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REVIEW

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 said to have a 'hypertrophic' morphology, whereas adipose with many adipocytes of a smaller size is said to have a 'hyperplastic' morphology. Hypertrophic adipose morphology is positively associated with insulin resistance, diabetes and cardiovascular disease. By contrast, hyperplastic morphology is associated with improved metabolic parameters. These phenotypic associations suggest that adipose morphology influences risk of cardiometabolic disease. Intriguingly, monozygotic twin studies have determined that adipose morphology is in part determined genetically. Therefore, identifying the genetic regulation of adipose morphology may help us to predict, prevent and ameliorate insulin resistance and associated metabolic diseases. Here, we review the current literature regarding adipose morphology in relation to: (1) metabolic and medical implications; (2) the methods used to assess adipose morphology; and (3) transcriptional differences between morphologies. We further highlight three mechanisms that have been hypothesized to promote adipocyte hypertrophy and thus to regulate adipose morphology.

KEY WORDS: Adipose tissue, Adipose morphology, Adipogenesis, Hyperplasia, Hypertrophy

Introduction

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

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

Overweight and obesity are characterized by increased lipid accumulation in adipocytes, whereas weight loss is characterized by reduced lipid accumulation in AT (Eriksson-Hogling et al., 2015; Goodpaster and Sparks, 2017). Obesity is strongly correlated with metabolic disease – for every kg increase in body weight, diabetes rates increase linearly (Haffner, 2006). Although the rising prevalence of being overweight and obesity has led to an increased occurrence of metabolic diseases, including diabetes and cardiovascular disease (CVD) (Wilding, 2017), obesity is not synonymous with metabolic dysfunction. For example, in humans, insulin resistance is a major underlying cause of CVD (Ginsberg, 2000) and is associated with dyslipidemia (Reaven et al., 1967), hypertension (Welborn et al., 1966) and atherosclerosis (Howard et al., 1996). However, huge variation exists in the degree of insulin resistance across all values of body mass index (BMI; a surrogate measure of adiposity) (McLaughlin et al., 2004). Indeed, the degree of insulin resistance can vary sixfold at any given BMI (McLaughlin et al., 2004). Therefore, obesity per se is clearly not the sole driving force for metabolic dysfunction, and understanding which other factors are responsible for the unexplained variance in insulin resistance will have important consequences for public health. Multiple related factors have been proposed to explain the dysfunctional AT and the variability in insulin resistance during obesity, including adipose inflammation, fibrosis, impaired angiogenesis, hypoxia and body fat distribution (Blüher, 2016; Crewe et al., 2017; Divoux et al., 2010; Khan et al., 2009; Sun et al., 2011; Trayhurn, 2013; Weisberg et al., 2003). Here, we present evidence from the literature showing that adipose morphology is an additional factor that influences susceptibility to metabolic disease.

Regional variation in adipose morphology

To assess the role of adipose morphology in metabolic disease, it is first essential to review how adipose morphology can vary between regionally distinct ATs. Briefly, ATs are distributed throughout the human body, but are mainly categorized into subcutaneous ATs (SAT; ATs situated between muscle and skin) and visceral ATs (VAT; AT associated with internal visceral organs) (Fig. 1A) (Shen et al., 2003). The subcutaneous and visceral sites of adipose accumulation appear to be conserved in mouse (Bartelt and Heeren, 2014; Cinti, 2012; Shen et al., 2003) and, strikingly, most regional AT sites even appear to be conserved in zebrafish (Minchin and Rawls, 2017; Shen et al., 2003). The regional distribution of human AT is strongly associated with insulin resistance. A recent meta-analysis demonstrated that VAT was the strongest predictor of insulin resistance [measured by homeostatic model assessment–insulin resistance (HOMA–IR)] (Zhang et al., 2015); but total fat

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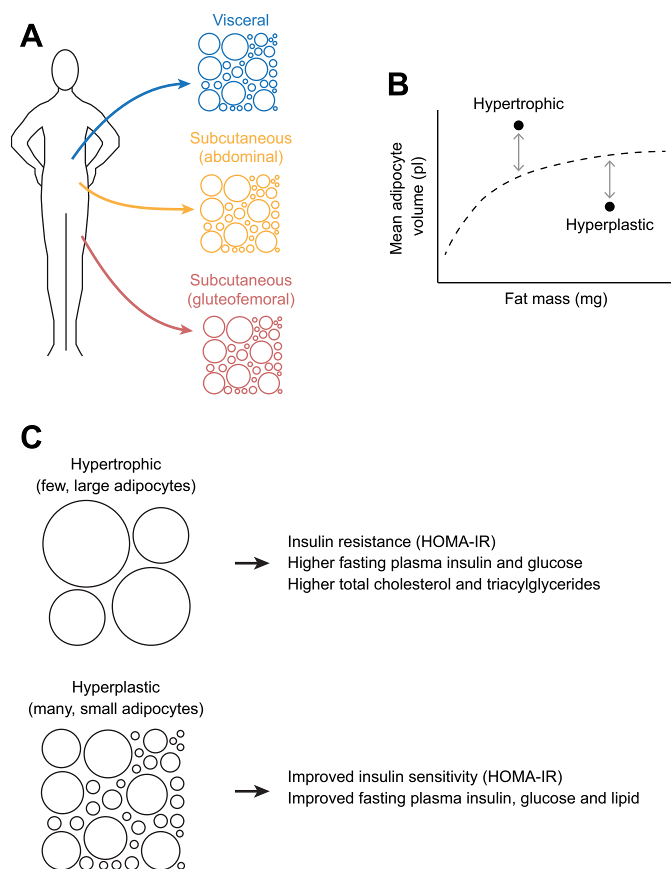

Fig. 1. Schematic illustrating regional adipose morphology. (A) This Review largely concentrates on three human adipose tissues (ATs): visceral AT (VAT; blue), abdominal subcutaneous AT (SAT) (yellow) and gluteofemoral SAT (red). (B) Adipose morphology can be categorized by finding a line of best fit 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) indicates hypertrophic morphology, whereas an adipocyte volume smaller than expected denotes hyperplastic morphology. (C) Adipose morphology can be hyperplastic, i.e. characterized by many small adipocytes, or hypertrophic, i.e. characterized by few large adipocytes. Each morphology is associated with distinct metabolic traits (Arner et al., 2010). HOMA-IR, homeostatic model assessment–insulin resistance.

mass, BMI, waist circumference, intra-abdominal fat and total abdominal fat were also significantly associated with insulin resistance (Zhang et al., 2015). By contrast, lower-body SAT is not correlated with insulin resistance and has been shown to protect against metabolic dysfunction (Snijder et al., 2005, 2004; Zhang et al., 2015). Many previous studies in humans have also linked the accumulation of lipid within abdominal SAT to insulin resistance and metabolic disease, suggesting that upper body fat distribution (or central adiposity) rather than lower body fat distribution (or peripheral adiposity) is an important factor in metabolic disease (Karpe and Pinnick, 2015; Porter et al., 2009).

