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REVIEW

Heterogeneity of adipose tissue in development and metabolic function

Theresa Schoettl[1,2,*], Ingrid P. Fischer[1,2,3,*] and Siegfried Ussar[1,2,‡]

ABSTRACT

Adipose tissue is a central metabolic organ. Unlike other organs, adipose tissue is compartmentalized into individual depots and distributed throughout the body. These different adipose depots show major functional differences and risk associations for developing metabolic syndrome. Recent advances in lineage tracing demonstrate that individual adipose depots are composed of adipocytes that are derived from distinct precursor populations, giving rise to different populations of energy-storing white adipocytes. Moreover, distinct lineages of energy-dissipating brown and beige adipocytes exist in discrete depots or within white adipose tissue depots. In this Review, we discuss developmental and functional heterogeneity, as well as sexual dimorphism, between and within individual adipose tissue depots. We highlight current data relating to the differences between subcutaneous and visceral white adipose tissue in the development of metabolic dysfunction, with special emphasis on adipose tissue expansion and remodeling of the extracellular matrix. Moreover, we provide a detailed overview of adipose tissue development as well as the consensus and controversies relating to adult adipocyte precursor populations.

KEY WORDS: Adipose tissue, Heterogeneity, Metabolic syndrome

Introduction

A sedentary lifestyle paired with the continuous availability of high caloric food options has initiated a pandemic of obesity, with higher prevalence in women than in men, and its associated co-morbidities such as cardiovascular disease, certain types of cancer and type 2 diabetes (GBD 2015 Obesity Collaborators et al., 2017; WHO fact sheet: Obesity and overweight 2016; www.who.int/mediacentre/factsheets/fs311/en/). White adipose tissue (WAT) is the primary site for energy storage and plays a role in thermal insulation as well as in protection from mechanical damage (Frayn et al., 2003). Moreover, since the discovery of the hormone leptin in the 1990s (Zhang et al., 1994), adipose tissue has been recognized as an important endocrine organ, secreting a large number of bioactive peptides (adipokines) and other metabolites (Blüher and Mantzoros, 2015; Fasshauer and Blüher, 2015; Kershaw and Flier, 2004; Samad et al., 2011; Trujillo and Scherer, 2006), thereby regulating whole body energy and glucose homeostasis (Ouchi et al., 2011; Sell et al., 2012). Thus, adipose tissue is crucial to balance fluctuations in food availability and to maintain whole body metabolism. However, an excessive accumulation of triglycerides in adipose tissue can result in impaired adipose tissue function, characterized by altered adipokine secretion, tissue inflammation, and the spillover of lipids into other organs, such as skeletal muscle and liver, as well as local and systemic insulin resistance. These alterations frequently result in the development of metabolic syndrome. Therefore, many pharmacological approaches aim to either reduce adiposity or to maintain adipose tissue function, preventing the development of metabolic syndrome. Over the past years, brown adipose tissue (BAT) has been the focus of much attention. Unlike white fat, BAT dissipates energy through mitochondrial uncoupling and could provide ways to reduce body mass and restore glucose and lipid homeostasis. By contrast, the complexity of WAT has only recently been recognized owing to our increased understanding of the distinct functions of individual WAT depots and of the differences between adipocytes within depots. These findings are substantiated by the fact that not all obese subjects are alike, as the site of energy storage as well as the amount of available BAT are crucial to determining the metabolic outcome of obesity. Gender is an important factor determining the distribution and function of the different adipose depots. Furthermore, to make an already complex subject even more complex, recent studies have suggested that developmental and functional differences in adipocytes within distinct adipose tissue depots influence the overall behavior of these depots. To this end, we provide an overview of the differential roles of individual adipose tissue depots in the regulation of metabolism and a detailed description of the current understanding of adipose tissue depot heterogeneity, as well as the controversies, and its implications for adipose function.

