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1 **Role of adipocyte-derived extracellular vesicles in vascular**
2 **inflammation**

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11 *Abstract*

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

21

22 *Introduction*

23 In contrast to the wealth of data available regarding the roles of extracellular vesicles (EVs)
24 originating from most cell types in health and disease, our knowledge of the functional
25 characteristics of adipocyte-derived EVs (ADEVs) is less well established. However, the
26 field has recently begun to gain traction and adipocytes are now recognised as important
27 sources of EVs, particularly in obesity, metabolic syndrome, cardiovascular disease (CVD),
28 and certain cancers. This bears a resemblance to the historic consideration of adipose tissue
29 as simply a latent store of excess energy as opposed to the endocrine organ we know it as
30 today. This lag in interest may also be due in part to the relative inaccessibility of adipocytes
31 and adipose tissue as a potential source of EVs, though we and others have recently
32 established robust evidence for the presence of ADEVs in the circulation (Connolly et al.,
33 2018; Flaherty et al., 2019; Thomou et al., 2017). Functional ADEV generation was first
34 identified in rat primary adipocytes where “adiposomes” were presented as potential
35 autocrine communicators of adipocyte lipid storage status (Müller et al., 2009). ADEVs are
36 now known to participate in crosstalk with numerous different cell types, perhaps reflecting
37 the diverse role of adipocytes in a range of physiological and pathophysiological processes
38 and in the context of CVD, an area of research that has received relatively little attention.

