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1 **Adipose morphology and metabolic disease**

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10 Keywords: adipose tissue, adipose morphology, adipogenesis, hyperplasia,
11 hypertrophy

12 **Abstract**

13 Adipose morphology is defined as the number and size distribution of adipocytes (fat cells)
14 within adipose tissue. Adipose tissue with fewer, but larger adipocytes is termed as having a
15 'hypertrophic' morphology, whereas adipose with many adipocytes, of a smaller size, is termed a
16 'hyperplastic' morphology. Hypertrophic adipose morphology is positively associated with insulin
17 resistance, diabetes and cardiovascular disease. Contrastingly, hyperplastic morphology is
18 associated with improved metabolic parameters. These phenotypic associations suggest that
19 adipose morphology influences risk for cardiometabolic disease. Intriguingly, monozygotic twin
20 studies have determined that adipose morphology is in part genetically determined. Therefore,
21 identifying the genetic regulation of adipose morphology may help predict, prevent and ameliorate
22 insulin resistance and associated metabolic diseases. Here we review the current literature
23 regarding adipose morphology in relation to; (i) metabolic and medical implications, (ii) methods
24 used to assess adipose morphology, and (iii) transcriptional differences between morphologies.
25 We further highlight three mechanisms hypothesized to promote adipocyte hypertrophy and thus
26 regulate adipose morphology.

27 Adipose tissue (AT) is a morphologically unique organ that accumulates lipid in response
28 to an organism's energy status. During periods of caloric excess, AT sequesters circulating lipid
29 which accumulates mainly as triacylglyceride (TAG) in cytoplasmic lipid droplets (LDs) within
30 adipocytes (fat cells). Conversely, during periods of caloric need, AT mobilizes lipid from LDs into
31 the circulation to act as an energy source for peripheral tissues. As such, AT functions as an
32 energy buffer to protect an individual from adverse physiological demands. The ability to expand
33 and contract to such extreme degrees, is unique to AT among other adult tissues. For example,
34 in an individual whose weight increased from 70 to 150 kg, the AT mass quadrupled relative to
35 changes in skeletal or muscle mass (Prins and O'Rahilly, 1997). Fluctuation in AT mass is largely
36 due to changes in lipid volume and, to accommodate such dynamic variation, AT expands via
37 increases in adipocyte size (hypertrophy) and adipocyte number (hyperplasia) and contracts via
38 decreases in adipocyte size (hypotrophy) (Salans et al., 1973; Spalding et al., 2008). The balance
39 between these growth and regression states establishes and maintains AT morphology: AT with
40 fewer, but very large adipocytes is termed a hypertrophic morphology; whereas, AT with many,
41 smaller adipocytes is termed hyperplastic (**Fig. 1A**). In this review, we highlight how adipose
42 morphology is associated with metabolic and physiological derangements, and present
43 hypotheses for how adipose morphology may be regulated.

44

45 **Obesity is not synonymous with metabolic dysfunction: a role for adipose**
46 **morphology?**

47 Overweight and obesity are characterized by increased lipid accumulation in adipocytes;
48 whereas, weight loss is characterized by reduced lipid accumulation in AT (Eriksson-Hogling et
49 al., 2015; Goodpaster and Sparks, 2017). Obesity is strongly correlated with metabolic disease –
50 for every kg increase in body weight, diabetes rates increase linearly (Haffner, 2006). Although
51 the rising prevalence of overweight and obesity has led to an increased occurrence of metabolic
52 diseases including diabetes and cardiovascular disease (CVD) (Wilding, 2017), obesity is not
53 synonymous with metabolic dysfunction. For example, in humans insulin resistance is a major
54 underlying cause of CVD (Ginsberg, 2000), and is associated with dyslipidemia (Reaven et al.,
55 1967), hypertension (Welborn et al., 1966) and atherosclerosis (Howard et al., 1996). However,
56 huge variation exists in the degree of insulin resistance across all values of body mass index
57 (BMI; a surrogate measure of adiposity) (McLaughlin et al., 2004). Indeed, the degree of insulin
58 resistance can vary six fold at any given BMI (McLaughlin et al., 2004). Therefore, obesity *per se*
59 is clearly not the sole driving force for metabolic dysfunction, and understanding which other

60 factors are responsible for the unexplained variance in insulin resistance will have important
61 consequences for public health. Multiple related factors have been proposed to explain
62 dysfunctional AT, and the variability in insulin resistance, during obesity; including, adipose
63 inflammation, fibrosis, impaired angiogenesis, hypoxia and body fat distribution (Bluher, 2016;
64 Crewe et al., 2017; Divoux et al., 2010; Khan et al., 2009; Sun et al., 2011; Trayhurn, 2013;
65 Weisberg et al., 2003). Here, we present evidence from the literature that adipose morphology is
66 an additional factor that influences susceptibility to metabolic disease.

67

68 **Regional variation in adipose morphology**

69 To assess the role of adipose morphology in metabolic disease, it is first essential to
70 review how adipose morphology can vary between regionally-distinct ATs. Briefly, ATs are
71 distributed throughout the human body, but are mainly categorized into subcutaneous ATs (SAT;
72 AT situated between muscle and skin) and visceral ATs (VAT; AT associated with internal visceral
73 organs) (**Fig. 1A**) (Shen et al., 2003). The subcutaneous and visceral sites of adipose
74 accumulation appear conserved to mouse (Bartelt and Heeren, 2014; Cinti, 2012; Shen et al.,
75 2003), and strikingly, most regional AT sites even appear conserved to zebrafish (Minchin and
76 Rawls, 2017; Shen et al., 2003). The regional distribution of human AT is strongly associated with
77 insulin resistance. A recent meta-analysis demonstrated that VAT was the strongest predictor of
78 insulin resistance (measured by HOMA-IR) (Zhang et al., 2015); however, total fat mass, BMI,
79 waist circumference, intra-abdominal fat, abdominal fat were also significantly associated with
80 insulin resistance (Zhang et al., 2015). By contrast, lower-body SAT was not correlated with
81 insulin resistance and has been shown to protect against metabolic dysfunction (Snijder et al.,
82 2005; Snijder et al., 2004; Zhang et al., 2015). Many previous studies in humans have also linked
83 accumulation of lipid within abdominal SAT to insulin resistance and metabolic disease, thus
84 suggesting that upper body (or central adiposity) vs lower body (or peripheral adiposity) body fat
85 distribution is an important factor in metabolic disease (Karpe and Pinnick, 2015; Porter et al.,
86 2009).