Adipose tissue depots

Essentially every animal species has evolved to store energy in the form of triglycerides. Generally, adipose tissue can be differentiated as either WAT or BAT, which are morphologically and functionally distinct. WAT is mainly composed of unilocular cells, with a high capacity to store triglycerides, whereas BAT contains multilocular adipocytes, which are rich in mitochondria. Most importantly, brown adipocytes dissipate energy in the form of heat upon β-adrenergic stimulation. This process is mediated by uncoupling protein 1 (UCP1), a protein residing in the inner mitochondrial membrane, uncoupling the proton motive force of the respiratory chain, thereby combusting energy as heat (Klingenspor et al., 2008).

In most species, including fish, rodents and humans, adipocytes are mostly organized in anatomically distinct adipose tissue depots, which can be broadly divided into intra-abdominal (around abdominal organs) and subcutaneous (under the skin) depots (Pond, 1992). The primary site of energy storage varies among species, with invertebrates, such as amphibians and reptiles preferentially storing energy intra-abdominally, whereas mammals accumulate fat within the abdomen and subcutaneously (Cinti, 2007; Pond, 1992) (Fig. 1).

[1]JRG Adipocytes and Metabolism, Institute for Diabetes and Obesity, Helmholtz Diabetes Center at Helmholtz Center Munich, 85748 Garching, Germany. [2]German Center for Diabetes Research (DZD), 85764 Neuherberg, Germany. [3]Division of Metabolic Diseases, Department of Medicine, Technische Universität München, 80333 Munich, Germany.
*These authors contributed equally to this work

‡Author for correspondence (siegfried.ussar@helmholtz-muenchen.de)

S.U., 0000-0001-7575-0920

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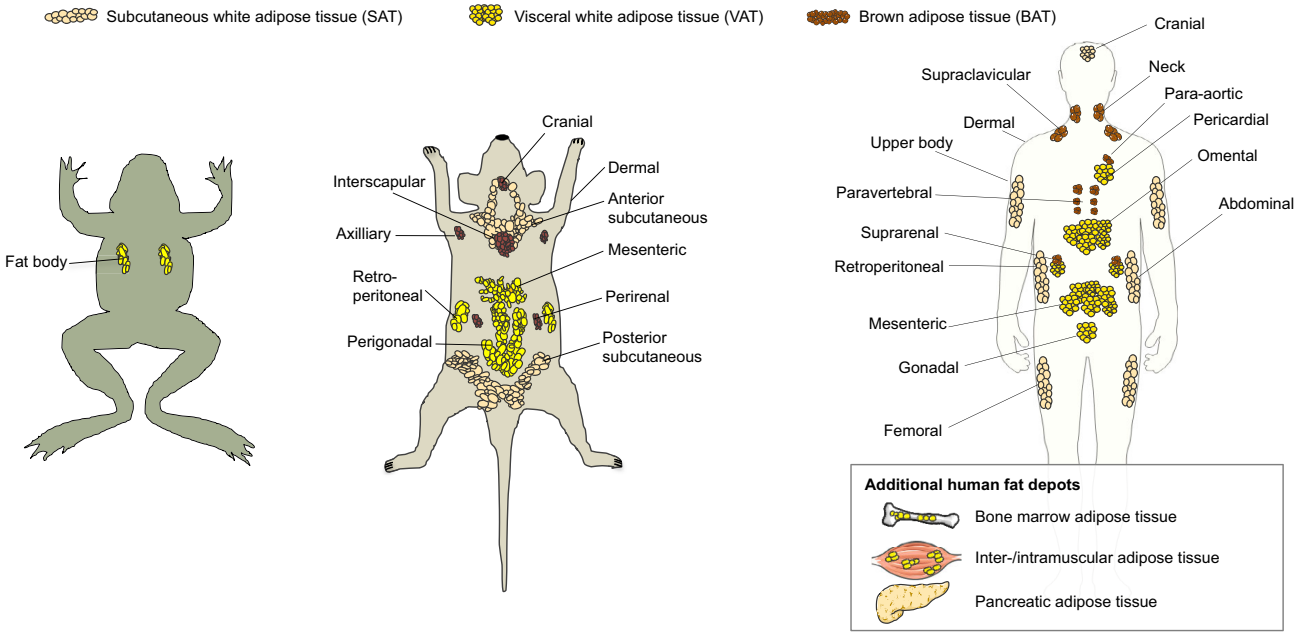

