potential source of EVs, though we and others have recently established robust evidence for the presence of ADEVs in the circulation (Connolly et al., 2018; Flaherty et al., 2019; Thomou et al., 2017). Functional ADEV generation was first identified in rat primary adipocytes where “adiposomes” were presented as potential autocrine communicators of adipocyte lipid storage status (Müller et al., 2009). ADEVs are now known to participate in crosstalk with numerous different cell types, perhaps reflecting the diverse role of adipocytes in a range of physiological and pathophysiological processes and in the context of CVD, an area of research that has received relatively little attention.

Content of ADEV

The composition of EVs generally depends on their cell of origin and their route of biogenesis. Here, we use "EV" to denote vesicles produced by both the classical (endocytic) pathway and direct budding of the plasma membrane with the goal of capturing the full spectrum of ADEV research. As such, ADEVs have been shown to contain an array of EV markers associated with these biogenesis pathways including tetraspanins such as CD63, Alix and TSG101 (Connolly et al., 2015) in addition to more adipocyte-specific proteins, lipids and RNAs (outlined below and in **Figure 1**).

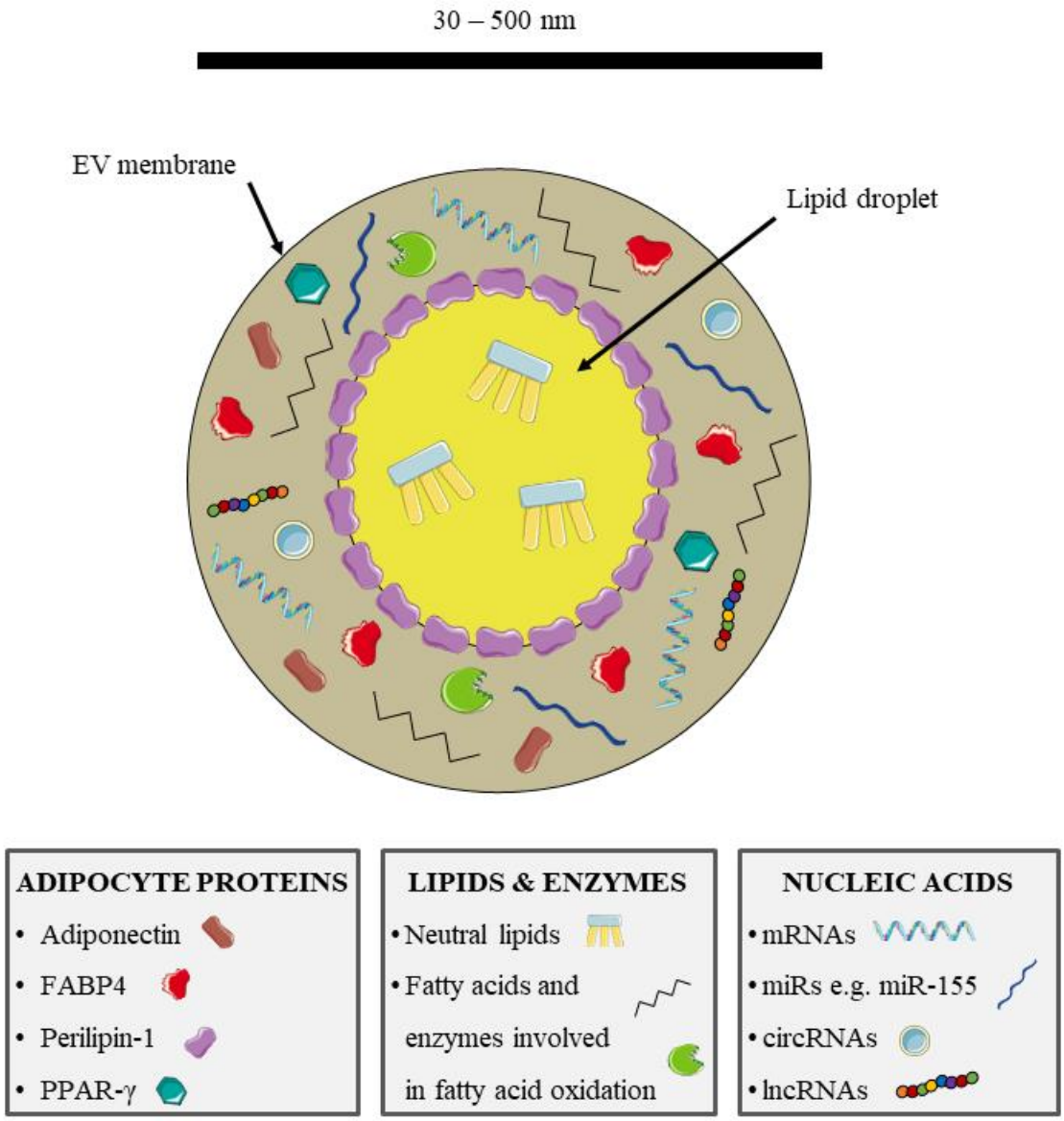


Figure 1: Proposed ADEV composition. A simplified representation of the potential protein, lipid, and RNA content of ADEVs. Scale bar represents the range in which the majority of ADEVs are

likely to fall. Image created using Servier Medical Art. FABP4; fatty acid binding protein-4, PPAR-γ; peroxisome proliferator activated receptor-γ, mRNA; messenger RNA, miR; microRNA, circRNA; circular RNA, lncRNA; long non-coding RNA.

Much of our knowledge of ADEVs stems from research centred on the use of the murine adipocyte cell line, 3T3-L1. These studies have highlighted adipocyte-specific markers that have allowed identification of ADEVs in complex, heterogeneous sources, such as plasma. Several adipocyte protein markers have been detected in circulating EV populations, including adiponectin (Kranendonk, Visseren, van Herwaarden, et al., 2014; Phoonsawat et al., 2014), fatty acid binding protein (FABP)-4 (Gustafson et al., 2015; Witczak et al., 2018), perilipin-1 (Eguchi et al., 2015) and peroxisome proliferator activated receptor (PPAR)-γ (Looze et al., 2009). However, caution must be exercised when using these markers to identify ADEVs in the circulation as many of these markers are soluble adipokines, capable of non-selective co-isolation with EV populations during sample processing (Connolly et al., 2018), and in some cases may not be specific to adipocytes alone.