Adipocytes from regionally distinct ATs can have significantly different sizes. For example, comparison of adipocyte size between three distinct human SATs (gluteal, anterior abdominal wall and triceps) revealed significant differences (gluteal>abdominal>triceps) (Salans et al., 1973, 1971). Indeed, intra-individual site-to-site variability in adipocyte size was greater than same site variability between individuals (Salans et al., 1971). In general, across studies, SAT adipocytes were significantly larger than VAT adipocytes, irrespective of BMI or metabolic state (Liu et al., 2009; Tchernof et al., 2006), suggesting that SAT undergoes greater hypertrophy relative to VAT. However, previous studies have suggested that SAT is inherently more hyperplastic than VAT, although these observations were based on *in vitro* data from mouse and humans showing that SAT-derived cells have greater adipogenic capacity than VAT-derived cells (Baglioni et al., 2012; Macotela et al., 2012; Tchkonia et al., 2006). More recent *in vivo* data, from the transgenic AdipoChaser mouse line, have suggested the opposite: following diet-induced obesity, VAT (epidydimal) underwent waves of hyperplastic growth, whereas SAT (inguinal) did not (Wang et al., 2013). Although these data conflict with the *in vitro* observations of higher SAT hyperplasia, they confirm the larger adipocyte size and presumed greater degree of hypertrophic growth in SAT. Thus, after exposure to a high-fat diet, SAT appears to undergo hypertrophic growth preferentially, leading to larger adipocytes relative to those in VAT. The contrasting growth dynamics of VAT and SAT are of biomedical importance because the reduced expandability of SAT is associated with insulin resistance (Gealekman et al., 2011; Virtue and Vidal-Puig, 2008). In line with these observations, the treatment of obese diabetics with thiazolidinidiones (TZDs) leads to greater weight gain, preferential lipid deposition in SAT and improved insulin sensitivity (Fonseca, 2003; Nichols and Gomez-Caminero, 2007). Thus, understanding the differential growth mechanisms of SAT may reveal therapeutic targets for treating obesity-associated metabolic disease.

Using a ‘morphology value’ to quantify adipose morphology

To quantify adipose morphology, Arner et al. (2010) described a ‘morphology value’ – the difference between measured adipocyte volume and expected adipocyte volume (relative to total adipose mass) (Arner et al., 2010; Spalding et al., 2008) (Fig. 1B). This metric was further utilized by Veilleux et al. (2011), and facilitated the categorization of individuals according to whether their adipose exhibits a hypertrophic or hyperplastic morphology (Arner et al., 2010; Veilleux et al., 2011). To determine the morphology value, AT biopsies were first taken, then a single-cell adipocyte suspension was produced by collagenase digestion and buoyancy separation, and the sizes of individual adipocytes were then measured using image-analysis software. A curvilinear line was then fitted to the data to best describe the relationship between adiposity and mean adipocyte size (Spalding et al., 2008). Each individual was then categorized in relation to the fitted line: individuals exhibiting a positive residual were hypertrophic (mean adipocyte volume larger than expected), whereas individuals exhibiting a negative residual were hyperplastic (mean adipocyte volume smaller than expected) (Arner et al., 2010; Veilleux et al., 2011) (Fig. 1B). Importantly, lower morphology values (hyperplasticity) were associated with an increased number of small adipocytes, whereas high morphology values (hypertrophicity) were associated with fewer larger adipocytes (Arner et al., 2010; Veilleux et al., 2011). Within a population, adipose morphology appears highly variable. Arner et al. (2010) found that hyperplastic and hypertrophic morphologies were present at equal frequencies in both males and females, and in obese and non-obese people (Arner et al., 2010). Strikingly, at comparable BMI, women typically present with ∼10% higher body fat, which is characterized by greater SAT in the abdomen and gluteofemoral regions (Camhi et al., 2011; Jackson et al., 2002; Karastergiou et al., 2012; Womersley, 1977). Body fat distribution is linked to health in both males and females, but the protective peripheral distribution is mainly seen in females (Krotkiewski et al., 1983). Large inter-individual variation in adipocyte number and

size within equivalent ATs was also observed, and this variation was independent of adipose mass (Arner et al., 2011; Salans et al., 1973, 1971). Furthermore, estimates of adipocyte number varied by as much as 85% between individuals (Salans et al., 1973), suggesting a high-level of inter-individual variability in adipose morphology. Intriguingly, adipocyte number and size were similar in monozygotic twins who had concordant BMI, suggesting that these traits have a strong genetic basis (Heinonen et al., 2014). Therefore, understanding how genetics drives variation in adipose morphology, with subsequent consequences for disease risk, is a central research question.

Hypertrophic morphology is associated with insulin resistance and increased risk of cardiovascular disease

The association between SAT morphology and metabolic disease has been extensively characterized. In a cohort of 764 subjects exhibiting a wide adiposity range (BMI 18–60 kg m^{-2}), Arner et al. (2010) found that hypertrophic morphology was positively correlated with insulin resistance (measured by HOMA-IR) and fasting plasma insulin in humans (Arner et al., 2010). Furthermore, in women, hypertrophic morphology was associated with a metabolic syndrome-like state, characterized by increased insulin resistance and by increases in circulating plasma insulin, total cholesterol and TAG (Arner et al., 2010). In addition, abdominal SAT adipocyte size itself was positively associated with insulin resistance, independently of BMI, in non-diabetic humans (Lundgren et al., 2007). Furthermore, SAT adipocyte size was positively associated with raised plasma insulin and blood glucose, and with insulin-induced glucose disposal and insulin sensitivity in humans (Hoffstedt et al., 2010). Finally, in humans, the average volume of adipocytes within abdominal SAT was correlated with insulin resistance (Yang et al., 2012). By contrast, hyperplastic morphology was associated with significantly better blood glucose, insulin and lipid profiles when compared with these profiles in subjects with hypertrophic morphology (Hoffstedt et al., 2010). Taken together, these data demonstrate that hypertrophic SAT morphology is associated with metabolic dysfunction, and metabolic risk factors for diabetes and CVD.