Fig. 1. Adipose tissue depots in different species. Invertebrates as well as reptiles and amphibians store fat in the intra-abdominal cavity as so-called fat bodies. In contrast, mammals such as mice and humans store fat in distinct adipose depots within the abdomen and under the skin (subcutaneous). These adipose compartments can be grouped into subcutaneous white adipose tissue, visceral white adipose tissue, dermal adipose tissue, bone marrow adipose tissue, intermuscular adipose tissue and brown adipose tissue and have individual metabolic functions and risks. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

In humans, the subcutaneous adipose tissue depots (SAT) comprise cranial, facial, abdominal, femoral and gluteal depots and are believed to serve as protection against mechanical damage and heat loss, although recent studies have demonstrated that dermal adipose tissue is primarily responsible for insulation (Alexander et al., 2015; Kasza et al., 2014). Intra-abdominal triglycerides are stored in the omentum as well as retroperitoneally and viscerally (visceral white adipose tissue, VAT), where VAT functions to shield organs. However, the individual ratio of SAT to VAT varies depending on age, sex, nutrition and the energy homeostasis of specific depots as well as individual genetics (Bjørndal et al., 2011; Shungin et al., 2015; Wajchenberg et al., 2002). Meta-analysis identified sexual dimorphism in the genetic basis of fat distribution (Heid et al., 2010; Randall et al., 2013). In the obese state, women typically show an enhanced accumulation of SAT, leading to a so-called pear-shaped body form associated with a lower metabolic risk for type 2 diabetes and cardiovascular diseases. In contrast, men classically accumulate more VAT, resulting in an apple-shape associated with a higher metabolic risk (Gesta et al., 2007).

In mice, SAT includes the posterior subcutaneous (psWAT, including inguinal, dorso-lumbal and gluteal WAT) and anterior subcutaneous white adipose tissues (asWAT). Dermal adipocytes form a stand-alone depot, distinct from SAT (Fig. 1) (Driskell et al., 2014; Festa et al., 2011), with potential functions in hair follicle cycling, wound healing and bacterial infection (Alexander et al., 2015; Festa et al., 2011; Marangoni et al., 2015; Zhang et al., 2015). Further studies are needed to elucidate the metabolic role of dermal adipose tissue.

Murine VAT mainly comprises mesenteric (mWAT), retroperitoneal (rpWAT), perirenal (prWAT) and perigonadal white adipose tissues (pgWAT) (Cinti, 2007; Tran and Kahn, 2010).

In the majority of the above-mentioned adipose depots, white adipocytes are the predominant cell type; however, brown-like adipocytes are found sporadically in the SAT and VAT of mice. BAT depots in rodents and human infants are primarily found in the inter- and subscapular (iBAT/sBAT), axillary (aBAT) and cervical (cBAT) areas (Cinti, 2007; de Jong et al., 2015) (Fig. 1). BAT activity can be increased upon cold exposure and noradrenergic stimulation, and also pharmacologically (Giordano et al., 2016) and by fasting (Migliorini et al., 1997). In humans, active BAT was believed to be only present in the neck and shoulder of newborns and small children, where its function was attributed to be maintenance of body temperature upon cold exposure to compensate for the lack of shivering-thermogenesis (Lean, 1989). However, in 2009, multiple studies observed the presence of metabolically active BAT in adult humans using ^{18}F-fluorodeoxyglucose-positron emission tomography-computed tomography scans (Cypess et al., 2009; Van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Human BAT is found in the anterior neck to the thorax (Cypess et al., 2009); the highest levels of activity are found in the paracervical and supraclavicular regions (Van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009) (Fig. 1). However, the amount of detectable active BAT in humans varies depending on age, body mass and season (Lee et al., 2010; Wang et al., 2015), and shows higher prevalence in young, lean women than in men (Lee et al., 2010).