Despite forming an estimated two-thirds of the total EV volume (Kreimer et al., 2015), lipids are often overlooked as potential signalling mediators within EVs. Indeed, the lipid volume of ADEVs is likely to be greater than other EV subtypes given the functional role of adipocytes in lipid storage. We have previously found that the lipid content of ADEVs increases to reflect that of the parent cell as preadipocytes undergo differentiation to mature adipocytes (Connolly et al., 2015). More in-depth analysis revealed that though ADEVs share a large degree of similarity to their parent cell, the composition of certain fatty acids and phospholipids were unique to ADEVs. For example, oleic acid and phosphatidylserine were enriched in ADEVs compared to adipocytes, suggesting that though there is significant crossover in the composition of adipocytes and corresponding ADEVs, there are also marked differences, indicating specific packaging of lipids within ADEVs (Connolly et al., 2015). ADEVs have recently been shown to confer a novel mechanism for local lipid release from adipocytes, harbouring and transferring triglycerides to local macrophages thereby driving their differentiation to adipose tissue macrophages (ATMs) (Flaherty et al., 2019). As outlined above, ADEVs also contain the lipid droplet-associated protein perilipin-1 (Connolly et al., 2018; Eguchi et al., 2015) and others, including CD73 (Müller et al., 2011b), suggesting adipocytes are able to use ADEVs to safely transfer lipid droplets to other cells within adipose tissue. ADEVs have also been shown to promote the migration and invasion

of melanoma cells by delivering metabolic enzymes that enable tumour cells to shift their metabolism towards fatty acid oxidation (Lazar et al., 2016). Interestingly, a further increase in migration is observed in obesity as ADEVs supply fatty acids in addition to metabolic enzymes to melanoma cells thereby providing tumour cells with the complete metabolic toolset required to facilitate tumour progression (Clement et al., 2020). Gu et al., (2021) have also shown that ADEVs can transfer metabolic enzymes to the liver, altering hepatic metabolism. In obesity, adipocytes produced EVs that were able to modulate hepatic metabolism, leading to steatosis (Gu et al., 2021). Together, these studies show that ADEVs can convey and alter metabolic properties in a variety of cells in a paracrine and endocrine fashion.