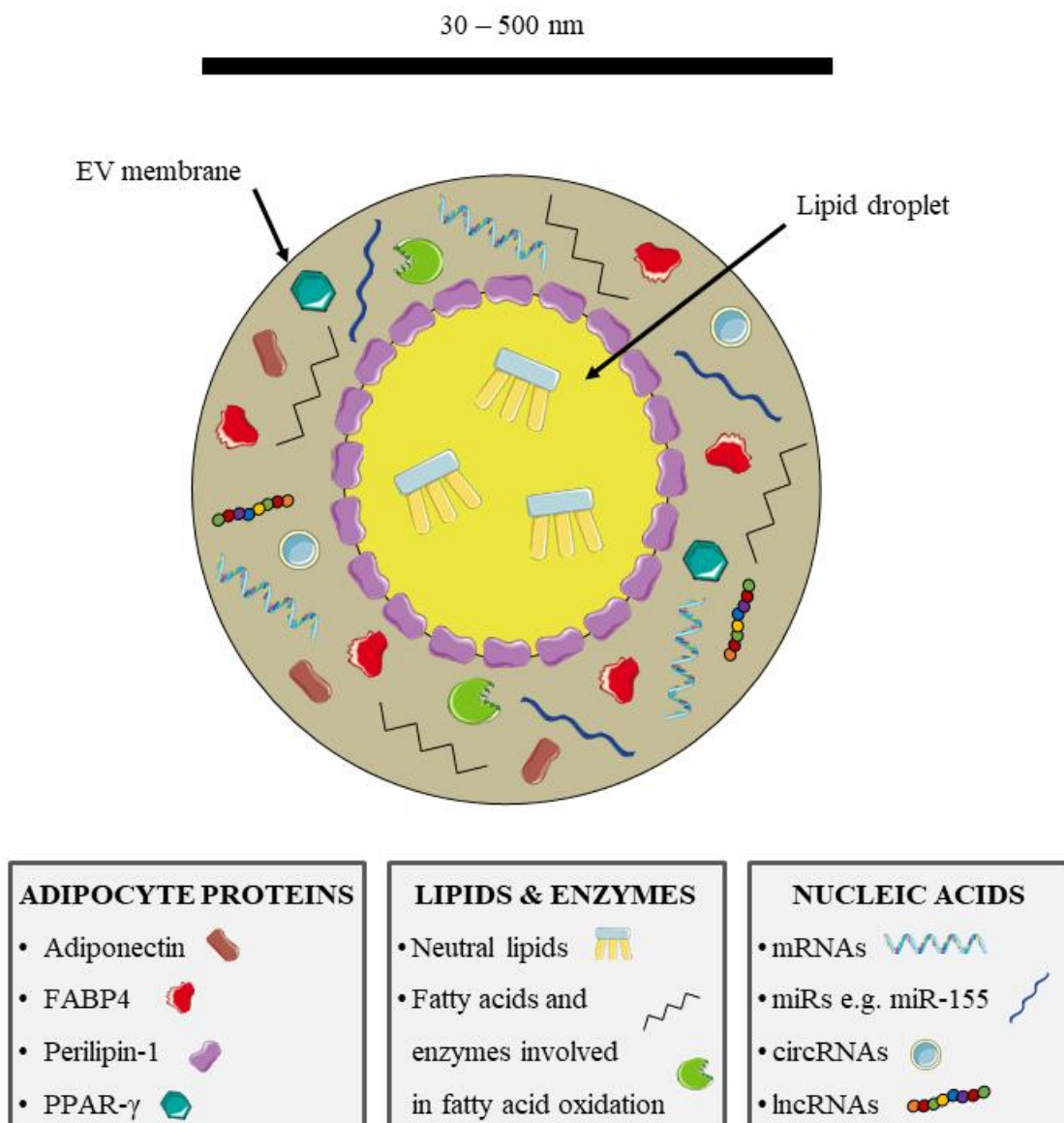
39

40

41 *Content of ADEV*

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

49



50

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

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

56

57 Much of our knowledge of ADEVs stems from research centred on the use of the murine
58 adipocyte cell line, 3T3-L1. These studies have highlighted adipocyte-specific markers that
59 have allowed identification of ADEVs in complex, heterogeneous sources, such as plasma.
60 Several adipocyte protein markers have been detected in circulating EV populations,
61 including adiponectin (Kranendonk, Visseren, van Herwaarden, et al., 2014; Phoonsawat et
62 al., 2014), fatty acid binding protein (FABP)-4 (Gustafson et al., 2015; Witczak et al., 2018),
63 perilipin-1 (Eguchi et al., 2015) and peroxisome proliferator activated receptor (PPAR)- γ
64 (Looze et al., 2009). However, caution must be exercised when using these markers to
65 identify ADEVs in the circulation as many of these markers are soluble adipokines, capable
66 of non-selective co-isolation with EV populations during sample processing (Connolly et al.,
67 2018), and in some cases may not be specific to adipocytes alone.

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

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

96 EVs are important sources of different functional RNAs, including messenger RNAs
97 (mRNA) and micro RNAs (miR), capable of being transferred to, and impacting the function
98 of, recipient cells (Valadi et al., 2007). Adipose tissue has been proposed as a major source of
99 exosomal miR in the circulation with multiple potential targets, as following adipocyte-
100 specific knockout of miR generation capacity, a reduction in circulating exosomal miR was
101 observed (Thomou et al., 2017). We and others have previously provided evidence to suggest
102 that the circulating population of ADEVs is relatively low in comparison to EVs from other
103 sources such as platelets (Connolly et al., 2018; Flaherty et al., 2019) though adipocytes may
104 be particularly enriched in miRs compared to other circulating EV populations. ADEVs have
105 previously been shown to transfer mRNA transcripts including, adiponectin and PPAR- γ 2,
106 and miRs such as miR-155 to macrophages (Ogawa et al., 2010; Ortega et al., 2015; Y.
107 Zhang et al., 2016), suggesting ADEVs can mediate local signalling within adipose tissue via
108 transfer of RNAs. There is also evidence to suggest that ADEVs can deliver functional RNA
109 to sites distal to adipose tissue; ADEVs were shown to promote hepatocellular carcinoma
110 proliferation through carriage of novel noncoding RNAs (circRNAs) (H. Zhang et al., 2019).
111 These circRNAs within ADEVs were able to suppress the actions of miR-34a and
112 subsequently promote tumorigenesis.

