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Adipose morphology and metabolic disease

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Abstract

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

Obesity is not synonymous with metabolic dysfunction: a role for adipose 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

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; Macotela et al., 2012; Tchkonia 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 (epididimal) 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

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

Hypertrophic morphology is associated with insulin resistance and increased risk 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/m^2),

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

Bi- and tri-modal adipocyte size distributions: a more complex relationship 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   **<u>Transcriptomic differences in distinct adipose morphologies</u>**

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 ±
247   3.54 μm) or large (mean diameter 100.1 ± 3.94 μm) 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 (*MSN*), 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 n non-obese hyperplasic individuals where

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 epidydimal 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   **<u>Cellular mechanisms hypothesized to regulate adipose morphology</u>**

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

A role for mTORC1 in regulating adipocyte size: a highly conserved signaling 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   (Laplante 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). Phosholipid

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

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 on 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 osmosensers and regulators is under intense investigation. Transient receptor potential cation

399 channel subfamily V member 4 (TRPV4) is a Ca^{2+}-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 osmosensers, 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

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 **<u>Tables</u>**

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

	Abdominal SAT	Negative	Increased VAT/VAT+SAT ratio	Kursawe**	20805387
Relative frequency of large adipocytes	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		

771 *Cohort was 28 obese individuals (mean age = ~50 years) stratified according to Insulin sensitivity
772 (Insulin resistant BMI = 30.6 kg/m^2; Insulin sensitive BMI = 29.4 kg/m^2). 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/m^2) 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/m^2) 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/m^2; mean = 25.7 kg/m^2) 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/m^2. 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/m^2.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
SELE	Selectin E	16754744
SPARCL1	SPARC-like 1	16754744
TM4SF1	Transmembrane 4 L six family member 1	16754744
DCN	Decorin	16754744
IL8	Interleukin 8	16754744
PALLD	Palladin	16754744, 24549139
SAA2	Serum amyloid A2	16754744
CLEC3B	C-type lectin domain family 3, member B	16754744
C1QR1	Complement component 1, q subcomponent, receptor 1	16754744
COL1A1	Collagen, type I, alpha 1	16754744
CXCL2	Chemokine (C-XC motif) ligand 1	16754744
COL1A2	Collagen, type I, alpha 2	16754744
FLJ14054		16754744
AQP1	Aquaporin 1	16754744
MSN	Moesin	24549139
NHEDC2	Na+/H+ exchanger domain containing 2	24549139
RP11-877E17.2	undefined	24549139
KIF3B	Kinesin family member 3B	24549139
NME5	Non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	24549139
IFT20	Intraflagellar transport 20 homolog (Chlamydomonas)	24549139
MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	24549139
SLC24A3	Solute carrier family 24 (sodium/potassium/calci um exchanger),member 3	24549139
C15orf59	Chromosome 15 open reading frame 59	24549139