EVs are important sources of different functional RNAs, including messenger RNAs (mRNA) and micro RNAs (miR), capable of being transferred to, and impacting the function of, recipient cells (Valadi et al., 2007). Adipose tissue has been proposed as a major source of exosomal miR in the circulation with multiple potential targets, as following adipocyte-specific knockout of miR generation capacity, a reduction in circulating exosomal miR was observed (Thomou et al., 2017). We and others have previously provided evidence to suggest that the circulating population of ADEVs is relatively low in comparison to EVs from other sources such as platelets (Connolly et al., 2018; Flaherty et al., 2019) though adipocytes may be particularly enriched in miRs compared to other circulating EV populations. ADEVs have previously been shown to transfer mRNA transcripts including, adiponectin and PPAR-γ2, and miRs such as miR-155 to macrophages (Ogawa et al., 2010; Ortega et al., 2015; Y. Zhang et al., 2016), suggesting ADEVs can mediate local signalling within adipose tissue via transfer of RNAs. There is also evidence to suggest that ADEVs can deliver functional RNA to sites distal to adipose tissue; ADEVs were shown to promote hepatocellular carcinoma proliferation through carriage of novel noncoding RNAs (circRNAs) (H. Zhang et al., 2019). These circRNAs within ADEVs were able to supress the actions of miR-34a and subsequently promote tumorigenesis.

The role of ADEV in adipose tissue dysfunction

The ability of adipose tissue to control endocrine functions facilitates its role in physiological processes such as the maintenance of vascular tone and control of metabolism. However, energy imbalances in obesity exert undue pressure on adipocytes leading to changes in

adipocyte number and structure, and changes to the adipose tissue environment (Choe et al., 2016). This results in dysregulation of endocrine signalling and promotes the transition of adipose tissue to a proinflammatory organ (Choe et al., 2016). Consequently, dysfunctional obese adipose tissue leads to an increased risk of CVD, diabetes and cancer, three of the leading causes of mortality worldwide (WHO, 2020). Several mechanisms for the initiation of adipose tissue dysfunction have been suggested, with adipocyte endoplasmic reticulum (ER) stress (Kawasaki et al., 2012) and adipose tissue hypoxia (Trayhurn et al., 2008) being among the most widely researched.

ER stress is a feature of obesity and has been shown to contribute to obesity-related insulin resistance and type 2 diabetes (Özcan et al., 2004) and adipose tissue inflammation (Kawasaki et al., 2012). ER stress occurs when certain conditions such as increases in demand for protein synthesis and increased levels of free fatty acids (both of which occur in obesity) interfere with ER function, thereby disrupting the folding of proteins within the ER (Özcan et al., 2004). Elevated levels of free fatty acids in adipose tissue can increase reactive oxygen species (ROS) production which can lead to misfolding of newly formed proteins within the ER either via direct oxidation or via indirect calcium depletion in the ER (Kawasaki et al., 2012; Malhotra & Kaufman, 2007). Misfolded or unfolded proteins activate the unfolded protein response which can lead to apoptosis and adipose tissue dysfunction (Kawasaki et al., 2012). ER stress has been shown to increase the production of EVs containing pro-inflammatory molecules in a model of pre-eclampsia (Collett et al., 2018). Recently, ADEV generated under conditions of ER stress were shown to deliver the metabolic enzyme aldo-keto reductase 1B7 to the liver which led to hepatic lipid accumulation and steatosis (Gu et al., 2021). Therefore, ER stress may be an important stimulator of ADEV production and these EVs may exacerbate obesity-related comorbidities, however; more research is required to establish the interplay between, ER stress, ROS production, ADEV generation and adipose tissue dysfunction in obesity.