87 Adipocytes from regionally distinct ATs can be significantly different sizes. For example,
88 comparison of adipocyte size between three distinct human SATs (gluteal, anterior abdominal
89 wall and triceps) revealed significant differences (gluteal > abdominal > triceps) (Salans et al.,
90 1973; Salans et al., 1971). Indeed, intra-individual site-to-site variability in adipocyte size was
91 greater than same site variability between individuals (Salans et al., 1971). In general, across

92 studies, SAT adipocytes were significantly larger than VAT adipocytes, irrespective of BMI or
93 metabolic state (Liu et al., 2009; Tchernof et al., 2006), thus suggesting that SAT undergoes
94 greater hypertrophy relative to VAT. However, previous studies have suggested that SAT is
95 inherently more hyperplastic than VAT; although, these observations were based on in vitro data
96 from mouse and humans showing that SAT-derived cells have greater adipogenic capacity than
97 VAT-derived cells (Baglioni et al., 2012; Macotella et al., 2012; Tchkonja et al., 2006). More recent
98 in vivo data using the transgenic AdipoChaser mouse line, have suggested the opposite; following
99 diet-induced obesity, VAT (epididymal) undergoes waves of hyperplastic growth, whereas SAT
100 (inguinal) did not (Wang et al., 2013). Although these data conflict with the in vitro observations
101 of higher SAT hyperplasia; these findings do conform to the larger adipocyte size, and presumed
102 greater degree of hypertrophic growth in SAT. Thus, after exposure to a high-fat diet, SAT appears
103 to preferentially undergo hypertrophic growth which leads to larger adipocytes relative to VAT.
104 The contrasting growth dynamics of VAT and SAT is of biomedical importance as reduced
105 expandability of SAT is associated with insulin resistance (Gealekman et al., 2011; Virtue and
106 Vidal-Puig, 2008). In line with these observations, treatment of obese diabetics with
107 thiazolidinidiones (TZDs) leads to greater weight gain, preferential lipid deposition in SAT and
108 improved insulin sensitivity (Fonseca, 2003; Nichols and Gomez-Caminero, 2007). Thus,
109 understanding the differential growth mechanisms of SAT may provide therapeutic targets for
110 treating obesity-associated metabolic disease.

111

112 **Using a 'morphology value' to quantify adipose morphology**

113 To quantify adipose morphology, Arner et al. (2010) described a 'morphology value' – the
114 difference between measured adipocyte volume and expected adipocyte volume (relative to total
115 adipose mass) (Arner et al., 2010; Spalding et al., 2008) (**Fig. 1B**). This metric was further utilized
116 by Veilleux et al. (2011), and facilitated the categorization of individuals according to whether
117 adipose exhibits a hypertrophic or hyperplastic morphology (Arner et al., 2010; Veilleux et al.,
118 2011). To determine the morphology value, AT biopsies were first taken, then a single-cell
119 adipocyte suspension was produced by collagenase digestion and buoyancy separation, and the
120 size of individual adipocytes was then measured using image analysis software. A curvilinear line
121 was then fitted to the data to best describe the relationship between adiposity and mean adipocyte
122 size (Spalding et al., 2008). Each individual was then categorized in relation to the fitted line:
123 individuals exhibiting a positive residual were hypertrophic (mean adipocyte volume larger than
124 expected); whereas, individuals exhibiting a negative residual were hyperplastic (mean adipocyte

125 volume smaller than expected) (Arner et al., 2010; Veilleux et al., 2011) (**Fig. 1B**). Importantly,
126 lower morphology values (hyperplastic) were associated with an increased number of small
127 adipocytes; whereas, high morphology values (hypertrophic) were associated with fewer, larger
128 adipocytes (Arner et al., 2010; Veilleux et al., 2011). Within a population, adipose morphology
129 appears highly variable. Arner et al. (2010) found that hyperplastic and hypertrophic morphologies
130 were present at equal frequencies in both males vs females, and obese vs non-obese (Arner et
131 al., 2010). Strikingly, at comparable BMI, women typically present with ~10% higher body fat,
132 characterized with greater SAT in the abdomen and gluteofemoral regions (Camhi et al., 2011;
133 Jackson et al., 2002; Karastergiou et al., 2012; Womersley, 1977). Body fat distribution is linked
134 to health in both males and females; however, the protective peripheral distribution is mainly seen
135 in females (Krotkiewski et al., 1983). Large inter-individual variation in adipocyte number and size
136 within equivalent ATs was also observed, which was independent of adipose mass (Arner et al.,
137 2011; Salans et al., 1973; Salans et al., 1971). Furthermore, estimates for adipocyte number
138 varied by as much as 85% between individuals (Salans et al., 1973), suggesting a high-level of
139 inter-individual variability in adipose morphology. Intriguingly, adipocyte number and size were
140 highly similar in monozygotic twins concordant for BMI, suggesting a strong genetic basis
141 (Heinonen et al., 2014). Therefore, understanding how genetics drives variation in adipose
142 morphology with subsequent consequences for disease risk is a central research question.

143

144 **Hypertrophic morphology is associated with insulin resistance and increased risk**
145 **for cardiovascular disease**

146 The association between SAT morphology and metabolic disease has been extensively
147 characterized. In a cohort of 764 subjects exhibiting a wide adiposity range (BMI 18-60 kg/m²),
148 Arner et al. (2010) found that hypertrophic morphology was positively correlated with insulin
149 resistance (measured by HOMA-IR) and fasting plasma Insulin in humans (Arner et al., 2010).
150 Furthermore, in women, hypertrophic morphology was associated with a metabolic syndrome-like
151 state, characterized by increased insulin resistance, and increases in circulating plasma Insulin,
152 total cholesterol and TAG (Arner et al., 2010). Additionally, abdominal SAT adipocyte size by itself
153 was positively associated with insulin resistance independent of BMI in non-diabetic humans
154 (Lundgren et al., 2007). Further, SAT adipocyte size was positively associated with plasma
155 Insulin, glucose, Insulin-induced glucose disposal and insulin sensitivity in humans (Hoffstedt et
156 al., 2010). Finally, in humans, the average volume of adipocytes within abdominal SAT was

157 correlated with insulin resistance (Yang et al., 2012). By contrast, hyperplastic morphology was
158 associated with significantly better blood glucose, Insulin and lipid profiles when compared to
159 subjects with hypertrophic morphology (Hoffstedt et al., 2010). Taken together, these data
160 demonstrate that hypertrophic SAT morphology is associated with metabolic dysfunction, and
161 metabolic risk factors for diabetes and CVD. VAT morphology has also been implicated in
162 metabolic disease. In a sample of 207 lean to severely obese females, subjects characterized by
163 hypertrophic omental VAT had higher plasma TAG, higher very low density lipoprotein (vLDL)-
164 TAG and higher vLDL-cholesterol when compared to subjects with hyperplastic VAT (Veilleux et
165 al., 2011). It was also estimated that a 10% enlargement of VAT adipocytes increased the risk of
166 hypertriacylglyceridemia 4-fold (Veilleux et al., 2011); whilst, a 10% increase in the number of
167 VAT adipocytes increased the risk of hypertriacylglyceridemia by 1.55-fold (Veilleux et al., 2011).
168 In morbidly obese females, and independent of age, BMI, body fat mass or body fat distribution,
169 VAT adipocyte size was positively associated with plasma apolipoprotein B, total cholesterol,
170 vLDL-cholesterol and triacylglycerides (Hoffstedt et al., 2010). Furthermore, large VAT adipocytes
171 (>75 μm diameter) were associated with insulin resistance in canines (Kabir et al., 2011). These
172 data demonstrate that hypertrophic VAT morphology is also associated with a metabolic-
173 syndrome-like state.