113

114 *The role of ADEV in adipose tissue dysfunction*

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

118 adipocyte number and structure, and changes to the adipose tissue environment (Choe et al.,
119 2016). This results in dysregulation of endocrine signalling and promotes the transition of
120 adipose tissue to a proinflammatory organ (Choe et al., 2016). Consequently, dysfunctional
121 obese adipose tissue leads to an increased risk of CVD, diabetes and cancer, three of the
122 leading causes of mortality worldwide (WHO, 2020). Several mechanisms for the initiation
123 of adipose tissue dysfunction have been suggested, with adipocyte endoplasmic reticulum
124 (ER) stress (Kawasaki et al., 2012) and adipose tissue hypoxia (Trayhurn et al., 2008) being
125 among the most widely researched.

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

144

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

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

161 Indeed, gene expression of the oxygen sensing transcription factor, hypoxia-inducible factor
162 (HIF)-1 α , is increased in obese adipose tissue and reduced upon weight loss (Canello et al.,
163 2005). Upregulation of HIF-1 α is associated with an altered adipokine profile that promotes
164 an inflammatory environment (B. Wang et al., 2007). In addition, activation of HIF-1 α is
165 known to induce reorganisation of the actin cytoskeleton (Weidemann et al., 2013); a key
166 requirement for EV formation (Burger et al., 2013). Indeed, we and others have shown that
167 hypoxia induces EV generation in several different cell types, including adipocytes (Sano et
168 al., 2014; Wadey et al., 2019) and silencing of HIF-1 α has been shown to abate hypoxia-
169 induced EV generation (Burnley-Hall et al., 2017; T. Wang et al., 2014). Importantly, not
170 only does hypoxia induce an approximate doubling of ADEV generation, the content of
171 hypoxic ADEVs exhibit signature components that differ from normoxic ADEVs. The fatty
172 acid and phospholipid composition of ADEVs were altered between normoxia and hypoxia;
173 for example, palmitoleic acid (widely considered a positive regulator of glucose homeostasis
174 in adipocytes (Bolsoni-Lopes et al., 2014) was decreased in hypoxic ADEVs (unpublished
175 observations). Furthermore, Sano et al., (2014) found that hypoxic ADEVs were enriched in
176 lipogenic enzymes, such as fatty acid synthase, suggesting ADEVs generated under hypoxic
177 conditions are able to influence metabolism in recipient cells.

178

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

182 2015; Flaherty et al., 2019) and decreased in response to low calorie diet intervention (Eguchi
183 et al., 2016). Therefore, ADEVs are likely to be key mediators of adipose tissue dysfunction
184 in obesity, potentially facilitating the initiation of an inflammatory environment both within
185 adipose tissue and peripherally. In fact, strong evidence is emerging for an interaction of
186 ADEVs with circulating monocytes and adipose tissue macrophages (ATMs) in obesity.
187 Plasma EVs from obese mice were shown to activate circulating monocytes and induce
188 infiltration of proinflammatory macrophages into adipose tissue (Eguchi et al., 2015). Several
189 studies have also shown ADEVs from obese subjects or models of obesity are capable of
190 modulating the differentiation of ATMs (Flaherty et al., 2019), promoting transition towards
191 an M1, proinflammatory phenotype (Deng et al., 2009; Eguchi et al., 2015; Kranendonk,
192 Visseren, van Balkom, et al., 2014; Ortega et al., 2015; Pan et al., 2019; M. Song et al., 2018;
193 Xie et al., 2018; Y. Zhang et al., 2016).

194 As previously mentioned, ADEVs may be important sources of miRs in the circulation
195 (Thomou et al., 2017) and certain miRs, including miR-155, miR-221 and miR-222 have
196 been found to be enriched in ADEVs, particularly in obesity (Ortega et al., 2015). Of
197 particular note, a 5-fold increase in miR-155 expression has been observed in ADEVs from
198 different models of obesity (Ortega et al., 2015; Y. Zhang et al., 2016). Zhang et al., (2016)
199 showed that miR-155 within ADEVs decreased the expression of suppressor of cytokine
200 signalling 1 (SOCS1) which was then able mediate M1 polarisation of macrophages through
201 the JAK/STAT signalling pathway. On the other hand, Pan et al., (2019) found that ADEVs
202 from obese adipose tissue were enriched in miR-34a which targeted Krüppel-like factor 4
203 (Klf4), a regulator of macrophage polarisation. ADEVs delivered miR-34a to macrophages
204 leading to suppression of Klf4 expression and a consequent inhibition of anti-inflammatory
205 M2 macrophage polarisation (Pan et al., 2019), thereby promoting adipose tissue
206 inflammation. Together, this suggests that miRs may be important components of ADEVs in
207 mediating the inflammatory effects of obesity within adipose tissue.