The controlled accommodation and storage of increases in energy intake is a key feature of adipose tissue. This is primarily achieved from hypertrophy of existing adipocytes before hyperplasia and differentiation of preadipocytes and adipocyte precursors if demand exceeds capacity. Interestingly, larger adipocytes have been shown to release EVs at a higher rate which contain lipid droplet-associated proteins and miRs capable of inducing lipogenesis in

smaller adipocytes (Müller et al., 2009, 2011a, 2011b). ADEVs may therefore shift the burden of triglyceride accumulation from large to small adipocytes, thereby ensuring safe adipocyte hypertrophy. However, this process may be overwhelmed with progressive obesity as single adipocytes hypertrophy up to 200 μm in diameter to manage the increasing demand for lipid storage (Skurk et al., 2007). Large diameter adipocytes may therefore push the limits for safe lipid accumulation, but also exceed the maximal diffusion distance of oxygen from surrounding capillaries, which is reported to be between 100-200 μm (Brahimi-Horn & Pouysségur, 2007). In addition, the perfusion of white adipose tissue is generally considered to be poor, and even poorer in obese white adipose tissue (Goossens et al., 2011). Therefore, obese adipose tissue is likely to contain regions of hypoxia where poor vascular supply is combined with large diameter adipocytes.

Indeed, gene expression of the oxygen sensing transcription factor, hypoxia-inducible factor (HIF)-1α, is increased in obese adipose tissue and reduced upon weight loss (Cancello et al., 2005). Upregulation of HIF-1α is associated with an altered adipokine profile that promotes an inflammatory environment (B. Wang et al., 2007). In addition, activation of HIF-1α is known to induce reorganisation of the actin cytoskeleton (Weidemann et al., 2013); a key requirement for EV formation (Burger et al., 2013). Indeed, we and others have shown that hypoxia induces EV generation in several different cell types, including adipocytes (Sano et al., 2014; Wadey et al., 2019) and silencing of HIF-1α has been shown to abate hypoxia-induced EV generation (Burnley-Hall et al., 2017; T. Wang et al., 2014). Importantly, not only does hypoxia induce an approximate doubling of ADEV generation, the content of hypoxic ADEVs exhibit signature components that differ from normoxic ADEVs. The fatty acid and phospholipid composition of ADEVs were altered between normoxia and hypoxia; for example, palmitoleic acid (widely considered a positive regulator of glucose homeostasis in adipocytes (Bolsoni-Lopes et al., 2014) was decreased in hypoxic ADEVs (unpublished observations). Furthermore, Sano et al., (2014) found that hypoxic ADEVs were enriched in lipogenic enzymes, such as fatty acid synthase, suggesting ADEVs generated under hypoxic conditions are able to influence metabolism in recipient cells.

ER stress and adipocyte hypoxia, both of which are known initiators of adipose tissue dysfunction, are therefore able to induce ADEV production with an altered bio-cargo and function. Circulating ADEVs have also been shown to be increased in obesity (Eguchi et al.,

2015; Flaherty et al., 2019) and decreased in response to low calorie diet intervention (Eguchi et al., 2016).Therefore, ADEVs are likely to be key mediators of adipose tissue dysfunction in obesity, potentially facilitating the initiation of an inflammatory environment both within adipose tissue and peripherally. In fact, strong evidence is emerging for an interaction of ADEVs with circulating monocytes and adipose tissue macrophages (ATMs) in obesity. Plasma EVs from obese mice were shown to activate circulating monocytes and induce infiltration of proinflammatory macrophages into adipose tissue (Eguchi et al., 2015). Several studies have also shown ADEVs from obese subjects or models of obesity are capable of modulating the differentiation of ATMs (Flaherty et al., 2019), promoting transition towards an M1, proinflammatory phenotype (Deng et al., 2009; Eguchi et al., 2015; Kranendonk, Visseren, van Balkom, et al., 2014; Ortega et al., 2015; Pan et al., 2019; M. Song et al., 2018; Xie et al., 2018; Y. Zhang et al., 2016).