174

175 **Bi- and tri-modal adipocyte size distributions: a more complex relationship**
176 **between adipose morphology and Insulin resistance?**

177 Many studies have concluded that adipocytes comprise a complex population of cells that
178 exhibit a bi- or tri-modal size distribution. In general, these studies have used osmium tetroxide
179 fixation of adipocytes, followed by size analysis of adipocytes using a Coulter counter (Cushman
180 and Salans, 1978; Etherton et al., 1977; Hirsch and Gallian, 1968). The advantages of this method
181 include the ability to analyze large numbers of adipocytes (~6000 cells from each subject), and
182 the automated and unbiased measurement of adipocyte size (Jo et al., 2012). To exclude the
183 possibility that the small adipocytes (within a bimodal population) were not artefactual 'debris', it
184 was confirmed by microscopy that these cells were comprised of intact, spherical small adipocytes
185 (McLaughlin et al., 2007). In addition to osmium tetroxide fixation, measurement by microscopy
186 has also indicated that adipocytes may form a bimodal size distribution (Fang et al., 2015).
187 Comparison of the size distributions obtained from these methods revealed a peak of small
188 adipocytes of ~25 μm diameter, and a peak of larger adipocytes of ~50 μm diameter (**Fig. 2**) (Jo

189 et al., 2012). Intriguingly, trimodal adipocyte size distributions in humans have also been observed
190 with peaks at ~25, ~50 and ~100 μm diameters (Yang et al., 2012). Recently, 3D reconstruction
191 of zebrafish VAT revealed a bimodal size distribution of adipocyte-localized LDs (Minchin et al.,
192 2015). However, it is likely that the smaller population of LDs (~1 μm in diameter) correspond to
193 additional LD 'locules' within multilocular adipocytes – a phenomenon also observed in human
194 and mouse white adipocytes (Chau et al., 2014; Cushman, 1970). Together, these studies
195 suggest that parametric statistics, such as mean adipocyte size and number, may not accurately
196 represent the true population mean, and should be used with caution when assessing adipose
197 morphology.

198 Multiple studies have confirmed that the size of larger adipocytes in a bimodal population
199 is positively associated with metabolic dysfunction. First, McLaughlin et al. (2014) found that
200 compared to BMI-matched insulin sensitive subjects, insulin resistant subjects had larger 'large'
201 adipocytes within abdominal SAT. Second, in 35 subjects (with BMI ranging from 18-34 kg/m^2)
202 the size of larger adipocytes within abdominal SAT was able to accurately predict insulin
203 resistance (Yang et al., 2012). Third, in insulin sensitive obese individuals, an increase in size of
204 the larger adipocyte fraction within abdominal SAT after feeding a hypercaloric diet, predicted a
205 decline in Insulin-mediated glucose uptake (McLaughlin et al., 2016). In addition to insulin
206 resistance, the size of the large adipocytes was also correlated with an increased VAT/VAT+SAT
207 ratio (Kursawe et al., 2010), an increased proportion of small adipocytes in both VAT and SAT
208 (Liu et al., 2009), and the normalization of insulin sensitivity in insulin resistant subjects after
209 treatment with rosiglitazone (Eliasson et al., 2014). A summary of these findings is provided in
210 **Table 1**. Taken together these studies show that hypertrophied adipocytes within the larger
211 fraction of adipocytes in a bimodal population is also associated with insulin resistance.

212 The proportion and size of small adipocytes within a bimodal size distribution is also
213 related to metabolic wellbeing. In moderately overweight/obese individuals, McLaughlin et al.
214 (2007 & 2014) found an increased proportion of small adipocytes in abdominal SAT to be
215 statistically associated with Insulin resistance, Further, an increased proportion of small
216 adipocytes was found in both abdominal SAT and omental VAT of diabetics (Fang et al., 2015).
217 Intriguingly, an increased proportion of small adipocytes in abdominal SAT also occurred in
218 subjects with high VAT/VAT+SAT ratio (Kursawe et al., 2010), after insulin sensitive subjects were
219 overfed (McLaughlin et al., 2016), or after diabetics were treated with rosiglitazone (Eliasson et
220 al., 2014). In diabetics, the size of small adipocytes was also found to be inversely correlated with
221 insulin sensitivity (Fang et al., 2015). Finally, an expanded nadir (the low point in frequency

222 between the small and large adipocyte populations) was found in insulin resistant subjects
223 (McLaughlin et al., 2007). A summary of these findings is provided in **Table 1**. Together, these
224 data show that insulin resistance is not only accompanied by hypertrophy of large adipocytes, but
225 in studies that detect a bimodal adipocyte size distribution, insulin resistance is also associated
226 with an increased proportion of small adipocytes. Related to the increased presence of small
227 adipocytes, the expression of genes related to adipogenesis was also lower in insulin resistant
228 individuals (McLaughlin et al., 2007). These findings are consistent with independent reports of
229 reduced adipogenesis in insulin resistant patients (Goedecke et al., 2011; Yang et al., 2004).
230 Further, these expression changes were also associated with modest increases in inflammatory
231 activity in insulin resistant AT (McLaughlin et al., 2008). As the presence of small adipocytes in
232 both VAT and SAT appears to be correlated with increased hypertrophy of larger adipocytes in
233 abdominal SAT (Liu et al., 2009), these findings could be interpreted to suggest that SAT is the
234 primary site for lipid accumulation; however, once a maximal SAT adipocyte size is reached,
235 hyperplastic growth is initiated in both VAT and SAT. In support, adipocytes have been shown to
236 expand to only a finite degree in both humans and rats (Faust et al., 1978; Kashiwagi et al., 1985).
237 A summary of these findings is provided in **Table 1**. Therefore, these data suggest that insulin
238 resistance is accompanied by an inability of small adipocytes to undergo hypertrophic expansion,
239 suggestive of defective adipogenesis, resulting in a higher proportion of small adipocytes amongst
240 a more general population of hypertrophied adipocytes.

241

242 **Transcriptomic differences in distinct adipose morphologies**

243 To study adipose hypertrophy and hyperplasia, and to identify potential molecular
244 pathways that influence morphology, it is useful to analyze the transcriptional state underlying
245 distinct morphologies. Strikingly, adipocytes of different sizes have distinct gene expression
246 profiles. Jernas et al. (2006) fractionated human adipocytes into small (mean diameter $57.6 \pm$
247 $3.54 \mu\text{m}$) or large (mean diameter $100.1 \pm 3.94 \mu\text{m}$) groups. Subsequent microarray analysis of
248 gene expression on the 2 groups of adipocytes revealed 14 genes with 4-fold higher expression
249 in large adipocytes (**Table 2**) (Jernas et al., 2006). Strikingly, some transcripts exhibited 19-fold
250 and 22-fold higher expression in large adipocytes, suggesting relatively large-scale differences
251 between adipocytes based on size (Jernas et al., 2006). In an additional study, Heinonen et al.
252 (2014) analyzed whether adipocyte size or number correlated with changes in the AT
253 transcriptome. RNA was extracted from whole-adipose biopsies (including both adipocyte and
254 stromal-vascular fractions), and gene expression changes positively correlating with adipocyte