As briefly mentioned above, batches of brown-like adipocytes can accumulate in WAT upon cold exposure, noradrenergic and various other endocrine stimuli (Harms and Seale, 2013). These brown-like adipocytes are termed beige or brite (‘brown in white’) (Seale et al., 2008; Wu et al., 2012) adipocytes. Even though beige adipocytes have characteristics of brown adipocytes, such as the same morphology, and express most of the brown adipocyte-specific genes, such as UCP1, Cidea, Pgc1α and PAT2 (Harms and Seale, 2013; Ussar et al., 2014), beige and brown adipocytes appear to develop from different embryonic precursor populations (Seale et al., 2008) with a unique gene expression signature (Ussar et al., 2014; Xue et al., 2007). However, these findings are controversial because different research groups have reached different

conclusions regarding the similarities and differences between brown and beige adipocytes (Harms and Seale, 2013; Kajimura et al., 2015; Lee et al., 2017b; Waldén et al., 2012). The development and function of BAT has been discussed extensively elsewhere (Cannon, 2004; Kajimura et al., 2015), therefore, here, we focus on the developmental and functional heterogeneity of WAT depots and white adipocyte populations.

Metabolic risk is associated with individual adipose tissue depots

In 1981, the paradoxical phenomenon of individuals who are lean but show obesity-related perturbations, such as hyperinsulinemia and hypertriglyceridemia, was described for the first time by Ruderman and colleagues (Ruderman et al., 1981). Since then, these so-called subgroups of 'metabolically obese' patients have been studied: the accumulation of fat in VAT has been shown to contribute to metabolic disturbances observed in normal-weight individuals (Dvorak et al., 1999; Ruderman et al., 1998). A second phenomenon describing the opposite state of 'obese but metabolically healthy' individuals is associated with high fat mass but unimpaired insulin sensitivity, lipid and inflammatory profiles (Primeau et al., 2011; Stefan et al., 2008). Although the mechanisms underlying this favorable obesity phenotype are still not completely understood, preferential accumulation of fat in lower subcutaneous depots, such as those detected in women, positively correlates with healthy obesity (Kim et al., 2016; Primeau et al., 2011).

A number of different hypotheses have been developed to understand the mechanisms underlying these different risk associations. The different anatomical locations of VAT and SAT form the basis of the 'portal theory', which states that VAT, especially omental fat, secretes metabolites and adipokines into the portal vein, facilitating direct delivery to the liver (Björntorp, 1990). Thus, functional alterations in VAT would directly impact on hepatic function and contribute to overall higher (cardio)-metabolic risk (Wajchenberg et al., 2002). However, the observed improvement of glucose metabolism after a combination of VAT removal and bariatric surgery (Thörne et al., 2002) was predominantly caused by bariatric surgery rather than VAT removal (Andersson et al., 2014; Lima et al., 2013). In addition, removal of SAT also showed little effects on metabolism (Mohammed et al., 2008; Seretis et al., 2015). Furthermore, mice and rats preferentially accumulate fat in pgWAT, unlike humans, which accumulate fat in omental adipose tissue, which further complicates the mechanistic analysis of this hypothesis. This difference is important because even though pgWAT, unlike omental fat, does not have direct access to the portal vein, the accumulation of pgWAT also results in metabolic disturbances in mice. Moreover, studies in mice have shown that transplantation of SAT from lean mice into the visceral cavity has beneficial effects on body weight, glucose metabolism and insulin sensitivity (Tran and Kahn, 2010) in lean (Tran et al., 2008) and obese (Foster et al., 2013; Hocking et al., 2015) mice. However, the transplantation of pgWAT to subcutaneous sites has resulted in either no (Barrera et al., 2012; Tran et al., 2008) or some metabolic advantages (Satoor et al., 2011; Tran and Kahn, 2010). Thus, the accumulation of VAT is more harmful than peripheral, subcutaneous obesity, increasing the risk of the development of non-alcoholic fatty liver disease and type 2 diabetes (Fox et al., 2007). However, the reasons for this difference can only be partially explained, if at all, by the direct access of VAT to the portal vein.