VAT morphology has also been implicated in metabolic disease. In a sample of 207 lean to severely obese females, subjects characterized by hypertrophic omental VAT had higher plasma TAG, higher very low density lipoprotein (vLDL)-TAG and higher vLDL cholesterol when compared with subjects with hyperplastic VAT (Veilleux et al., 2011). It was also estimated that a 10% enlargement of VAT adipocytes was associated with a fourfold increase in the risk of hypertriacylglyceridemia (Veilleux et al., 2011), whereas a 10% increase in the number of VAT adipocytes was associated with a 1.55-fold increase in the risk of hypertriacylglyceridemia (Veilleux et al., 2011). In morbidly obese females (and independent of age, BMI, body fat mass or body fat distribution), VAT adipocyte size was positively associated with plasma apolipoprotein B, total cholesterol, vLDL cholesterol and triacylglycerides (Hoffstedt et al., 2010). Furthermore, large VAT adipocytes (>75 µm diameter) were associated with insulin resistance in canines (Kabir et al., 2011). These data demonstrate that hypertrophic VAT morphology is also associated with a metabolic-syndrome-like state.

Bi- and tri-modal adipocyte size distributions: a more complex relationship between adipose morphology and insulin resistance?

Many studies have concluded that adipocytes comprise a complex population of cells that exhibit a bi- or tri-modal size distribution. In general, these studies have used osmium tetroxide fixation of adipocytes, followed by size analysis of adipocytes using a Coulter counter (Cushman and Salans, 1978; Etherton et al., 1977; Hirsch and Gallian, 1968). The advantages of this method include the ability to analyze large numbers of adipocytes (∼6000 cells from each subject), and the automated and unbiased measurement of adipocyte size (Jo et al., 2012). To exclude the possibility that the small adipocytes (within a bimodal population) were not artefactual 'debris', microscopy was used to confirm that these cells comprised intact, spherical small adipocytes (McLaughlin et al., 2007). Measurement by microscopy has also indicated that adipocytes may form a bimodal size distribution (Fang et al., 2015). Comparison of the size distributions obtained from these methods revealed a peak of small adipocytes of ∼25 µm diameter and a peak of larger adipocytes of ∼50 µm diameter (Fig. 2) (Jo et al., 2012). Intriguingly, tri-modal adipocyte size distributions in humans have also been observed with peaks at ∼25, ∼50 and ∼100 µm diameters (Yang et al., 2012). Recently, three-dimensional reconstruction of zebrafish VAT revealed a bimodal size distribution of adipocyte-localized LDs (Minchin et al., 2015). It is likely, however, that the smaller population of LDs in zebrafish (∼1 µm in diameter) corresponds to additional LD 'locules' within multilocular adipocytes – a phenomenon also observed in human and mouse white adipocytes (Chau et al., 2014; Cushman, 1970). Together, these studies suggest that parametric statistics, such as mean adipocyte size and number, may not accurately represent the true population mean, and should be used with caution when assessing adipose morphology.

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

The proportion and size of small adipocytes within a bimodal size distribution is also related to metabolic wellbeing. In moderately overweight and obese individuals, McLaughlin et al. (2007, 2014) found that an increased proportion of small adipocytes in abdominal SAT was statistically associated with insulin resistance. Furthermore, an increased proportion of small adipocytes was found in both the abdominal SAT and the omental VAT of diabetics (Fang et al., 2015). Intriguingly, an increased proportion of small adipocytes in abdominal SAT also occurred in subjects with high VAT/VAT+SAT ratio (Kursawe et al., 2010), after insulin-sensitive subjects were overfed (McLaughlin et al., 2016), or after diabetics were treated with rosiglitazone (Eliasson et al., 2014). In diabetics, the size of small adipocytes was also inversely correlated with insulin sensitivity (Fang et al., 2015). Finally, an expanded nadir

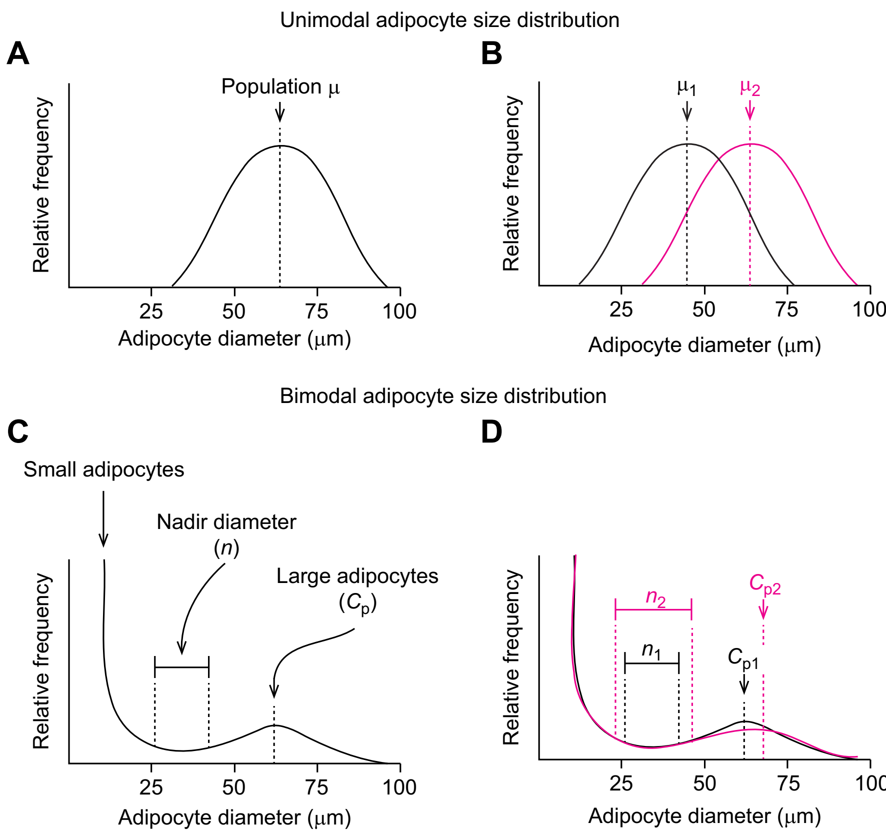

Fig. 2. Schematic illustrating common adipocyte size distributions. (A,B) A unimodal adipocyte size distribution is often found, and in obesity or after exposure to a high-fat diet (magenta) the population mean (μ) shifts to a larger size. (C,D) A bimodal adipocyte size distribution can be evaluated by (1) the value for the nadir (n) and (2) the centre of peak (C_p) of large adipocytes. In obesity, the nadir and centre of peak for the large adipocytes are increased.