255 size included, genes implicated in cell cytoskeleton and membrane modifications (*MSN*,
256 *NHEDC2*, *KIF3B*, *PALLD*), oxidative stress and apoptosis (*MSM*), cell mediated immunity (*MIF*)
257 and cancer (*NMES*) (**Tables 2 & 3**) (Heinonen et al., 2014). Genes inversely correlated with
258 adipocyte size include *FDFT1* (mevalonate pathway, cholesterol biosynthesis), *ADH1B*
259 (metabolism of a wide range of substrates, including hydroxysteroids and lipid peroxidation
260 products), *EIF1B* (unknown function) (**Tables 2 & 3**) (Heinonen et al., 2014). Significant Gene
261 Ontology (GO) terms, used to describe shared relationships between sets of genes, revealed that
262 leukocyte migration and immune system processes were significantly enriched terms in genes
263 upregulated in large adipocytes (**Table 4**). Adiponectin mRNA was also found to be negatively
264 associated with size in isolated adipocytes (Bambace et al., 2011); whereas, Leptin mRNA was
265 positively associated with adipocyte volume in isolated adipocytes (Guo et al., 2004). However,
266 Leptin mRNA per unit of fat mass decreased at more extreme levels of obesity (Guo et al., 2004).
267 Skurk et al. (2007) analyzed the relationship between adipocyte size and secreted factors and
268 found that Leptin, IL6, IL8, MCP1 and G-CSF were significantly increased in large adipocytes,
269 supportive of altered immune signaling following hypertrophy (Skurk et al., 2007). Recently, Gao
270 et al (2014) utilized adipose biopsies from a cohort of 56 healthy males and females, subdivided
271 into obese or non-obese individuals with hyperplastic or hypertrophic morphologies (ie, obese
272 hyperplastic, obese hypertrophic, non-obese hyperplastic and non-obese hypertrophic) (Gao et
273 al., 2014). This analysis identified 619 genes differentially altered by morphology in non-obese
274 subjects (Gao et al., 2014). Genes increased in non-obese hypertrophy were associated with pro-
275 inflammatory pathways; whereas, genes increased in non-obese hyperplastic individuals were
276 involved in carbohydrate and lipid metabolism (Gao et al., 2014). The transcriptome of adipocytes
277 in bimodal size distributions have also been analyzed. Liu et al. (2010) characterized small
278 adipocytes from epididymal AT (VAT) of Zucker Obese (ZO) and Lean (ZL) rats and found that
279 small adipocytes had a 3-fold decrease in Adiponectin and Pparg in ZO versus ZL rats (Liu et al.,
280 2010), along with a 2.5-fold increase in IL-6 (Liu et al., 2010). These data suggest that both
281 hypertrophied adipocytes, and the small adipocytes from a bimodal population have pro-
282 inflammatory characteristics. Altogether, these data support the conclusion that small and large
283 adipocytes have distinct transcriptional profiles, and that large adipocytes are characterized by
284 altered immune/inflammatory activity.

285

286 **Cellular mechanisms hypothesized to regulate adipose morphology**

287 Understanding the cell and molecular mechanisms that underpin adipose morphology is
288 likely to provide new therapeutic targets for combating obesity-associated disease. For simplicity,
289 we have separated the factors that are likely to influence adipose morphology into two categories:
290 (i) factors that regulate adipocyte number and (ii) factors that regulate adipocyte size. As the
291 regulation of adipocyte number (adipogenesis) is a well-studied subject with many in-depth
292 reviews (Berry et al., 2016; Berry et al., 2014; Hepler et al., 2017), we will focus on mechanisms
293 hypothesized to regulate adipocyte size. Surprisingly, relatively few studies have identified cellular
294 mechanisms that regulate adipocyte size. Therefore, we first review highly conserved
295 mechanisms for regulating cell size across multiple cell types, and investigate whether these
296 conserved mechanisms may also regulate adipocyte size. We then focus on two interesting,
297 adipocyte-specific pathways that regulate adipocyte size: how the phospholipid monolayer on the
298 surface of LDs controls their expansion, and the emerging field of osmolarity sensors in regulating
299 adipocyte hypertrophy.

300

301 **A role for mTORC1 in regulating adipocyte size: a highly conserved signaling**
302 **pathway that control cell size across multiple diverse cell types.**

303 Highly conserved homeostatic mechanisms regulate cell size in eukaryotes (Lloyd, 2013).
304 As adipocyte size is also highly regulated we reasoned that understanding the core pathways that
305 maintain cell size, across multiple diverse cell types, has the potential to shed light on
306 mechanisms controlling adipocyte hypertrophy. Central to the control of cell size across the
307 animal kingdom is the Insulin Growth Factor (IGF), Phosphoinositide 3-kinase (PI3K), Protein
308 kinase B (AKT), and Mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway.
309 IGF/PI3K/AKT/mTORC1 coordinates nutrition with cell growth, and acts as a node to integrate
310 external signals, including Insulin signalling, with biogenic pathways (Edgar, 2006). mTORC1
311 responds to multiple inputs, including; amino acids, energy, stress, oxygen and growth factors,
312 and regulates downstream anabolic processes that promote cell growth, including; protein and
313 lipid synthesis (through SREBP1/2), mitochondria biogenesis, and ATP production (Cunningham
314 et al., 2007; Duvel et al., 2010; Ma and Blenis, 2009; Porstmann et al., 2008). In addition,
315 mTORC1 also promotes cell growth by negatively regulating autophagy (Hosokawa et al., 2009).
316 Strikingly, artificial activation of the mTORC1 pathway promotes dramatic increases in cell size
317 (Laplane and Sabatini, 2012). Altogether, these data suggest that activation of mTORC1
318 signaling may induce and augment adipocyte hypertrophy. In accordance, *Raptor* KO mice

319 (Raptor is an mTOR binding protein essential for formation, and activity of the mTORC1 complex)
320 have smaller adipocytes (and a reduced number), suggesting that mTORC1 may promote
321 adipocyte hypertrophy (Polak et al., 2008). Indeed, *Raptor* KO mice, with specific loss of Raptor
322 and mTORC1 in adipocytes, develop lipodystrophy with age, suggesting that mTORC1 is
323 essential for maintaining a hypertrophic state in mature adipocytes (Lee et al., 2016).
324 Furthermore, adipocyte-specific *Raptor* KO led to the induction of a bimodal ‘polarized’ adipocyte
325 size distribution, characterized by the addition of a small population of adipocytes, further
326 suggesting that mTORC1 is essential for maintaining adipose morphology (Lee et al., 2016). Such
327 a polarized adipocyte size distribution is reminiscent of fat-specific insulin receptor knockout
328 (FIRKO) mice (Bluher et al., 2002), suggesting that Insulin signalling maybe critically important
329 for maintaining adipose morphology. Elevated mTORC1 signaling, produced after deletion of
330 tuberous sclerosis complex 2 (*Tsc2*) – a complex made up of *Tsc1* & 2 proteins that inhibits
331 mTORC1 signaling, led to increased adipogenesis in mouse fibroblasts and 3T3-L1 adipocytes
332 (Zhang et al., 2009). However, in vivo constitutive activation of mTORC1 in adipocytes by
333 tuberous sclerosis complex 1 (*Tsc1*) deletion, did not induce adipocyte hypertrophy but instead
334 led to reduced VAT mass, VAT adipocyte number and diameter without affecting SAT, pointing
335 to the complex nature of mTORC1 signaling in adipocytes (Magdalon et al., 2016). Taken
336 together, mTORC1 depletion leads to adipose atrophy; however, conclusive evidence for a role
337 for mTORC1 in adipocyte hypertrophy has not been fully elucidated.