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

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

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

230

231 *Role of ADEV in mediating vascular changes*

232 In contrast to the extensive evidence of pro-inflammatory crosstalk of ADEVs with
233 macrophages in obesity, far less is known regarding the exchange of EVs between adipocytes
234 and endothelial cells in obesity. This is despite obesity being a long-established independent
235 risk factor for the development of CVD (Hubert et al., 1983) and adipose tissue inflammation
236 being strongly linked to the development of endothelial dysfunction (Chudek & Wiecek,
237 2006).

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

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

257 Crewe et al., (2018) recently highlighted the role of EVs in facilitating crosstalk between cell
258 populations within adipose tissue. Knockdown of an adipocyte-specific form of the caveolin-
259 1 (cav-1) protein ablated gene expression of cav-1 but not the adipocyte protein expression.
260 Local endothelial cells were shown to be transferring the cav-1 protein to adipocytes via EVs
261 and interestingly, this process was sensitive to metabolic status of endothelial cells both *in*
262 *vitro* and *in vivo* (Crewe et al., 2018). During fasting conditions, EV production from
263 endothelial cells (and subsequent uptake by adipocytes) was enhanced whereas this was
264 reversed during the fed state, with adipocytes readily shedding EVs containing cav-1. These
265 EVs were then largely taken up by surrounding endothelial cells and ATMs indicating the
266 close cross talk between cells of adipose tissue in communicating the metabolic state.