(the low point in frequency between the small and large adipocyte populations) was found in insulin-resistant subjects (McLaughlin et al., 2007). A summary of these findings is provided in Table 1. Together, these data show that insulin resistance is not only accompanied by the hypertrophy of large adipocytes, but is also associated with an increased proportion of small adipocytes in studies that detect a bimodal adipocyte size distribution. Related to the increased presence of small adipocytes, the expression of genes related to adipogenesis was also lower in insulin-resistant individuals (McLaughlin et al., 2007). These findings are consistent with independent reports of reduced adipogenesis in insulin-resistant patients (Goedecke et al., 2011; Yang et al., 2004). Furthermore, these expression changes were also associated with modest increases in inflammatory activity in insulin-resistant AT (McLaughlin et al., 2008). As the presence of small adipocytes in both VAT and SAT appears to be correlated with increased hypertrophy of larger adipocytes in abdominal SAT (Liu et al., 2009), these findings could be interpreted as suggesting that SAT is the primary site for lipid accumulation; however, once a maximal SAT adipocyte size is reached, hyperplastic growth is initiated in both VAT and SAT. Furthermore, adipocytes have been shown to expand to only a finite degree in both humans and rats (Faust et al., 1978; Kashiwagi et al., 1985). A summary of these findings is provided in Table 1. Therefore, these data suggest that insulin resistance is accompanied by an inability of small adipocytes to undergo hypertrophic expansion, suggesting defective adipogenesis that results in a higher proportion of small adipocytes among a more general population of hypertrophied adipocytes.

Transcriptomic differences in distinct adipose morphologies

To study adipose hypertrophy and hyperplasia, and to identify potential molecular pathways that influence morphology, it is useful to analyze the transcriptional state underlying distinct morphologies. Strikingly, adipocytes of different sizes have distinct gene expression profiles. Jernas et al. (2006) fractionated human adipocytes into small (mean diameter 57.6±3.54 μm) or large (mean diameter 100.1±3.94 μm) groups. Subsequent microarray analysis of gene expression in the two groups of adipocytes revealed 14 genes with fourfold higher expression in large adipocytes (Table 2) (Jernas et al., 2006). Strikingly, some transcripts exhibited 19-fold and 22-fold higher expression in large adipocytes, suggesting relatively large-scale differences between adipocytes of different sizes (Jernas et al., 2006). In an additional study, Heinonen et al. (2014) analyzed whether adipocyte size or number correlated with changes in the AT transcriptome. RNA was extracted from whole-adipose biopsies (including both adipocyte and stromal-vascular fractions) and the genes whose expression positively correlated with adipocyte size included genes implicated in cell cytoskeleton and membrane modifications (*MSN*, *NHEDC2*, *KIF3B* and *PALLD*), oxidative stress and apoptosis (*MSN*), cell-mediated immunity (*MIF*) and cancer (*NMES*) (Tables 2 and 3) (Heinonen et al., 2014). Genes whose expression inversely correlated with adipocyte size included *FDFT1* (mevalonate pathway, cholesterol biosynthesis), *ADH1B* (metabolism of a wide range of substrates, including hydroxysteroids and lipid peroxidation products) and *EIF1B* (unknown function) (Tables 2 and 3) (Heinonen et al., 2014). Significant Gene Ontology (GO) terms, which are used to describe shared relationships between sets of genes, revealed that leukocyte migration and immune system processes were significantly enriched terms for genes that were upregulated in large adipocytes (Table 4). Adiponectin mRNA was also found to be negatively associated with the size of isolated adipocytes (Bambace et al., 2011), whereas leptin mRNA was positively associated with adipocyte volume in isolated adipocytes

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

Morphology trait	AT	Direction of association	Metabolic trait	Study
Relative frequency of small adipocytes	Abdominal SAT	Positive	Insulin resistance	McLaughlin et al. (2007)*
	Abdominal SAT	Positive	Increased VAT/VAT+SAT ratio	Kursawe et al. (2010)**
	Abdominal SAT	Positive	Insulin resistance	McLaughlin et al. (2014)****
	Abdominal SAT	Positive	Type 2 diabetics treated with rosiglitazone	Eliasson et al. (2014)‡
	Abdominal SAT	Positive	Diabetics versus non-diabetics	Fang et al. (2015)‡‡
	Omental VAT	Positive	Diabetics versus non-diabetics	Fang et al. (2015)‡‡
	Abdominal SAT	Negative	Overfed insulin-sensitive subjects	McLaughlin et al. (2016)‡‡‡
Change in diameter of small adipocytes	Abdominal SAT	Negative	Diabetics versus non-diabetics	Fang et al. (2015)‡‡
	Omental VAT	Negative	Diabetics versus non-diabetics	Fang et al. (2015)‡‡
Nadir diameter (n)	Abdominal SAT	Positive	Insulin resistance	McLaughlin et al. (2007)*
Relative frequency of large adipocytes	Abdominal SAT	Negative	Increased VAT/VAT+SAT ratio	Kursawe et al. (2010)**
	Abdominal SAT	Negative	Insulin resistance	McLaughlin et al. (2014)****
	Abdominal SAT	Negative	Diabetics versus non-diabetics	Fang et al. (2015)‡‡
	Omental VAT	Negative	Diabetics versus non-diabetics	Fang et al. (2015)‡‡
Large adipocyte size (C_p)	Abdominal SAT	Positive	Increased small adipocytes in omental VAT and abdominal SAT	Liu et al. (2009)***
	Abdominal SAT	Positive	Increased VAT/VAT+SAT ratio	Kursawe et al. (2010)**
	Abdominal SAT	Positive	Insulin resistance	Yang et al. (2012)****
	Abdominal SAT	Positive	Insulin resistance	McLaughlin et al. (2014)****
	Abdominal SAT	Positive	Type 2 diabetics treated with rosiglitazone	Eliasson et al. (2014)‡
	Abdominal SAT	No change	Overfed insulin-resistant subjects	McLaughlin et al. (2016)‡
	Abdominal SAT	Positive	Overfed insulin-resistant subjects	McLaughlin et al. (2016)‡‡‡

*Cohort was 28 obese individuals (mean age ∼50 years) stratified according to insulin sensitivity (insulin-resistant BMI=30.6 kg m^{-2}; insulin-sensitive BMI=29.4 kg m^{-2}). No statistical differences between insulin-resistant and -sensitive groups were found for age, gender, reported levels of exercise, blood pressure, fasting glucose, total cholesterol and LDL cholesterol. HDL cholesterol was lower in the insulin-resistant group.