338

339 **The availability of lipid as a rate-limiting step for adipocyte hypertrophy**

340 The single defining feature of white adipocytes is the presence of large cytoplasmic LDs
341 that can reach ~200 μm in diameter (Walther and Farese, 2009). This feature is unique to white
342 adipocytes and, therefore, we reasoned that understanding how LD growth is regulated may also
343 allow us to elucidate cellular mechanisms underlying adipocyte hypertrophy. LD size reflects two
344 processes: (i) lipid incorporation into LDs and (ii) lipid mobilization from LDs. However, each of
345 these processes is highly complex and can be regulated at multiple levels. For example, at a
346 minimum, lipid incorporation into LDs depends on (i) circulating levels of lipid (i.e. availability of
347 lipid to adipocytes), (ii) lipid uptake into adipocytes, (iii) re-esterification of non-esterified fatty
348 acids (NEFAs) into TAG, and (iv) de-novo lipogenesis in adipocytes, (v) incorporation of TAG into
349 LDs (**Fig. 3A**). Lipid synthesis, transport and metabolism in adipocytes is a large subject area
350 beyond the scope of this review; however, we recommend the following reference for further
351 reading on the subject (Large et al., 2004). Briefly, however, and as described above, hypertrophic

352 VAT was associated with higher plasma TAG, vLDL TAG and cholesterol, total plasma
353 cholesterol, higher total-to-HDL cholesterol and increased plasma apolipoprotein B when
354 compared to hyperplastic VAT (Hoffstedt et al., 2010; Veilleux et al., 2011). Together, these data
355 suggest that increased circulating lipid may promote hypertrophic growth of adipocytes.
356 Accordingly, treatment of 3T3-L1 adipocytes with saturated or monounsaturated NEFAs resulted
357 in adipocyte hypertrophy (Kim et al., 2015). Regarding uptake of lipid into adipocytes, the first
358 step is often hydrolysis of TAG from circulating lipoproteins by Lipoprotein lipase (LPL). In adipose
359 tissue, LPL is expressed on vascular endothelial cells and adipocytes (Gonzales and Orlando,
360 2007; Merkel et al., 2002), and hydrolyses TAG (from lipoproteins) to form glycerol and NEFAs
361 for uptake into adipocytes (Geldenhuys et al., 2017). In SAT, higher levels of LPL activity were
362 associated with adipocyte hypertrophy (Serra et al., 2015). Further, LPL deficiency in mice results
363 in lipodystrophy and elevated plasma lipid levels (Weinstock et al., 1995). Following their
364 production by LPL, NEFAs are taken up by adipocytes using specialized fatty acid transporters,
365 including Fatty acid transport proteins (FATPs), the scavenger receptor CD36, and the
366 mitochondrial aspartate amino transferase (FABPpm). In particular, isolated adipocytes from
367 CD36 KO mice have impaired NEFA uptake (Coburn et al., 2000). Further, CD36-deficient mice
368 do not develop diet-induced obesity, suggesting that adipocyte hypertrophy is impaired (Hajri et
369 al., 2007; Koonen et al., 2010; Vroegrijk et al., 2013). We speculate that increased circulating lipid
370 causes adipocyte hypertrophy and adipose growth; however, there is currently limited evidence
371 that circulating lipid levels induce a hypertrophic morphology as defined by Arner et al. (2010).
372 Altogether, these data suggest that lipid availability, in the form of plasma lipid levels, LPL activity
373 and lipid uptake into cells, is key to promoting adipocyte hypertrophy.

374

375 **Specialized pathways for regulating lipid droplet size in adipocytes**

376 Large cells, such as neurons and ova, often have specialized mechanisms that allow them
377 to grow to extreme sizes (Lloyd, 2013). Therefore, it is likely that adipocytes have adipocyte-
378 specific pathways that govern their hypertrophic capacity. Multiple intriguing mechanisms regulate
379 LD growth. First, a genome-wide screen in yeast identified 10 mutants that produced “supersized”
380 LDs, capable of forming LDs >50 times larger than wild-type LDs (Fei et al., 2011). The genes
381 identified include yeast homologs of Seipin (Fei et al., 2008; Fei et al., 2011), regulators of
382 phospholipid metabolism, and multiple subunits of casein kinase 2 (Fei et al., 2011). Phospholipid
383 metabolism was a shared feature of the genes identified from the screen, and the surface layer
384 of LDs are coated with a phospholipid monolayer (Walther and Farese, 2009). Phosphatidic acid

385 (PA), a cone-shaped lipid common in phospholipids which alters the curvature of membranes,
386 and promotes membrane fusion events, was a key factor in formation of supersized LDs (Fei et
387 al., 2011; Marchesan et al., 2003). Further, supersized LDs could be formed by PA-stimulated
388 fusion of LDs (Fei et al., 2011), suggesting that the phospholipid monolayer of LDs is important
389 for LD growth and may mediate LD fusion to facilitate LD hypertrophy.

390

391 **Regulation of adipocyte size by osmolarity-sensing ion channels**

392 Cells respond to changes in size by generating osmotic gradients using plasma membrane
393 ion channels and transporters to manipulate the osmolarity of the surrounding environment.
394 Utilizing of these ion channels can create a hypotonic environment leading to cell swelling (RVI;
395 regulatory volume increase), or a hypertonic environment leading to cell shrinkage (RVD;
396 regulatory volume decrease) (**Fig. 3B**) (Hoffmann et al., 2009; Jentsch, 2016). As cell size
397 regulation is central to the dynamic growth and regression of adipocytes, the role of such
398 osmosensors and regulators is under intense investigation. Transient receptor potential cation
399 channel subfamily V member 4 (TRPV4) is a Ca²⁺-permeable, nonselective cation channel
400 involved in the regulation of osmotic pressure (Harteneck and Reiter, 2007), and is activated by
401 cellular swelling and stretch (Liedtke et al., 2000; Mochizuki et al., 2009; Strotmann et al., 2000;
402 Thodeti et al., 2009). Adipocytes from TRPV4 KO mice do not undergo hypertrophy and
403 underwent increased oxidative metabolism (Ye et al., 2012) (**Fig. 3C**). Additionally, the TRPV4
404 KO mice were protected from diet-induced obesity, adipose inflammation and Insulin resistance
405 (Ye et al., 2012). Thus, TRPV4 promotes adipocyte hypertrophy, and may contribute to the Insulin
406 resistance inherent to hypertrophied adipocytes. Recently the voltage-regulated anion channel
407 (VRAC), SWELL1 (LRCC8A), was shown to regulate adipocyte size, Insulin signaling and glucose
408 homeostasis (Zhang et al., 2017). VRACs export chloride ions (Cl⁻) and other small organic
409 osmolytes, and thus generate a hypertonic environment that induces cell shrinkage (RVD)
410 (Jentsch, 2016; Qiu et al., 2014). Zhang et al. (2017) utilized patch-clamp recordings of ionic
411 currents in freshly isolated adipocytes to identify that hypertrophic adipocytes exhibit an increased
412 'swell-activated' Cl⁻ current relative to smaller adipocytes (Zhang et al., 2017). Furthermore, the
413 increased current observed in hypertrophied adipocytes was dependent on SWELL1. Although
414 activation of VRACs has generally been shown to induce RVD and decrease cell volume (Jentsch,
415 2016), the authors propose that SWELL1-mediated expansion acts as a feed-forward amplifier
416 for further adipocyte hypertrophy (**Fig. 3C**). Further, SWELL1 knockout (KO) adipocytes were
417 Insulin resistant with reduced GLUT4 translocation to the adipocyte plasma membrane after

418 stimulation with Insulin (Zhang et al., 2017). This effect was found to be mediated by PI3K-AKT
419 signaling, and SWELL1 KO adipocytes had reduced phosphorylation of AKT (Zhang et al., 2017).
420 Thus, taken together, these data suggest that osmosensing is active in adipocytes during
421 hypertrophy, and may modulate adipocyte hypertrophy and Insulin sensitivity. In addition to ion
422 channel osmosensors, the adipocyte plasma membrane contains abundant caveolae, small flask-
423 shaped invaginations of the plasma membrane enriched in cholesterol and sphingolipids, which
424 disassemble in response to osmotic and mechanical stress (Sinha et al., 2011). Caveolae are
425 present at a high density in cells that experience mechanical stress, and cover ~30% of the
426 adipocyte surface (Le Lay et al., 2015). Caveolae formation is driven by the assembly of 3 distinct
427 Caveolin proteins (Cav1-3), and deletion of individual Cav genes leads to loss of caveolae (Le
428 Lay and Kurzchalia, 2005). Caveolae mediate the response of several cell types to mechanical
429 stress (Boyd et al., 2003; Sedding et al., 2005), and intriguingly, loss of caveolae induces
430 lipodystrophy in mice and humans (Kim et al., 2008; Razani et al., 2002). Further, overexpression
431 of Cav1 in adipocytes induced an increase in caveolae density, but also stimulated the
432 accumulation of larger LDs (**Fig. 3C**) (Briand et al., 2014). No role for caveolae as an
433 osmosensor/regulator during adipocyte hypertrophy is known; however, it is clear that caveolae
434 are essential for lipid storage fluctuations in adipocytes.