Another anatomical feature of VAT, which is discussed as a risk factor for developing obesity-associated metabolic complications, is the restricted ability of VAT to expand owing to limited space in the abdominal cavity. In lean humans, subcutaneous adipocytes are larger than visceral adipocytes (Pellegrinelli et al., 2016), whereas the opposite is the case in mice (Sackmann-Sala et al., 2012; Schöttl et al., 2015) (Fig. 2). Moreover, SAT fat cells are larger in men than in women; however, this gender effect is diminished in obese subjects (Andersson et al., 2017). However, fat cell numbers in SAT depots are larger in women than in men (Andersson et al., 2017). In humans and mice, initial weight gain results in increased adipocyte cell size (hypertrophy) owing to enhanced triglyceride storage in individual adipocytes (Martinsson, 1969) (Fig. 2). Triglyceride synthesis is facilitated by lipoprotein lipase (LPL)-mediated free fatty acid (FFA) uptake from (very) low-density lipoprotein and chylomicron and *de novo* lipogenesis via insulin-dependent glucose uptake by Glut4. Overall ∼10–15% of ingested glucose is transported into adipocytes (Kahn, 1996; Rosen and Spiegelman, 2006), of which ∼50% is used to synthesize glycerol and fatty acids (Festuccia et al., 2009). Interestingly, both LPL activity, especially in obese men (Boivin et al., 2007), and glucose uptake are higher in VAT than in SAT (Perrini et al., 2008; Veilleux et al., 2009; Virtanen et al., 2002), even though Glut 4 expression is lower (Lefebvre et al., 1998). Moreover, the rate of lipolysis facilitated by a cascade of adipose triglyceride lipase, hormone-sensitive lipase and monoacylglyceride lipase is higher in human VAT than SAT in the steady state (Arner, 1995; Engfeldt and Amer, 1988) and upon β-adrenergic stimulation by catecholamines (Hellmér et al., 1992). Furthermore, women exhibit enhanced sensitivity compared with men (Ramis et al., 2006), mainly owing to differences in the expression of adrenoceptors (Hellmér et al., 1992; Hoffstedt et al., 1997; Ramis et al., 2006; Reynisdottir et al., 1997).

In addition, excessive accumulation of visceral fat induces local insulin resistance, decreasing its sensitivity to the anti-lipolytic effects of insulin (Engfeldt and Amer, 1988; Gastaldelli et al., 2002; Wajchenberg et al., 2002). This further enhances the release of FFA, resulting in a direct correlation of FFA release from VAT with portal FFA concentrations (Nielsen et al., 2004; Votruba and Jensen, 2007) and metabolic dysfunction in obese subjects (Hoffstedt et al., 2010). Thus, in men (Geer and Shen, 2009), elevated postprandial levels of insulin, FFA and triglycerides (Geer and Shen, 2009) parallel the previously mentioned higher visceral adiposity.

In addition to adipocyte hypertrophy, continued weight gain results in increased adipocyte numbers (hyperplasia) (Fig. 2), which some studies have shown occurs primarily in the visceral fat depots of mice (Kim et al., 2014; Wang et al., 2013). Other studies have shown an association between hyperplasia and subcutaneous fat depots (Tchkonia et al., 2013), with a higher capacity for adipogenesis observed in femoral rather than in abdominal subcutaneous depots in humans (Tchoukalova et al., 2010). However, a study by van Beek and colleagues demonstrated that pgWAT in mice has a limited capacity to store lipids, which is reached when mice achieve an average body weight of 40 g (van Beek et al., 2015). A prolonged high-fat diet caused adipocyte cell death in mice, as indicated by the formation of macrophages containing crown-like structures, resulting in ectopic lipid accumulation in the liver and the development of insulin resistance (van Beek et al., 2015). In contrast, psWAT and mWAT show a slower rate of tissue expansion but continue to accumulate lipids for a prolonged time without signs of inflammation (Strissel et al., 2007; van Beek et al., 2015) (Fig. 2).