As previously mentioned, ADEVs may be important sources of miRs in the circulation (Thomou et al., 2017) and certain miRs, including miR-155, miR-221 and miR-222 have been found to be enriched in ADEVs, particularly in obesity (Ortega et al., 2015). Of particular note, a 5-fold increase in miR-155 expression has been observed in ADEVs from different models of obesity (Ortega et al., 2015; Y. Zhang et al., 2016). Zhang et al., (2016) showed that miR-155 within ADEVs decreased the expression of suppressor of cytokine signalling 1 (SOCS1) which was then able mediate M1 polarisation of macrophages through the JAK/STAT signalling pathway. On the other hand, Pan et al., (2019) found that ADEVs from obese adipose tissue were enriched in miR-34a which targeted Krüppel-like factor 4 (Kfl4), a regulator of macrophage polarisation. ADEVs delivered miR-34a to macrophages leading to suppression of Kfl4 expression and a consequent inhibition of anti-inflammatory M2 macrophage polarisation (Pan et al., 2019), thereby promoting adipose tissue inflammation. Together, this suggests that miRs may be important components of ADEVs in mediating the inflammatory effects of obesity within adipose tissue.

In one of the earliest studies assessing the effect of ADEVs on ATMs, Deng et al., (2009) showed ADEVs from visceral adipose tissue of obese mice were enriched in FABP4 which stimulated the differentiation of macrophages *in vitro* to a pro-inflammatory phenotype, secreting IL-6 and TNF-α. Circulating levels of FABP4 show a strong positive correlation with BMI and hallmarks of metabolic syndrome (Xu et al., 2006). In addition, data from our own group have indicated a strong association of FABP4 with circulating EVs which was altered following bariatric surgery, reflecting the changes in metabolic status of adipose

tissue after significant weight loss (Witczak et al., 2018). ADEVs containing FABP4 therefore may be important endocrine mediators of obese adipose tissue. In support of this, ADEVs containing FABP4 from obese mice were primarily taken up by monocytes *in vivo* and enhanced the development of insulin resistance (Deng et al., 2009).

Interestingly, EVs from adipose-derived stem cells (ADSCs) were able to promote transition of ATMs to an anti-inflammatory phenotype and mitigate adipose tissue inflammation, highlighting ADSC-derived EVs as important regulators of adipocyte dysfunction (Zhao et al., 2018). Furthermore, administration of brown adipose tissue (BAT)-derived EVs to obese mice reversed the hallmarks of metabolic syndrome and reduced the overall mass and adipocyte size of white adipose tissue (WAT) (Zhou et al., 2020). In support of this, Thomou et al., (2017) found BAT-EVs were more efficient at regulating crosstalk with the liver, improving glucose tolerance and reducing circulating insulin levels than EVs from WAT. This suggests that ADEVs from WAT depots may play a role in the initiation of adipose tissue dysfunction and inflammation in obesity whereas ADEVs from other adipose tissue depots may offer some form of protection of adipose tissue homeostasis.

Role of ADEV in mediating vascular changes

In contrast to the extensive evidence of pro-inflammatory crosstalk of ADEVs with macrophages in obesity, far less is known regarding the exchange of EVs between adipocytes and endothelial cells in obesity. This is despite obesity being a long-established independent risk factor for the development of CVD (Hubert et al., 1983) and adipose tissue inflammation being strongly linked to the development of endothelial dysfunction (Chudek & Wiecek, 2006).

Our group has previously used conditions of hypoxia and inflammation to mimic adipose tissue dysfunction, and to test the effect of ADEVs generated from obese-like conditions on properties of endothelial cell function (Wadey et al., 2019). Hypoxia and inflammation increased the production of EVs synergistically from adipocytes and caused dysregulation of adipokine content of both the parent cells and resulting EVs. ADEVs generated from hypoxic, inflammatory adipocytes also increased the expression of endothelial vascular cell adhesion molecule (VCAM)-1 and subsequent leukocyte attachment to endothelial cells, an effect that was largely mediated by TNF-α. This suggests that adipocytes can confer their dysregulated state to ADEVs which may then in turn, initiate leukocyte adhesion to the

vascular endothelium and exacerbate vascular disease in obesity (Wadey et al., 2019). ADEVs from visceral adipose tissue have been shown to stimulate formation of macrophage foam cells and to enhance the formation of atherosclerotic lesions *in vivo* (Xie et al., 2018). Furthermore, ADEVs derived from a model of insulin resistance were able to induce angiogenesis via transfer of pro-angiogenic sonic hedgehog glycoprotein (shh) (F. Wang et al., 2018). In addition, when these ADEVs enriched in shh were delivered to an *in vivo* diabetic mouse model, vasa vasorum angiogenesis was enhanced which consequently impaired atherosclerotic plaque stability. Taken together, these studies suggest that in obesity, ADEVs may play an important role in the decline of vascular function by promoting an inflammatory phenotype that can initiate and exacerbate atherosclerosis.