435

436 **Conclusion**

437 At a population level, adipose morphology is highly varied, genetically determined and associated
438 with cardiometabolic disease susceptibility. However, the precise genetic determinants of adipose
439 morphology are largely unknown. In this Review, we find strong evidence in the literature that a
440 hypertrophic morphology, adipose characterized by few but large adipocytes, is associated with
441 a range of metabolic perturbances including plasma glucose, lipid and Insulin levels, insulin
442 resistance and susceptibility to disease. We further review whether distinct morphologies have
443 unique transcriptomic signatures, and identify that hypertrophic morphology is characterized by a
444 pro-inflammatory expression profile across multiple studies and methodologies. Finally, we
445 explore some intriguing cellular mechanisms that are predicted to regulate adipocyte cell size and
446 morphology; including (i) how the phospholipid monolayer covering lipid droplets regulates their
447 growth, and (ii) how osmolarity sensing in adipocytes can stimulate hypertrophy.

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767

768 **Tables**

769 **Table 1. Human adipose morphology and metabolic parameter associations – bimodal**
 770 **adipocyte size distribution.**

Morphology trait	AT	Direction of association	Metabolic Trait	Study	PMID
Relative frequency of small adipocytes	Abdominal SAT	Positive	Insulin resistance	McLaughlin*	17549449
	Abdominal SAT	Positive	Increased VAT/VAT+SAT ratio	Kursawe**	20805387
	Abdominal SAT	Positive	Insulin resistance	McLaughlin****	23666871
	Abdominal SAT	Positive	Type 2 diabetics treated with rosiglitazone	Eliasson^	26317056
	Abdominal SAT	Positive	Diabetics vs non-diabetics	Fang^^	26451283
	Omental VAT	Positive	Diabetics vs non-diabetics	Fang^^	26451283
	Abdominal SAT	Negative	Overfed Insulin sensitive subjects	McLaughlin^^^	26884438
Change in diameter of small adipocytes	Abdominal SAT	Negative	Diabetics vs non-diabetics	Fang^^	26451283
	Omental VAT	Negative	Diabetics vs non-diabetics	Fang^^	26451283
Nadir diameter (n)	Abdominal SAT	Positive	Insulin resistance	McLaughlin*	17549449

Relative frequency of large adipocytes	Abdominal SAT	Negative	Increased VAT/VAT+SAT ratio	Kursawe**	20805387
	Abdominal SAT	Negative	Insulin resistance	McLaughlin****	23666871
	Abdominal SAT	Negative	Diabetics vs non-diabetics	Fang^^	26451283
	Omental VAT	Negative	Diabetics vs non-diabetics	Fang^^	26451283
Large adipocyte size (Cp)	Abdominal SAT	Positive	Increased small adipocytes in Omental VAT and abdominal SAT	Liu***	19711137
	Abdominal SAT	Positive	Increased VAT/VAT+SAT ratio	Kursawe**	20805387
	Abdominal SAT	Positive	Insulin resistance	Yang****	22240722
	Abdominal SAT	Positive	Insulin resistance	McLaughlin****	23666871
	Abdominal SAT	Positive	Type 2 diabetics treated with rosiglitazone	Eliasson^	26317056
	Abdominal SAT	No change	Overfed Insulin resistant subjects	McLaughlin^^^	26884438
	Abdominal SAT	Positive	Overfed Insulin	McLaughlin^^^	26884438

			resistant subjects		
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771 *Cohort was 28 obese individuals (mean age = ~50 years) stratified according to Insulin sensitivity
 772 (Insulin resistant BMI = 30.6 kg/m²; Insulin sensitive BMI = 29.4 kg/m²). No statistical differences
 773 between Insulin resistant and sensitive groups were found for age, gender, reported levels of
 774 exercise, blood pressure, fasting glucose, total cholesterol, LDL-cholesterol. HDL-cholesterol
 775 were lower in Insulin resistant group.

776 **Cohort was 38 adolescents (~ 15 years old) with similar degrees of obesity (mean BMI = ~37
 777 kg/m²) were divided into 2 groups: low VAT/VAT+SAT ratio (<0.11) and high VAT/VAT+SAT ratio
 778 (>0.11). None of the participants were on any medication or had any known disease.

779 ***Cohort was 11 obese (mean BMI = 45.3 kg/m²) Insulin resistant, but non-diabetic women.
 780 Patients were excluded if they had coronary heart disease, hepatic or renal disease, cancer, or
 781 use medications for weight loss.

782 ****Cohort was 35 subjects with a range of BMI (range = 18-34 kg/m²; mean = 25.7 kg/m²) and
 783 age (range = 28-49 years; mean = 41 years). The subjects were non-diabetic, but had a known
 784 family history of diabetes, with at least 2 first-degree relatives with type 2 diabetes.

785 ^Cohort was 12 patients with type 2 diabetes (11 male, 1 female). Patients had a mean BMI of ~
 786 28 kg/m². Patients were on diet or oral hypoglycemic treatments including sulfonylurea,
 787 repaglinide and metformin). Rosiglitazone (8 mg QD) was added to the treatment regimen.
 788 Subjects were excluded if they exhibited clinically significant disease. Adipose biopsies were
 789 taken before and after rosiglitazone treatment.

790 ^Cohort was 30 subjects with morbid obesity. Adipose biopsies were taken from subcutaneous,
 791 omental and mesenteric locations.

792 ^^Cohort consisted of healthy overweight adults, aged 30-60 years. BMI = 25-35 kg/m². Subjects
 793 had a stable body weight during the prior 3 months, and fasting plasma glucose <126 mg/dL.
 794 Subjects were given a hypercaloric diet to induce 3.2 kg weight gain over 4 weeks, followed by 1
 795 week of weight stabilization.