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

798

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

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

Figure legends

Figure 1. Schematic illustrating regional adipose morphology. A. This review largely concentrates on 3 human ATs; visceral (VAT; blue), abdominal SAT (yellow) and gluteofemoral SAT (red). **B.** Adipose morphology can be categorized by finding a line-of-best-fit to describe the relationship between fat mass (mg) and mean adipocyte volume (pl). Such a fitted line produces a curvilinear relationship (dotted line). AT from individuals (black circles) is assessed relative to the fitted line (dotted line). A positive residual (adipocyte volume greater than expected) indicated hypertrophic morphology, whereas an adipocyte volume smaller than expected denotes hyperplastic morphology. **C.** Adipose morphology can be hyperplastic characterized by many, small adipocytes. Or, hyperptrophic, characterized by few, large adipocytes. Each morphology is associated with distinct metabolic parameters (Arner et al. 2010).
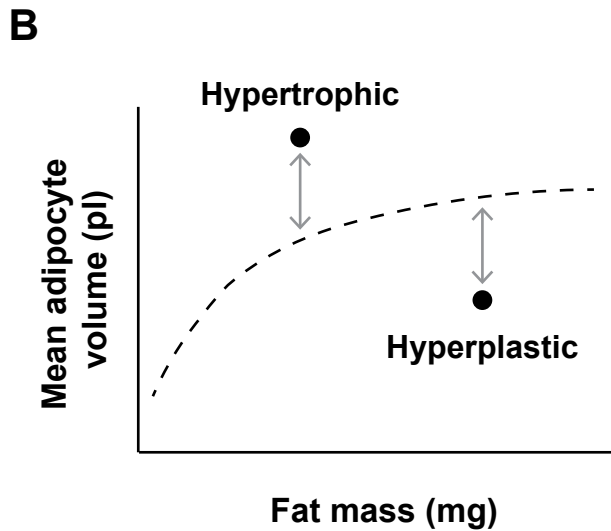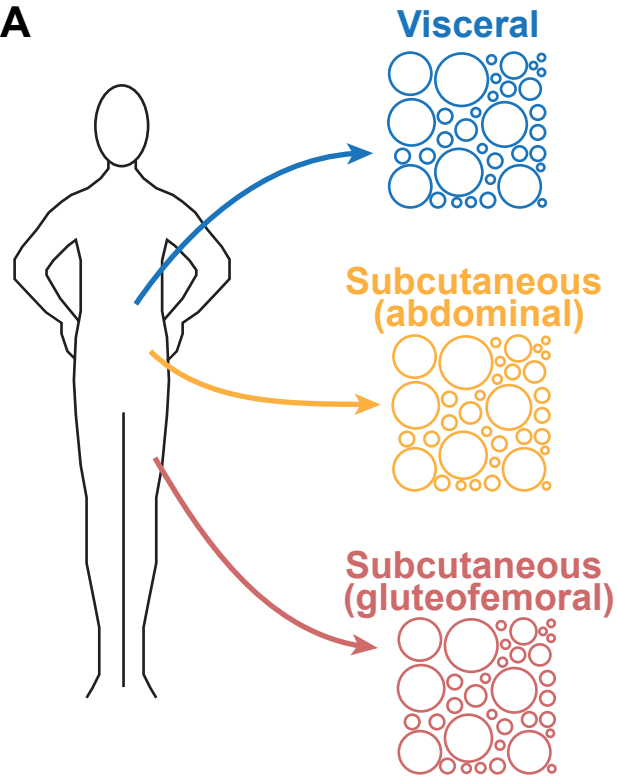
Figure 2. Schematic illustrating common adipocyte size distributions. A,B. A unimodal adipocyte size distribution is often found and in obesity, or after exposure to a high-fat diet (magenta) the population mean (μ) shifts to a larger size. **C,D.** A bimodal adipocyte size distribution can be evaluated by; (i) the value for the nadir (n), (ii) centre of peak (Cp) of large adipocytes. In obesity, the nadir and centre of peak for the large adipocytes is increased.

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

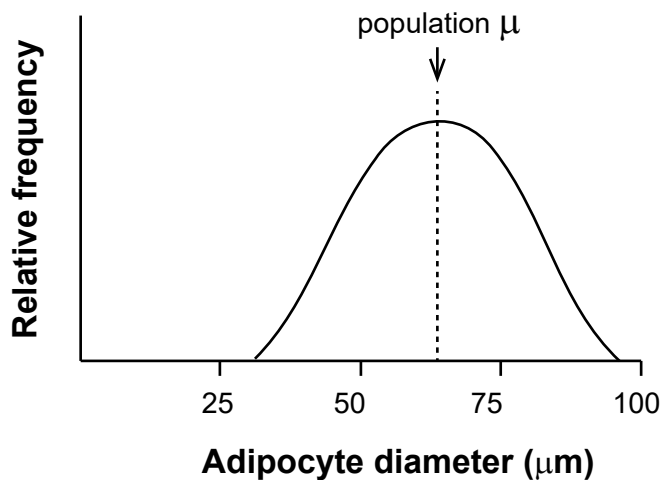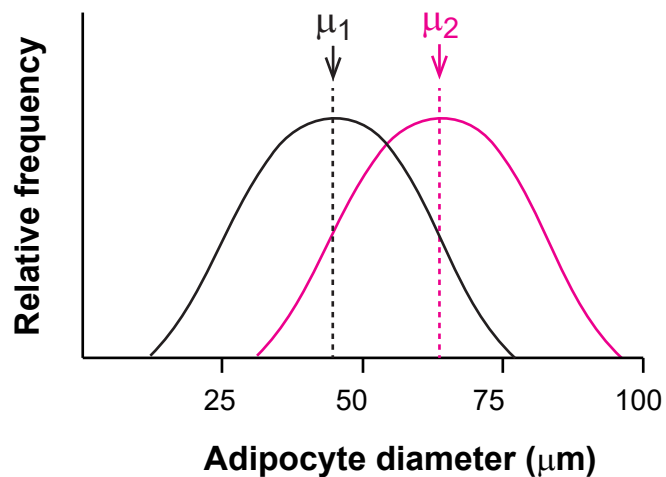841   osmosensing may regulate adipocyte hypertrophy.