These data not only indicate that VAT and SAT have different abilities to store lipids but also show that lipid storage cannot be shifted from VAT to SAT upon exceeding its storage capacity. In contrast, reaching the lipid storage capacity of VAT promotes ectopic lipid accumulation outside of adipose tissues, promoting

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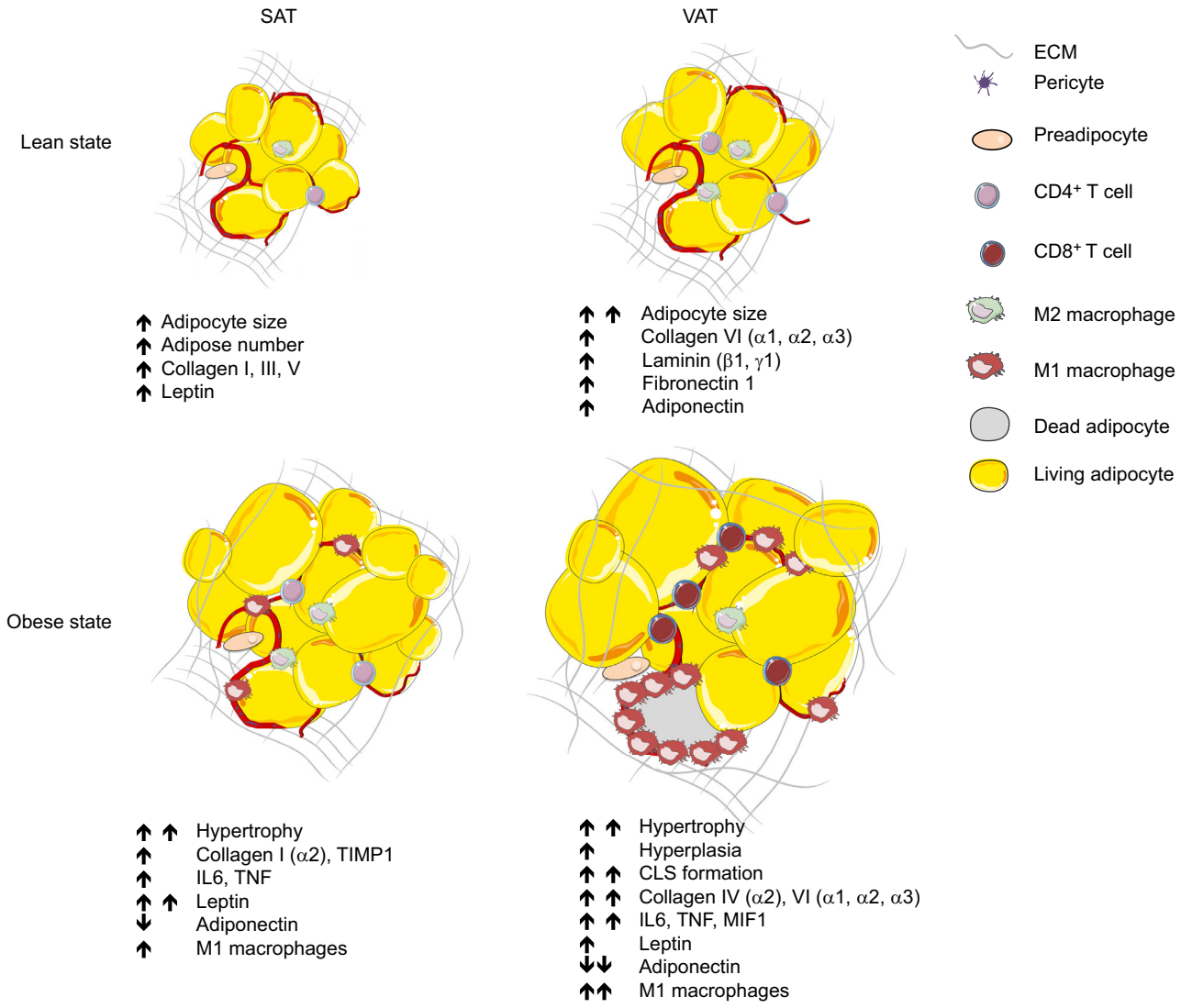