**Cohort was 38 adolescents (∼15 years old) with similar degrees of obesity (mean BMI ∼37 kg m^{-2}) divided into two groups: low VAT/VAT+SAT ratio (<0.11) and high VAT/VAT+SAT ratio (>0.11). None of the participants was on any medication or had any known disease.

***Cohort was 11 obese (mean BMI=45.3 kg m^{-2}) insulin-resistant but non-diabetic women. Patients were excluded if they had coronary heart disease, hepatic or renal disease, or cancer, or used medications for weight loss.

****Cohort was 35 subjects with a range of BMIs (range=18–34 kg m^{-2}; mean=25.7 kg m^{-2}) and age (range=28–49 years; mean=41 years). The subjects were non-diabetic, but had a known family history of diabetes, with at least two first-degree relatives with type 2 diabetes.

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

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

‡‡‡Cohort consisted of healthy overweight adults, aged 30–60 years. BMI=25–35 kg m^{-2}. Subjects had a stable body weight during the prior 3 months and a fasting plasma glucose of less than 126 mg dl^{-1}. Subjects were given a hypercaloric diet to induce 3.2 kg weight gain over 4 weeks, followed by 1 week of weight stabilization.

(Guo et al., 2004). However, leptin mRNA per unit of fat mass decreased at more extreme levels of obesity (Guo et al., 2004). Skurk et al. (2007) analyzed the relationship between adipocyte size and secreted factors and found that leptin, interleukin (IL) 6, IL8, Monocyte chemotactic protein 1 (MCP1) and granulocyte-colony stimulating factor (G-CSF) were significantly increased in large adipocytes, supporting the possibility of altered immune signaling following hypertrophy (Skurk et al., 2007). Recently, Gao et al. (2014) utilized adipose biopsies from a cohort of 56 healthy males and females, subdivided into obese or non-obese individuals with hyperplastic or hypertrophic morphologies (i.e. obese hyperplastic, obese hypertrophic, non-obese hyperplastic and non-obese hypertrophic) (Gao et al., 2014). This analysis identified 619 genes whose expression was differentially altered by morphology in non-obese subjects (Gao et al., 2014). Genes whose expression was increased in non-obese hypertrophy were associated with pro-inflammatory pathways, whereas genes whose expression was increased in non-obese hyperplasic individuals where involved in carbohydrate and lipid metabolism (Gao et al., 2014). The transcriptome of adipocytes in populations with bimodal size distributions has also been analyzed. Liu et al. (2010) characterized small adipocytes from epidydimal AT (VAT) of Zucker Obese (ZO) and Lean (ZL) rats and found that small adipocytes had a threefold decrease in adiponectin and Pparg expression in ZO versus ZL rats (Liu et al., 2010), along with a 2.5-fold increase in IL6 expression (Liu et al., 2010). These data suggest that both hypertrophied adipocytes and the small adipocytes from a bimodal population have pro-inflammatory characteristics. Altogether, these data support the conclusion that small and large adipocytes have distinct transcriptional profiles, and that large adipocytes are characterized by altered immune or inflammatory activity.

Cellular mechanisms hypothesized to regulate adipose morphology

Understanding the cell and molecular mechanisms that underpin adipose morphology is likely to provide new therapeutic targets for combating obesity-associated disease. For simplicity, we have separated the factors that are likely to influence adipose morphology into two categories: (1) factors that regulate adipocyte number and (2) factors that regulate adipocyte size. As the regulation of adipocyte number (adipogenesis) is a well-studied subject with many in-depth reviews (Berry et al., 2016, 2014; Hepler et al., 2017), we will focus on mechanisms that are hypothesized to regulate adipocyte size. Surprisingly, relatively few studies have identified cellular mechanisms that regulate adipocyte size. Therefore, we first review highly conserved mechanisms for regulating cell size across multiple cell types, and investigate whether these conserved mechanisms may also regulate adipocyte

Table 2. Genes positively correlated with adipocyte size

Gene symbol	Gene name	References
SELE	Selectin E	Jernas et al. (2006)
SPARCL1	SPARC-like 1	Jernas et al. (2006)
TM4SF1	Transmembrane 4L six family member 1	Jernas et al. (2006)
DCN	Decorin	Jernas et al. (2006)
IL8	Interleukin 8	Jernas et al. (2006)
PALLD	Palladin	Jernas et al. (2006), Heinonen et al. (2014)
SAA2	Serum amyloid A2	Jernas et al. (2006)
CLEC3B	C-type lectin domain family 3, member B	Jernas et al. (2006)
C1QR1	Complement component 1, q subcomponent, receptor 1	Jernas et al. (2006)
COL1A1	Collagen, type I, alpha 1	Jernas et al. (2006)
CXCL2	Chemokine (C-XC motif) ligand 1	Jernas et al. (2006)
COL1A2	Collagen, type I, alpha 2	Jernas et al. (2006)
FLJ14054	Undefined	Jernas et al. (2006)
AQP1	Aquaporin 1	Jernas et al. (2006)
MSN	Moesin	Heinonen et al. (2014)
NHEDC2	Na^+/H^+ exchanger domain containing 2	Heinonen et al. (2014)
RP11-877E17.2	Undefined	Heinonen et al. (2014)
KIF3B	Kinesin family member 3B	Heinonen et al. (2014)
NME5	Non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	Heinonen et al. (2014)
IFT20	Intraflagellar transport 20 homolog (*Chlamydomonas*)	Heinonen et al. (2014)
MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	Heinonen et al. (2014)
SLC24A3	Solute carrier family 24 ($Na^+/K^+/Ca^+$ exchanger), member 3	Heinonen et al. (2014)
C15orf59	Chromosome 15 open reading frame 59	Heinonen et al. (2014)
CD248	CD248 molecule, endosialin	Heinonen et al. (2014)
SLC46A3	Solute carrier family 46, member 3	Heinonen et al. (2014)
XPO6	Exportin 6	Heinonen et al. (2014)
FAT1	FAT tumor suppressor homolog 1 (*Drosophila*)	Heinonen et al. (2014)
GNG2	Guanine nucleotide binding protein (G protein), gamma 2	Heinonen et al. (2014)
LPCAT1	Lysophosphatidylcholine acyltransferase 1	Heinonen et al. (2014)
TCTA	T-cell leukemia translocation altered gene	Heinonen et al. (2014)
CLTB	Clathrin, light chain B	Heinonen et al. (2014)
SPTAN1	Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	Heinonen et al. (2014)
CYBASC3	Cytochrome b, ascorbate dependent 3	Heinonen et al. (2014)