Crewe et al., (2018) recently highlighted the role of EVs in facilitating crosstalk between cell populations within adipose tissue. Knockdown of an adipocyte-specific form of the caveolin-1 (cav-1) protein ablated gene expression of cav-1 but not the adipocyte protein expression. Local endothelial cells were shown to be transferring the cav-1 protein to adipocytes via EVs and interestingly, this process was sensitive to metabolic status of endothelial cells both *in vitro* and *in vivo* (Crewe et al., 2018). During fasting conditions, EV production from endothelial cells (and subsequent uptake by adipocytes) was enhanced whereas this was reversed during the fed state, with adipocytes readily shedding EVs containing cav-1. These EVs were then largely taken up by surrounding endothelial cells and ATMs indicating the close cross talk between cells of adipose tissue in communicating the metabolic state.

In addition to adipocyte-endothelial crosstalk, ADEVs have also been shown to mediate changes in vascular smooth muscle cells (Li et al., 2019). EVs isolated from mesenteric perivascular adipose tissue were increased in obesity and were able to induce a phenotypic switch in vascular smooth muscle cells from a contractile to a synthetic phenotype. This effect was enhanced in obese/inflamed perivascular adipose tissue due to high expression of miR-221-p3 in perivascular ADEVs (Li et al., 2019). Enrichment of miR-221-p3 in ADEVs was shown to induce mitochondrial dysfunction in vascular smooth muscle cells through suppression of the mitochondrial regulator, PPAR-γ coactivator (PGC)-1α. Not only does this suggest a role for ADEVs locally within the vessel wall in mediating obesity-related vascular remodelling, but also highlights the potential involvement of ADEVs in the disruption of mitochondrial function. Mitochondrial dysfunction can result in the production of ROS, and mitochondria within obese adipocytes are known to generate higher amounts of ROS

(Chattopadhyay et al., 2015). Therefore, ADEVs may also contribute to oxidative stress in obese adipose tissue.

A number of studies have also indicated a protective role for ADSC-EVs via mediation of adipocyte-endothelial cross talk. EVs from ADSCs have been shown to induce growth of endothelial cells and angiogenesis in *in vivo* models of wound healing through up-regulation of several genes involved in proliferation and growth, such as cyclins and VEGF-A (Liu et al., 2019; Ren et al., 2019). Additionally, exosomes harvested from ADSCs overexpressing the long noncoding RNA, SNHG9, were recently shown to protect against TNF receptor type-1 associated death domain protein (TRADD)-mediated inflammation and apoptosis in endothelial cells, thereby protecting against endothelial dysfunction (Y. Song et al., 2020). Furthermore, SNHG9 was decreased in plasma exosomes from obese patients and further so in obese patients with endothelial dysfunction (Y. Song et al., 2020), suggesting that beneficial components of adipose tissue-derived EVs (such as SNHG9) may be lost in obesity in addition to the gain of a more pathological cargo, the combination of which may lead to impaired vascular function.

These varying roles of ADEVs in the development and maintenance of adipose tissue inflammation and the consequent effects on the vasculature are summarised in **Figure 2**. Clearly, ADEVs facilitate close crosstalk of adipocytes with different cell types within adipose tissue including ATMs and endothelial cells, to maintain the metabolic health of lean adipose tissue and to propagate an inflammatory, dysfunctional environment in obesity.