Fig. 2. Heterogeneity of subcutaneous and visceral white adipose tissue depots in lean and obese conditions. In the lean state, subcutaneous white adipose tissue (SAT) is characterized by a smaller cell size but an increased number of adipocytes as well as differential adipokine secretion compared with visceral white adipose tissue (VAT). Weight gain triggers adipocyte hypertrophy and remodeling of the extracellular matrix (ECM), with the cell size of VAT adipocytes increasing more rapidly. This is associated with reduced adiponectin expression in VAT. Expansion of VAT is accompanied by increased cytokine [interleukin 6 (IL-6) and tumor necrosis factor (TNF)] secretion, which can fuel crown-like structure (CLS) formation, and augmented hypoxia-inducible factor 1α expression to promote angiogenesis. Excessive VAT expansion triggers proinflammatory cytokine expression as well as the recruitment of immune cells. In SAT, hypertrophy results in lower levels of expression of proinflammatory cytokines and less M1 macrophage infiltration. Collagen I and metalloproteinase inhibitor 1 (TIMP1) are increased and contribute to a 'healthier' expansion of the tissue. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

insulin resistance and glucose intolerance. Moreover, we and others have reported that even after weight loss, a history of obesity leaves a specific inflammatory fingerprint within pgWAT, together with persistent hypertrophy (Fischer et al., 2017), macrophage infiltration and secretion of pro-inflammatory cytokines (Schmitz et al., 2016; Zamarron et al., 2017), whereas there is little difference between the psWAT of obese mice and that of lean mice. This suggests that factors intrinsic to adipose tissue regulate the capacity to store lipids and to maintain adipocyte function. Thus, it is important to understand the molecular changes in VAT and SAT upon weight gain to fully understand the differential risk association.

Beyond VAT and SAT – other white adipose tissue depots

In addition to the major WAT depots, multiple other smaller depots exist, with distinct functions and metabolic disease association. Among these, the best-studied depots are inter-/intramuscular adipose tissue (IMAT), bone marrow adipose tissue (BMAT) and pancreatic fat.

IMAT is found beneath the fascia of skeletal muscle (Gallagher et al., 2005) and includes adipocytes between muscle groups (intermuscular), as well as adipocytes located between muscle fibers (intramuscular) (Addison et al., 2014; Gallagher et al., 2005; Karampinos et al., 2012) (Fig. 1). IMAT accounts for ∼2–3% of total body fat in healthy lean women (Gallagher et al., 2005) and increases upon aging (Song et al., 2004), obesity (Gallagher et al., 2005) and physical inactivity and sedentariness (Elder et al., 2004; Gorgey and Dudley, 2007; Manini et al., 2007; Ryan et al., 2011, 2002). Thus, it is not surprising that increased IMAT correlates with an increased risk for metabolic disease (Addison et al., 2014; Boettcher et al., 2009; Goodpaster et al., 2003, 1997; Leskinen

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et al., 2013; Ryan and Nicklas, 1999). Similarly, the appearance of adipocytes in the pancreas has been observed in obese subjects (Lee et al., 2010b; Pinnick et al., 2008). The anatomical location of IMAT (Yim et al., 2007) and pancreatic adipocytes suggests a direct interaction with skeletal muscle or pancreatic islets, respectively. This modulation could occur through variation in blood flow to the muscle (Goodpaster et al., 2000), or through the secretion of proinflammatory cytokines in skeletal muscle (Addison et al., 2014; Beasley et al., 2009) and the pancreas (Catanzaro et al., 2016).