Table 3. Genes inversely correlated with adipocyte size

Gene symbol	Gene name	Study PMID
NPEPPS	Aminopeptidase puromycin sensitive	Heinonen et al. (2014)
GLYCTK	Glycerate kinase	Heinonen et al. (2014)
PKP2	Plakophilin 2	Heinonen et al. (2014)
AZGP1	Alpha-2-glycoprotein 1, zinc-binding	Heinonen et al. (2014)
CIDEA	Cell death-inducing DFFA-like effector a	Heinonen et al. (2014)
FAM184A	Family with sequence similarity 184, member A	Heinonen et al. (2014)
NUP98	Nucleoporin 98 kDa	Heinonen et al. (2014)
WHSC2	Wolf-Hirschhorn syndrome candidate 2	Heinonen et al. (2014)
FAM161A	Family with sequence similarity 161, member A	Heinonen et al. (2014)
SLC27A2	Solute carrier family 27 (fatty acid transporter), member 2	Heinonen et al. (2014)
ZFAND1	Zinc-finger, AN1-type domain 1	Heinonen et al. (2014)
MACROD1	MACRO domain containing 1	Heinonen et al. (2014)
PPARA	Peroxisome proliferator-activated receptor alpha	Heinonen et al. (2014)
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like	Heinonen et al. (2014)
BBC3	BCL2 binding component 3	Heinonen et al. (2014)
CYP3A7	Cytochrome P450, family 3, subfamily A, polypeptide 7	Heinonen et al. (2014)
TOE1	Target of EGR1, member 1 (nuclear)	Heinonen et al. (2014)
EIF1B	Eukaryotic translation initiation factor 1B	Heinonen et al. (2014)
ADH1B	Alcohol dehydrogenase 1B (class I), beta polypeptide	Heinonen et al. (2014)
FDFT1	Farnesyl-diphosphate farnesyltransferase 1	Heinonen et al. (2014)

size. We then focus on two interesting, adipocyte-specific pathways that have a role in regulating adipocyte size: first, the phospholipid monolayer on the surface of LDs controls their expansion; second, osmolarity sensors are emerging as regulators of adipocyte hypertrophy.

A role for mTORC1 in regulating adipocyte size

Highly conserved homeostatic mechanisms regulate cell size in eukaryotes (Lloyd, 2013). As adipocyte size is also highly regulated, we reasoned that understanding the core pathways that maintain cell size across multiple diverse cell types has the potential to shed light on the mechanisms controlling adipocyte hypertrophy. Central to the control of cell size across the animal kingdom is the signaling pathway involving insulin growth factor (IGF), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT) and mechanistic target of rapamycin complex 1 (mTORC1). IGF–PI3K–AKT–mTORC1 coordinates nutrition with cell growth, and acts as a node to integrate external signals, including insulin signaling, with biogenic pathways (Edgar, 2006). mTORC1 responds to multiple inputs, including amino acids, energy, stress, oxygen and growth factors, and regulates downstream anabolic processes that promote cell growth, including protein and lipid synthesis (through SREBP1/2), mitochondrial biogenesis and ATP production (Cunningham et al., 2007; Düvel et al., 2010; Ma and Blenis, 2009; Porstmann et al., 2008). In addition, mTORC1 also promotes cell growth by negatively regulating autophagy (Hosokawa et al., 2009). Strikingly, artificial activation of the mTORC1 pathway promotes dramatic increases in cell size (Laplante and Sabatini, 2012). Together, these data suggest that the activation of mTORC1 signaling may induce and augment adipocyte hypertrophy. In accordance, *Raptor* KO mice (Raptor is an mTO-binding protein that is essential for the formation and activity of the mTORC1 complex) have smaller adipocytes (and a reduced number of adipocytes), suggesting that mTORC1 may promote adipocyte hypertrophy (Polak et al., 2008). Indeed, *Raptor* KO mice with specific loss of Raptor and mTORC1 in adipocytes develop lipodystrophy with age, suggesting that mTORC1 is essential for maintaining a hypertrophic state in mature adipocytes (Lee et al., 2016). Furthermore, adipocyte-specific *Raptor* KO led to the induction of a bimodal 'polarized' adipocyte size distribution, characterized by the addition of a small population of adipocytes, further suggesting that mTORC1 is essential for maintaining adipose morphology (Lee et al., 2016). Such a polarized adipocyte size distribution is reminiscent of that occurring in fat-specific insulin receptor knockout (FIRKO) mice (Blüher et al.,

Table 4. Significant GO terms shared among genes whose expression is positively correlated with adipocyte size (Jernas et al., 2006; Heinonen et al., 2014)

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*

2002), suggesting that insulin signaling may be crucially important for maintaining adipose morphology. Elevated mTORC1 signaling resulting from the deletion of tuberous sclerosis complex 2 (Tsc2), a complex made up of Tsc1 and Tsc2 proteins that inhibits mTORC1 signaling, led to increased adipogenesis in mouse fibroblasts and 3T3-L1 adipocytes (Zhang et al., 2009). However, *in vivo* constitutive activation of mTORC1 in adipocytes by Tsc1 deletion did not induce adipocyte hypertrophy, but instead led to reduced VAT mass, reduced VAT adipocyte number and reduced VAT adipocytes diameter without affecting SAT, pointing to the complex nature of mTORC1 signaling in adipocytes (Magdalon et al., 2016). Taken together, these results show that mTORC1 depletion leads to adipose atrophy, although conclusive evidence of a role for mTORC1 in adipocyte hypertrophy has not been fully elucidated.