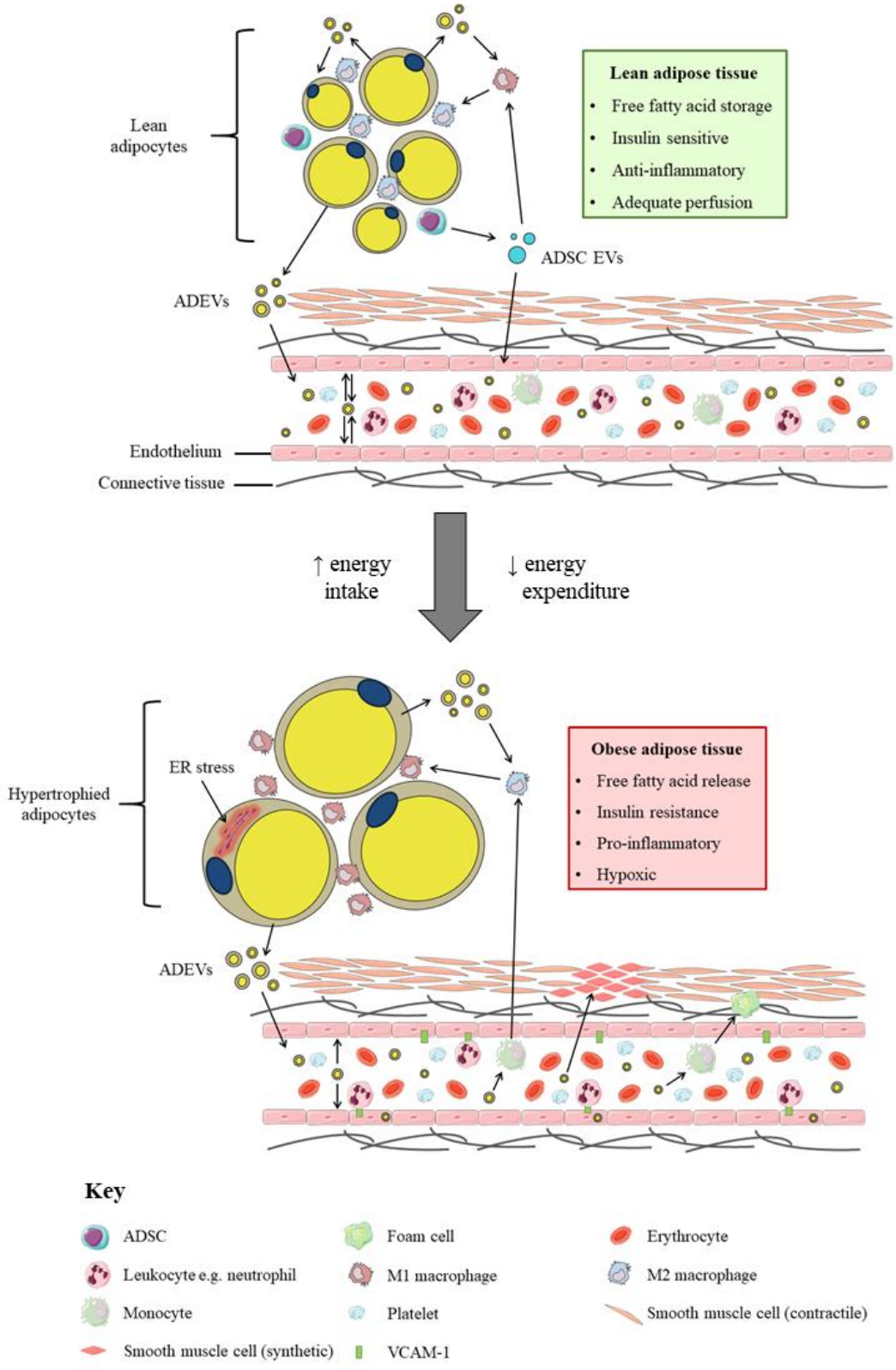
Redox potential role of ADEVs

It is well-established that adipocytes and adipose tissue are sensitive to oxidative stress, particularly in obesity (Furukawa et al., 2004). In fact, ROS such as H_2O_2, and oxidative enzymes such as NADPH oxidase, have been shown to be increased in obese adipose tissue (Chattopadhyay et al., 2015; Furukawa et al., 2004) and fat accumulation is positively correlated with systemic markers of oxidative stress (Furukawa et al., 2004; Keaney et al., 2003). Therefore, oxidative stress in combination with ER stress and regions of hypoxia can cause dysregulation of adipokine secretion and the development of metabolic syndrome.