796

797 **Table 2. Genes positively correlated with adipocyte size.**

Gene symbol	Gene name	Study PMID
<i>SELE</i>	Selectin E	16754744
<i>SPARCL1</i>	SPARC-like 1	16754744
<i>TM4SF1</i>	Transmembrane 4 L six family member 1	16754744
<i>DCN</i>	Decorin	16754744
<i>IL8</i>	Interleukin 8	16754744
<i>PALLD</i>	Palladin	16754744, 24549139
<i>SAA2</i>	Serum amyloid A2	16754744
<i>CLEC3B</i>	C-type lectin domain family 3, member B	16754744
<i>C1QR1</i>	Complement component 1, q subcomponent, receptor 1	16754744
<i>COL1A1</i>	Collagen, type I, alpha 1	16754744
<i>CXCL2</i>	Chemokine (C-XC motif) ligand 1	16754744
<i>COL1A2</i>	Collagen, type I, alpha 2	16754744
<i>FLJ14054</i>		16754744
<i>AQP1</i>	Aquaporin 1	16754744
<i>MSN</i>	Moesin	24549139
<i>NHEDC2</i>	Na ⁺ /H ⁺ exchanger domain containing 2	24549139
<i>RP11-877E17.2</i>	undefined	24549139
<i>KIF3B</i>	Kinesin family member 3B	24549139
<i>NME5</i>	Non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	24549139
<i>IFT20</i>	Intraflagellar transport 20 homolog (Chlamydomonas)	24549139
<i>MIF</i>	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	24549139
<i>SLC24A3</i>	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	24549139
<i>C15orf59</i>	Chromosome 15 open reading frame 59	24549139

<i>CD248</i>	CD248 molecule, endosialin	24549139
<i>SLC46A3</i>	Solute carrier family 46, member 3	24549139
<i>XPO6</i>	Exportin 6	24549139
<i>FAT1</i>	FAT tumor suppressor homolog 1 (Drosophila)	24549139
<i>GNG2</i>	Guanine nucleotide binding protein (G protein), gamma 2	24549139
<i>LPCAT1</i>	Lysophosphatidylcholine acyltransferase 1	24549139
<i>TCTA</i>	T-cell leukemia translocation altered gene	24549139
<i>CLTB</i>	Clathrin, light chain B	24549139
<i>SPTAN1</i>	Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	24549139
<i>CYBASC3</i>	Cytochrome b, ascorbate dependent 3	24549139

799 **Table 3. Genes inversely correlated with adipocyte size.**

Gene symbol	Gene name	Study PMID
<i>NPEPPS</i>	Aminopeptidase puromycin sensitive	24549139
<i>GLYCTK</i>	Glycerate kinase	24549139
<i>PKP2</i>	Plakophilin 2	24549139
<i>AZGP1</i>	Alpha-2-glycoprotein 1, zinc-binding	24549139
<i>CIDEA</i>	Cell death-inducing DFFA-like effector a	24549139
<i>FAM184A</i>	Family with sequence similarity 184, member A	24549139
<i>NUP98</i>	Nucleoporin 98kDa	24549139
<i>WHSC2</i>	Wolf-Hirschhorn syndrome candidate 2	24549139
<i>FAM161A</i>	Family with sequence similarity 161, member A	24549139
<i>SLC27A2</i>	Solute carrier family 27 (fatty acid transporter), member 2	24549139
<i>ZFAND1</i>	Zinc finger, AN1-type domain 1	24549139
<i>MACROD1</i>	MACRO domain containing 1	24549139
<i>PPARA</i>	Peroxisome proliferator-activated receptor alpha	24549139
<i>GPD1L</i>	Glycerol-3-phosphate dehydrogenase 1-like	24549139
<i>BBC3</i>	BCL2 binding component 3	24549139
<i>CYP3A7</i>	Cytochrome P450, family 3, subfamily A, polypeptide 7	24549139
<i>TOE1</i>	Target of EGR1, member 1 (nuclear)	24549139
<i>EIF1B</i>	Eukaryotic translation initiation factor 1B	24549139
<i>ADH1B</i>	Alcohol dehydrogenase 1B (class I), beta polypeptide	24549139
<i>FDFT1</i>	Farnesyl-diphosphate farnesyltransferase 1	24549139

800

801

802 **Table 4. Significant GO terms shared among genes positively correlated with adipocyte**
 803 **size (both studies).**

GO ID	Term	Corrected P-value	Annotated genes
GO:0048522	positive regulation of cellular process	0.003361691	CXCL2, IL8, KIF3B, MIF, NHEDC2, C1QR1, CD248, CLEC3B, COL1A1, AQP1
GO:0006928	movement of cell or subcellular component	0.004711785	CD248, CXCL2, IL8, NME5, KIF3B, MIF, COL1A1
GO:0050900	leukocyte migration	0.005651237	CXCL2, IL8, MIF, COL1A1
GO:0002376	immune system process	0.007058561	CD248, CXCL2, IL8, KIF3B, MIF, NHEDC2, COL1A1, C1QR1

804

805

806 **Figure legends**

807 **Figure 1. Schematic illustrating regional adipose morphology. A.** This review largely
 808 concentrates on 3 human ATs; visceral (VAT; blue), abdominal SAT (yellow) and gluteofemoral
 809 SAT (red). **B.** Adipose morphology can be categorized by finding a line-of-best-fit to describe the
 810 relationship between fat mass (mg) and mean adipocyte volume (pl). Such a fitted line produces
 811 a curvilinear relationship (dotted line). AT from individuals (black circles) is assessed relative to
 812 the fitted line (dotted line). A positive residual (adipocyte volume greater than expected) indicated
 813 hypertrophic morphology, whereas an adipocyte volume smaller than expected denotes
 814 hyperplastic morphology. **C.** Adipose morphology can be hyperplastic characterized by many,
 815 small adipocytes. Or, hypertrophic, characterized by few, large adipocytes. Each morphology is
 816 associated with distinct metabolic parameters (Arner et al. 2010).

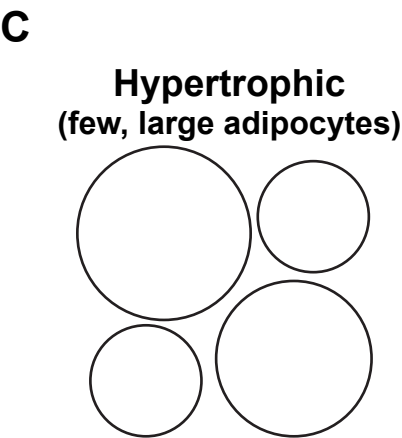
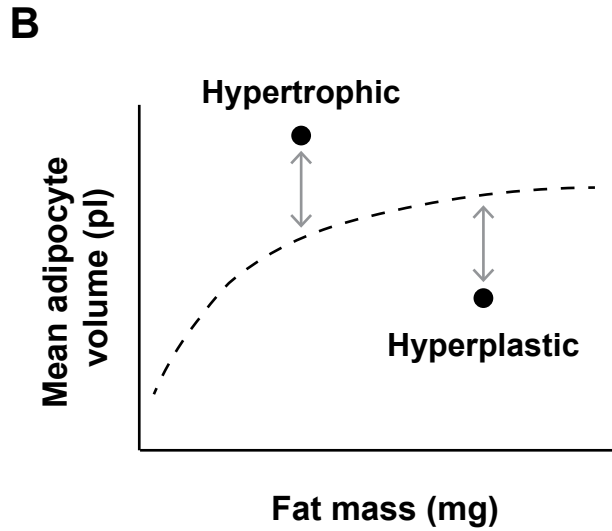
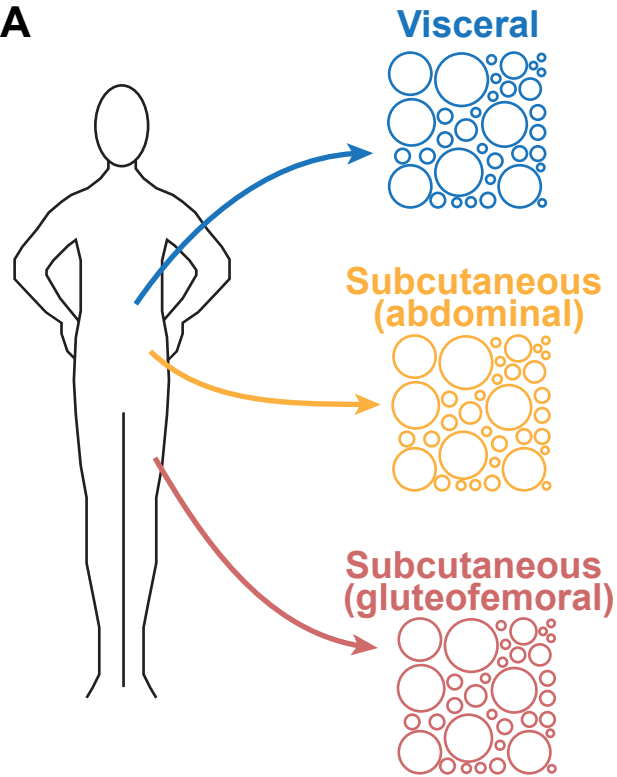
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818 **Figure 2. Schematic illustrating common adipocyte size distributions. A,B.** A unimodal
 819 adipocyte size distribution is often found and in obesity, or after exposure to a high-fat diet
 820 (magenta) the population mean (μ) shifts to a larger size. **C,D.** A bimodal adipocyte size
 821 distribution can be evaluated by; (i) the value for the nadir (n), (ii) centre of peak (Cp) of large
 822 adipocytes. In obesity, the nadir and centre of peak for the large adipocytes is increased.