Availability of lipid as a rate-limiting step for adipocyte hypertrophy

The single defining feature of white adipocytes is the presence of large cytoplasmic LDs that can reach ∼200 µm in diameter (Walther and Farese, 2009). This feature is unique to white adipocytes and, therefore, we reasoned that understanding how LD growth is regulated may also allow us to elucidate the cellular mechanisms underlying adipocyte hypertrophy. LD size reflects two processes: (1) lipid incorporation into LDs and (2) lipid mobilization from LDs. However, each of these processes is highly complex and can be regulated at multiple levels. For example, at a minimum, lipid incorporation into LDs depends on: (1) circulating levels of lipid (i.e. the availability of lipid to adipocytes), (2) lipid uptake into adipocytes, (3) re-esterification of non-esterified fatty acids (NEFAs) into TAG, (4) *de novo* lipogenesis in adipocytes and (5) incorporation of TAG into LDs (Fig. 3A). Lipid synthesis, transport and metabolism in adipocytes is a large subject area beyond the scope of this Review, and we recommend the review by Large et al. (2004) for further reading on the subject. Briefly, however, and as described above, hypertrophic VAT has been associated with higher plasma TAG, vLDL TAG and vLDL cholesterol, higher total plasma cholesterol, a higher total-to-HDL cholesterol ratio and increased plasma apolipoprotein B when compared with hyperplastic VAT (Hoffstedt et al., 2010; Veilleux et al., 2011). Together, these data suggest that increased circulating lipid may promote the hypertrophic growth of adipocytes. Accordingly, treatment of 3T3-L1 adipocytes with saturated or monounsaturated NEFAs resulted in adipocyte hypertrophy (Kim et al., 2015).

With regard to the uptake of lipid into adipocytes, the first step is often the hydrolysis of TAG from circulating lipoproteins by lipoprotein lipase (LPL). In adipose tissue, LPL is expressed on the surface of vascular endothelial cells and adipocytes (Gonzales and Orlando, 2007; Merkel et al., 2002), and hydrolyses TAG (from lipoproteins) to form glycerol and NEFAs, both of which are taken up into adipocytes (Geldenhuys et al., 2017). In SAT, higher levels of LPL activity are associated with adipocyte hypertrophy (Serra et al., 2015). Furthermore, LPL deficiency in mice results in lipodystrophy and elevated plasma lipid levels (Weinstock et al., 1995). Following their production by LPL, NEFAs are taken up by adipocytes using specialized fatty acid transporters, including fatty acid transport proteins (FATPs), the scavenger receptor CD36 and the mitochondrial aspartate amino transferase (FABPpm). Isolated adipocytes from CD36 KO mice have impaired NEFA uptake (Coburn et al., 2000). Furthermore, CD36-deficient mice do not develop diet-induced obesity, suggesting that adipocyte hypertrophy is impaired in these mice (Hajri et al., 2007; Koonen et al., 2010; Vroegrijk et al., 2013). We speculate that increased circulating lipid causes adipocyte hypertrophy and adipose growth, but there is currently limited evidence to show clearly that circulating lipid levels induce a hypertrophic morphology, as defined by Arner et al. (2010). Altogether, these data suggest that lipid availability, in the form of plasma lipid levels, LPL activity and lipid uptake into cells, is key to promoting adipocyte hypertrophy.

Specialized pathways for regulating lipid droplet size in adipocytes

Large cells, such as neurons and ova, often have specialized mechanisms that allow them to grow to extreme sizes (Lloyd, 2013). It is likely, therefore, that adipocytes have adipocyte-specific pathways that govern their hypertrophic capacity. Multiple intriguing mechanisms regulate LD growth. First, a genome-wide screen in yeast identified 10 mutants that produced 'supersized' LDs, in fact they were capable of forming LD over 50 times larger than those of wild-type cells (Fei et al., 2011). The genes that were affected in these mutants included those encoding yeast homologs of seipin (Fei et al., 2008, 2011), regulators of phospholipid metabolism and multiple subunits of casein kinase 2 (Fei et al., 2011). The surface layers of LDs are coated with a phospholipid monolayer, and phospholipid metabolism was a shared feature of the genes identified from this yeast screen (Walther and Farese, 2009). Phosphatidic acid (PA), a cone-shaped lipid common in phospholipids that alters the curvature of membranes and promotes membrane fusion events, was a key factor in the formation of supersized LDs (Fei et al., 2011; Marchesan et al., 2003). Further, supersized LDs could be formed by PA-stimulated fusion of LDs (Fei et al., 2011), suggesting that the phospholipid monolayer of LDs is important for LD growth and may mediate LD fusion to facilitate LD hypertrophy.

Regulation of adipocyte size by osmolarity-sensing ion channels

Cells respond to changes in size by generating osmotic gradients using plasma membrane ion channels and transporters to manipulate the osmolarity of the surrounding environment. Utilization of these ion channels can create a hypotonic environment, leading to cell swelling (RVI; regulatory volume increase), or a hypertonic environment, leading to cell shrinkage (RVD; regulatory volume decrease) (Fig. 3B) (Hoffmann et al., 2009; Jentsch, 2016). As cell-size regulation is central to the dynamic growth and regression of adipocytes, the role of such