A

Visceral

Subcutaneous
(abdominal)

Subcutaneous
(gluteofemoral)

B

Mean adipocyte volume (pl)

Hypertrophic

Hyperplastic

Fat mass (mg)

C

Hypertrophic
(few, large adipocytes)
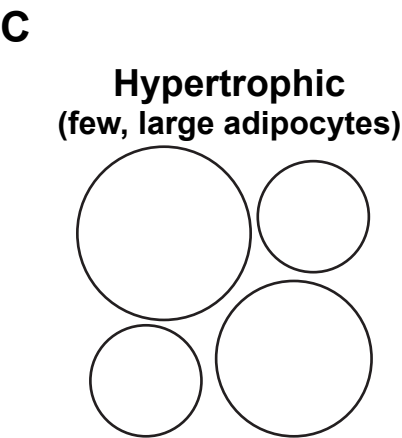
→ insulin resistance (HOMA-IR)
higher fasting plasma Insulin & glucose
higher total cholesterol & triacylglycerides
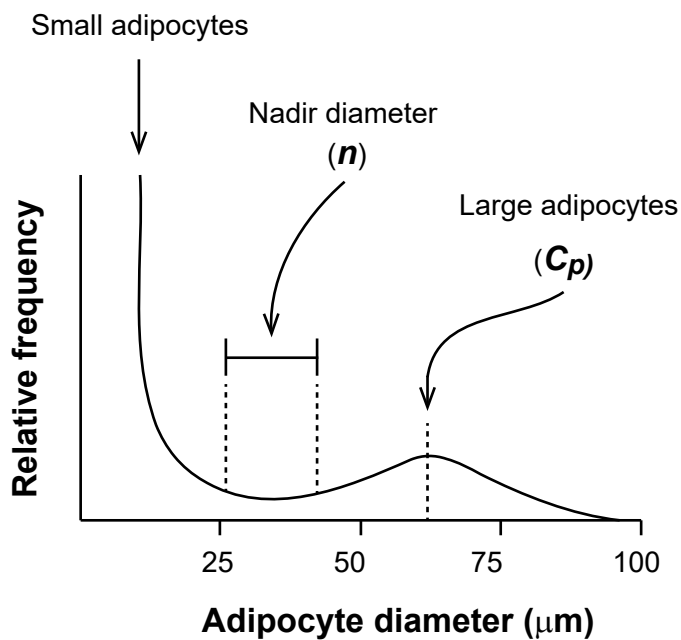
Hyperplastic
(many, small adipocytes)
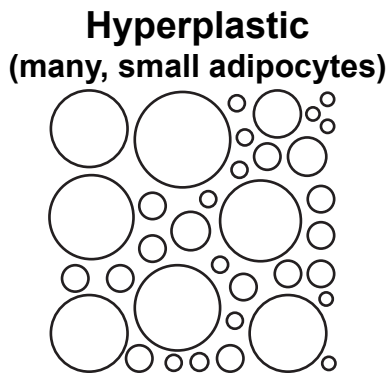
→ improved insulin sensitivity (HOMA-IR)
improved fasting plasma Insulin, glucose & lipid

Unimodal adipocyte size distribution

A

B

Bimodal adipocyte size distribution

C

D