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

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

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

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

299

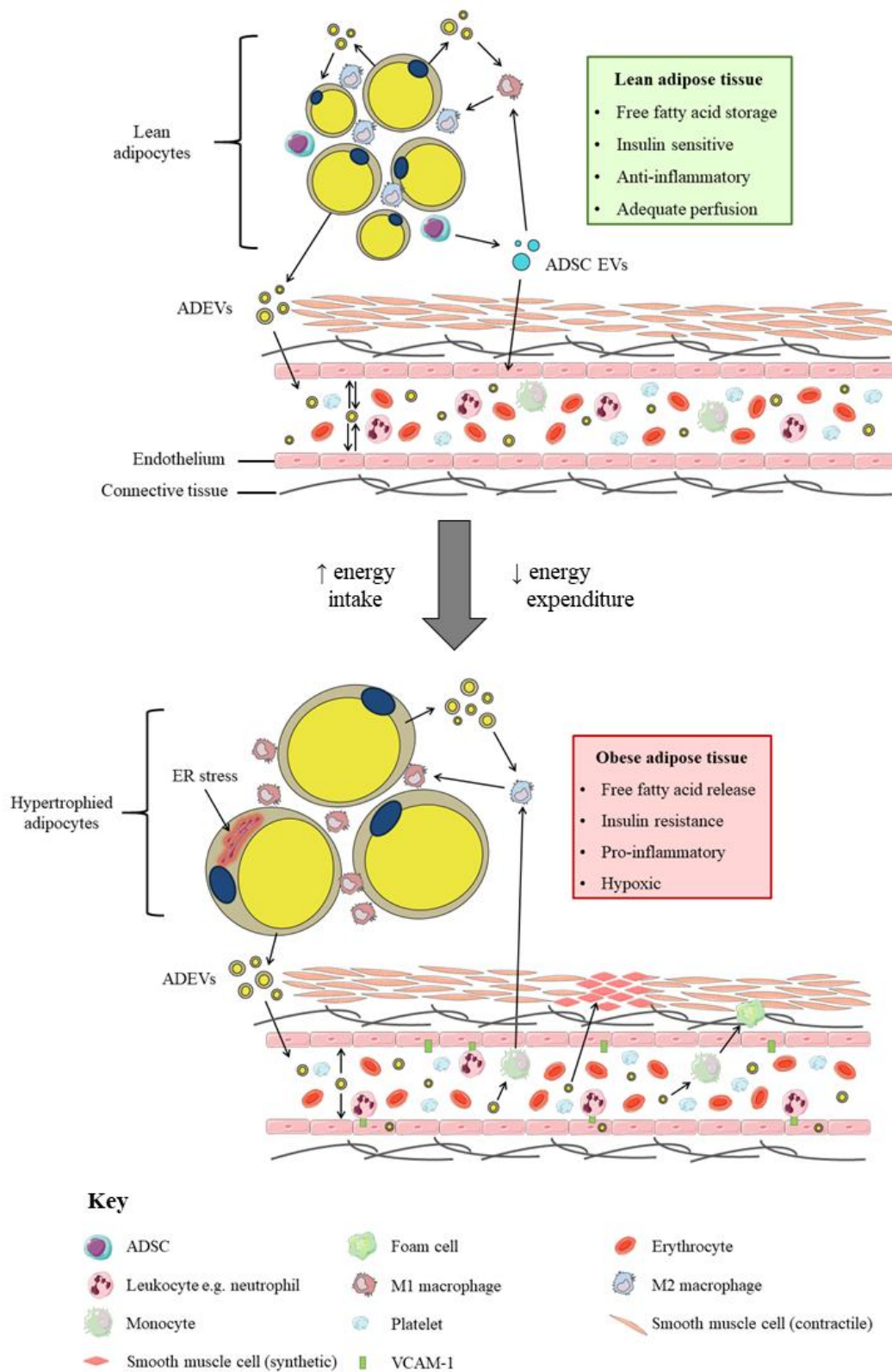
300 *Redox potential role of ADEVs*

301 It is well-established that adipocytes and adipose tissue are sensitive to oxidative stress,
302 particularly in obesity (Furukawa et al., 2004). In fact, ROS such as H₂O₂, and oxidative
303 enzymes such as NADPH oxidase, have been shown to be increased in obese adipose tissue
304 (Chattopadhyay et al., 2015; Furukawa et al., 2004) and fat accumulation is positively
305 correlated with systemic markers of oxidative stress (Furukawa et al., 2004; Keaney et al.,
306 2003). Therefore, oxidative stress in combination with ER stress and regions of hypoxia can
307 cause dysregulation of adipokine secretion and the development of metabolic syndrome.

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

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

320 Akin to the lack of research surrounding ADEVs in CVD, we could find no evidence of
321 direct research into the redox potential of ADEVs. This is despite evidence that individually,
322 adipocytes and EVs are involved in redox reactions and can promote oxidative stress.
323 Additionally, as outlined above, ADEVs contain components that are susceptible to oxidation
324 (including lipids, proteins and nucleic acids) which can be transferred to cells locally within
325 adipose tissue but also in an endocrine fashion. ADEV production is also sensitive to the
326 condition of the parent cell. Indeed, several conditions likely to disrupt redox signalling in
327 adipocytes have been shown to increase ADEV generation, for example, elevated levels of
328 free fatty acids (palmitic acid) (Eguchi et al., 2015), hypoxia and inflammation (Wadey et al.,
329 2019) and induction of ER stress (Gu et al., 2021). Furthermore, ADEVs from obese adipose
330 tissue were shown to induce mitochondrial dysfunction (Li et al., 2019), a key contributor to
331 oxidative stress. ADEVs are therefore likely to play an important role in redox signalling
332 within adipose tissue and in obesity, ADEVs may mediate the communication of adipocyte
333 oxidative stress in an autocrine and paracrine fashion but also systemically, potentially
334 facilitating the development of metabolic syndrome and associated CVD.



335

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

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

344

345 *Conclusion*

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

367

368 *Acknowledgement*

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