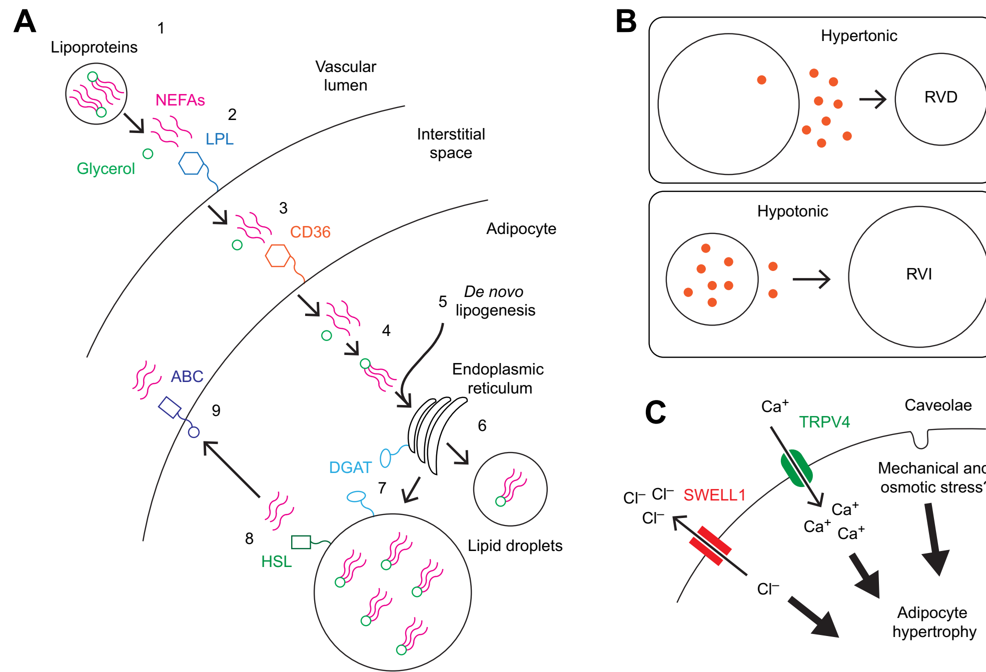

Fig. 3. Schematic illustrating putative mechanisms for the regulation of adipocyte hypertrophy. (A) Lipid accumulation is crucial to adipocyte hypertrophy, and can be split into multiple steps. (1) Circulating lipid in lipoproteins [for example, very low density lipoprotein (vLDL)] can contain triacylglyceride (TAG), and the plasma levels of lipoproteins are associated with adipocyte hypertrophy. (2) Hydrolysis of TAG from lipoproteins is performed by lipoprotein lipase (LPL) [and glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 (GPIHBP1)] on the surface of the adipocytes and vascular endothelial cells, and produces non-esterified fatty acids (NEFAs) and glycerol. The level and activity of LPL are 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. (5) *De novo* lipogenesis within adipocytes also contributes to the TAG pool. (6) Lipid droplets can grow to be large or small depending on the transfer of lipogenic enzymes [e.g. diglyceride acyltransferase (DGAT)] from the endoplasmic reticulum membrane to the lipid droplet (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 active transport mechanisms, including ABC transporters (Tarling et al., 2013). (B) Osmolarity is defined as either hypertonic, which induces regulated volume decreases (RVD), or hypotonic, which induces regulated volume increases (RVI). (C) Schematic of an adipocyte showing three proposed mechanisms that regulate adipocyte hypertrophy. First, the voltage-regulated anion channel (VRAC) SWELL1 (LRCC8A) was shown to regulate adipocyte size through export of chloride ions (Cl−). Second, transient receptor potential cation channel subfamily V member 4 (TRPV4) regulates adipocyte osmotic pressure and cell size. Third, caveolae respond to mechanical and osmotic stress to regulate adipocyte size.

osmosensors and regulators is under intense investigation. Transient receptor potential cation channel subfamily V member 4 (TRPV4) is a $Ca2^{+}$-permeable, non-selective cation channel that is involved in the regulation of osmotic pressure (Harteneck and Reiter, 2007) and is activated by cellular swelling and stretching (Liedtke et al., 2000; Mochizuki et al., 2009; Strotmann et al., 2000; Thodeti et al., 2009). Adipocytes from TRPV4 KO mice do not undergo hypertrophy and undergo increased oxidative metabolism (Ye et al., 2012) (Fig. 3C). In addition, the TRPV4 KO mice were protected from diet-induced obesity, adipose inflammation and insulin resistance (Ye et al., 2012). Thus, TRPV4 promotes adipocyte hypertrophy, and may contribute to the insulin-resistance inherent to hypertrophied adipocytes. Recently, the voltage-regulated anion channel (VRAC) SWELL1 (LRCC8A) was shown to regulate adipocyte size, insulin signaling and glucose homeostasis (Zhang et al., 2017). VRACs export chloride ions (Cl^-) and other small organic osmolytes, and thus generate a hypertonic environment that induces cell shrinkage (RVD) (Jentsch, 2016; Qiu et al., 2014). Zhang et al. (2017) utilized patch-clamp recordings of ionic currents in freshly isolated adipocytes to show that hypertrophic adipocytes exhibit an increased ‘swell-activated’ Cl^- current when compared with smaller adipocytes (Zhang et al., 2017). Furthermore, the increased current observed in hypertrophied adipocytes was dependent on SWELL1. Although activation of VRACs has generally been shown to induce RVD and decrease cell volume (Jentsch, 2016), Zhang et al. (2017) propose that SWELL1-mediated expansion acts as a feed-forward amplifier for further adipocyte hypertrophy (Fig. 3C). Furthermore, SWELL1 knockout (KO) adipocytes were insulin resistant with reduced GLUT4 translocation to the adipocyte plasma membrane after stimulation with insulin (Zhang et al., 2017). This effect was found to be mediated by PI3K–AKT signaling, and SWELL1 KO adipocytes had reduced phosphorylation of AKT (Zhang et al., 2017). Thus, taken together, these data suggest that osmosensing is active in adipocytes during hypertrophy, and may modulate adipocyte hypertrophy and insulin sensitivity. In addition to ion-channel osmosensors, the adipocyte plasma membrane contains abundant caveolae, small flask-shaped invaginations of the plasma membrane that are enriched in cholesterol and sphingolipids, which disassemble in response to osmotic and mechanical stress (Sinha et al., 2011). Caveolae are present at a high density in cells that experience mechanical stress, and cover ∼30% of the adipocyte surface (Le Lay et al., 2015). The formation of caveolae is driven by the assembly of three distinct caveolin proteins (Cav1–3), and the deletion of individual Cav genes leads to loss of caveolae (Le Lay and Kurzchalia, 2005). Caveolae mediate the response of several cell types to mechanical stress (Boyd et al., 2003; Sedding et al., 2005) and, intriguingly, loss of caveolae induces lipodystrophy in mice and humans (Kim et al., 2008; Razani et al., 2002). Furthermore, overexpression of *Cav1* in adipocytes not only

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induced an increase in the density of caveolae but also stimulated the accumulation of larger LDs (Fig. 3C) (Briand et al., 2014). No role for caveolae as osmosensors or regulators during adipocyte hypertrophy is known, but it is clear that caveolae play an essential role in lipid storage fluctuations in adipocytes.

Conclusions

At a population level, adipose morphology is highly varied, genetically determined and associated with cardiometabolic disease susceptibility. Nevertheless, the precise genetic determinants of adipose morphology are largely unknown. In this Review, we present strong evidence from the literature to show that a hypertrophic morphology, in which adipose tissues are characterized by few but large adipocytes, is associated with a range of metabolic perturbances, including changes to plasma glucose, lipid and insulin levels, insulin resistance and susceptibility to disease. Upon reviewing the evidence on whether distinct morphologies have unique transcriptomic signatures, we identify that hypertrophic morphology is characterized by a pro-inflammatory expression profile across multiple studies and methodologies. Finally, we explore some intriguing cellular mechanisms that are predicted to regulate adipocyte cell size and morphology, including (1) how the phospholipid monolayer covering lipid droplets regulates their growth and (2) how osmolarity sensing in adipocytes can stimulate hypertrophy.

Competing interests
The authors declare no competing or financial interests.

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