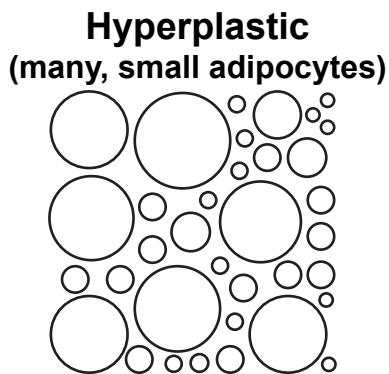
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824 **Figure 3. Schematic illustrating putative mechanisms that regulate adipocyte hypertrophy.**
 825 **A.** Lipid availability is critical to adipocyte hypertrophy, and can be split into multiple steps. 1)
 826 circulating lipid in lipoproteins (for example, very Low Density Lipoprotein (vLDL)) can contain
 827 TAG, and the plasma levels of lipoproteins is associated with adipocyte hypertrophy. 2) Hydrolysis
 828 of TAG from lipoproteins is performed by LPL (and GPIHBP1) on the surface of adipocytes of
 829 vascular endothelial cells and produces non-esterified fatty acids (NEFAs) and glycerol. Levels
 830 and activity of LPL is associated with adiposity levels and plasma lipid levels. 3) NEFAs are taken
 831 up into adipocytes by fatty acid transporters such as CD36 (and also FATPs). CD36 levels are
 832 associated with adipocyte hypertrophy. 4) Intracellular NEFAs are re-esterified into TAG in
 833 conjunction with the endoplasmic reticulum (6). De-novo lipogenesis within adipocytes also
 834 contributes to the TAG pool (5). 6) Lipid droplets can grow large or small based on transfer of
 835 lipogenic enzymes (eg, DGAT, diglyceride acyltransferase) from the endoplasmic reticulum
 836 membrane to the LD membrane (Wilfling et al., 2013). 7) TAG within LDs is mobilized (lipolysis)
 837 by lipases including HSL (hormone sensitive lipase). 8) NEFAs are released from adipocytes by

838 active transport mechanisms including by ABC transporters (Tarling et al., 2013). **B.** Osmolarity
839 is defined as either hypertonic which induces regulated volume decreases (RVD), or hypotonic
840 which induces regulated volume increases (RVI). **C.** 3 proposed mechanisms by which
841 osmosensing may regulate adipocyte hypertrophy.

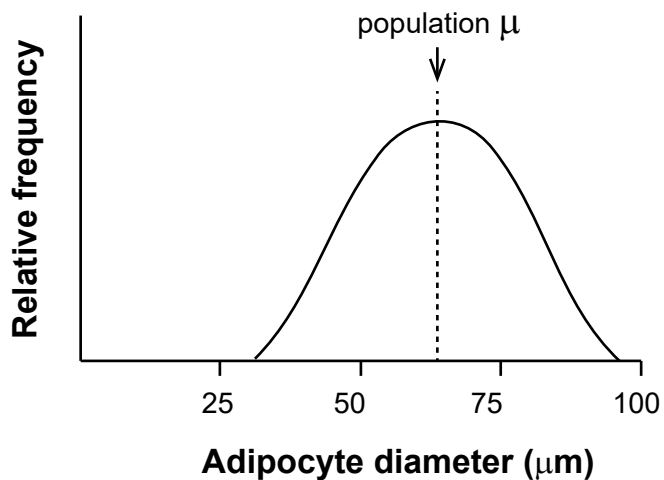
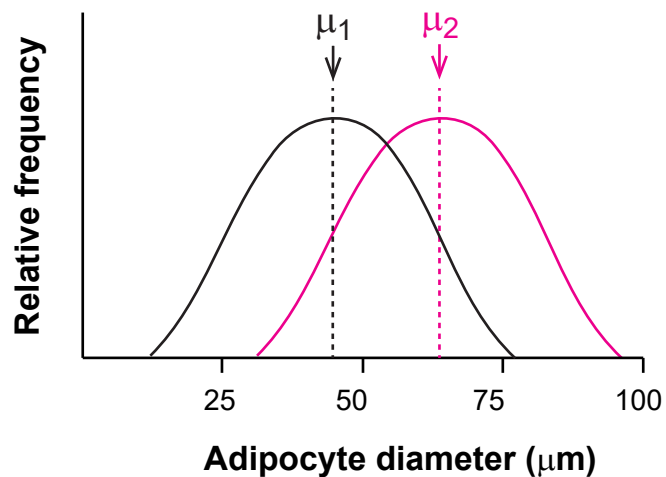


→ **insulin resistance (HOMA-IR)**
higher fasting plasma Insulin & glucose
higher total cholesterol & triacylglycerides

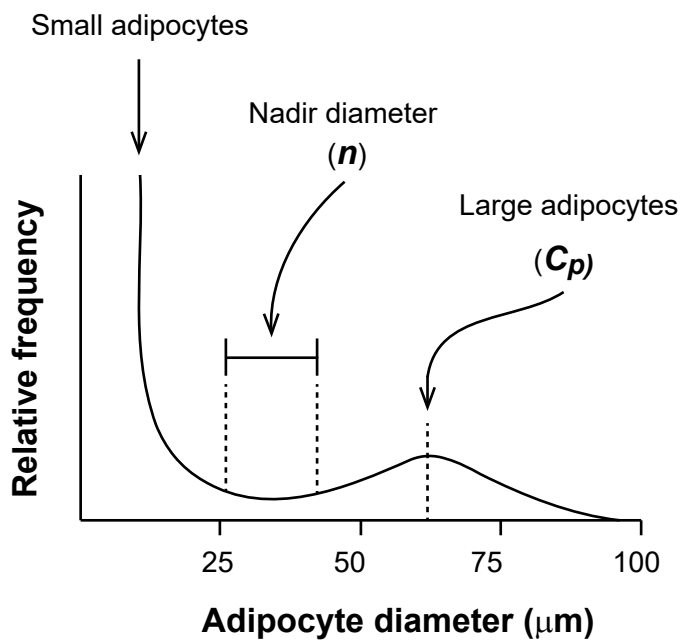


→ **improved insulin sensitivity (HOMA-IR)**
improved fasting plasma Insulin, glucose & lipid

Unimodal adipocyte size distribution

A**B**

Bimodal adipocyte size distribution

C**D**