EVs have been suggested as novel indicators of a cell's redox status, as oxidative damage accumulates in lipids, nucleic acids and proteins of the parent cell which may then be

packaged into EVs (Borras et al., 2020). Furthermore, when the parent cell is exposed to pro-oxidant conditions, EV production is altered. For example, angiotensin II induced oxidative stress in endothelial cells by increasing activation of NADPH oxidase and superoxide formation, but also increased EV generation (Burger et al., 2011). These EVs were then able to induce ROS formation and oxidative stress in naïve endothelial cells. Furthermore, circulating EVs were increased in patients with metabolic syndrome and these EVs were able to induce endothelial dysfunction *in vivo* (Agouni et al., 2008). EVs have also been shown to contain a number of different redox enzymes, including glutathione *S*-transferase, glutathione peroxidase (Jin et al., 2005) and endothelial nitric oxide synthase; the latter was found to be decreased in patients with endothelial dysfunction (Horn et al., 2013).

Akin to the lack of research surrounding ADEVs in CVD, we could find no evidence of direct research into the redox potential of ADEVs. This is despite evidence that individually, adipocytes and EVs are involved in redox reactions and can promote oxidative stress. Additionally, as outlined above, ADEVs contain components that are susceptible to oxidation (including lipids, proteins and nucleic acids) which can be transferred to cells locally within adipose tissue but also in an endocrine fashion. ADEV production is also sensitive to the condition of the parent cell. Indeed, several conditions likely to disrupt redox signalling in adipocytes have been shown to increase ADEV generation, for example, elevated levels of free fatty acids (palmitic acid) (Eguchi et al., 2015), hypoxia and inflammation (Wadey et al., 2019) and induction of ER stress (Gu et al., 2021). Furthermore, ADEVs from obese adipose tissue were shown to induce mitochondrial dysfunction (Li et al., 2019), a key contributor to oxidative stress. ADEVs are therefore likely to play an important role in redox signalling within adipose tissue and in obesity, ADEVs may mediate the communication of adipocyte oxidative stress in an autocrine and paracrine fashion but also systemically, potentially facilitating the development of metabolic syndrome and associated CVD.



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336 **Figure 2: The role of ADEVs in communicating adipose tissue dysfunction.** A simplified
337 summary of the known roles of ADEVs as local communicators of lean and obese adipose tissue.
338 ADEVs in lean adipose tissue promote safe lipid storage and an anti-inflammatory environment and
339 are involved in crosstalk with vascular endothelial cells. Conversely, hypertrophied adipocytes release

ADEVs that: encourage macrophage infiltration into adipose tissue; promote transition of ATMs to an M1 pro-inflammatory phenotype; increase leukocyte adhesion to the vascular endothelium; drive foam cell formation and induce contractile vascular smooth muscle cells to transition towards a synthetic phenotype. Image created using Servier Medical Art. Image is not drawn to scale.

Conclusion

In the past decade, research into the release of EVs from various cell types, into different biological fluids, and the roles of EVs in a wide range of conditions has increased dramatically (Théry et al., 2018). Until recently, relatively little of this research focused on ADEVs and consequently, we are only just beginning to understand the potential roles of ADEVs as paracrine and endocrine communicators of adipose tissue. As such, the roles and effects of ADEVs in the development of CVD in obesity remains largely unexplored. Evidence currently indicates that ADEVs are key mediators of adipocyte crosstalk, promoting adipose tissue homeostasis in lean adipose tissue, whilst facilitating adipose tissue inflammation and dysregulation of vascular function in obesity. However, much more research is required to fully elucidate the role ADEVs play in inflammation and vascular homeostasis; the interaction of ADEVs with platelets and the resulting effecting on coagulation for example, is not understood and the wider endocrine roles of ADEVs in obesity remain under-appreciated. Additionally, the potential of ADEVs to participate in redox signalling remains unexplored, despite the known involvement of adipocytes and other EV subtypes in communicating oxidative stress. Recent advancements in the isolation and purification of EVs alongside more sophisticated profiling methodologies (such as proteomics, lipidomics and miR analysis) will also allow for a more precise understanding of ADEV content and how this changes as adipocytes transition to a dysfunctional state. This combined with the current increase in momentum in the ADEV field will no doubt lead to a clearer understanding of the roles ADEVs play in obesity and associated co-morbidities, including CVD.

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