In contrast to IMAT and pancreatic adipocytes, the amount of BMAT does not correlate with total body fat (Di Iorgi et al., 2008; Hardouin et al., 2016; Justesen et al., 2001; Shen et al., 2007), even though a positive correlation between visceral adiposity and vertebral BMAT has been reported for obese premenopausal women (Bredella et al., 2011). Moreover, BMAT is not catabolized during acute starvation but increases during conditions of prolonged caloric restriction, including anorexia nervosa (Abella et al., 2002; Bredella et al., 2009; Cawthorn et al., 2016; Devlin et al., 2010; Ecklund et al., 2010; Moyer-Mileur et al., 2008). The metabolic role of BMAT is not well understood; however, aging-associated increases in BMAT result in reduced bone mineral density (Griffith et al., 2012; Kugel et al., 2001). Moreover, Ambrosi and colleagues recently showed that increased BMAT during aging and obesity negatively impacts on hematopoiesis and bone regeneration owing to a redirection of precursor populations toward the adipogenic lineage (Ambrosi et al., 2017).

Remodeling of the ECM is a prerequisite for adipose tissue expansion

The expansion of adipose tissue depends on the remodeling of the extracellular matrix (ECM) to provide sufficient space for the enlargement of adipocytes and the formation of new blood vessels to prevent hypoxia (Sun et al., 2011). The formation of new blood vessels is crucial for the healthy expansion of adipose tissue because failure to do so results in adipocyte death and the development of metabolic syndrome (Sun et al., 2011, 2013). Moreover, as discussed further below, blood vessels are thought to provide a source of adipocyte precursor populations, which is crucial for adipogenesis and adipose tissue hyperplasia (Fig. 3). The exact molecular mechanisms regulating neovascularization in adipose tissue have been extensively reviewed (Cao, 2007, 2013; Corvera and Gealekman, 2014); however, the role played by the ECM and cellular receptors, such as integrins, in the differential function and disease association of adipose tissue depots is less well understood.

The ECM is a complex structure consisting of a tissue-specific assortment of proteoglycans, polysaccharides, different types of collagens, elastins, fibronectins and laminins, each with a specific structural function and binding ability to cellular adhesion receptors (Leiss et al., 2008; Mariman and Wang, 2010; Schiller and Fässler, 2013). The ECM serves as a reservoir for various growth factors, such as bone morphogenetic proteins (Margoni et al., 2012; Schulz and Tseng, 2009), fibroblast growth factors (Ohta and Itoh, 2014) and other factors (Muruganandan et al., 2009) known to modulate adipocyte function. Thus, the ECM plays a crucial role in regulating the biophysical properties of tissues and in regulating intracellular signal transduction, either directly via binding to integrins and other proteoglycans or indirectly via regulation of the local microenvironment comprising growth factors, as well as other metabolites and macro- and micronutrients. Although the role of the ECM in regulating these latter factors has not been well studied, extracellular calcium is known to play an important role in regulating adipocyte differentiation and lineage commitment, most likely through the modulation of cell surface receptors such as integrins (Pramme-Steinwachs et al., 2017).

Fibronectin and collagens are the most abundant proteins of the ECM; however, the exact composition of the ECM in adipose tissue changes during development and is different between depots (Kubo et al., 2000; Mariman and Wang, 2010; Mori et al., 2014). Subcutaneous adipocytes of adult Wistar rats express high levels of type I, III and V collagen, whereas visceral adipocytes express high levels of collagen IIIIa1, laminin β1 (lamb1), laminin subunit γ1 (lamc1) and fibronectin 1 (FN1) (Mori et al., 2014) (Table 1).

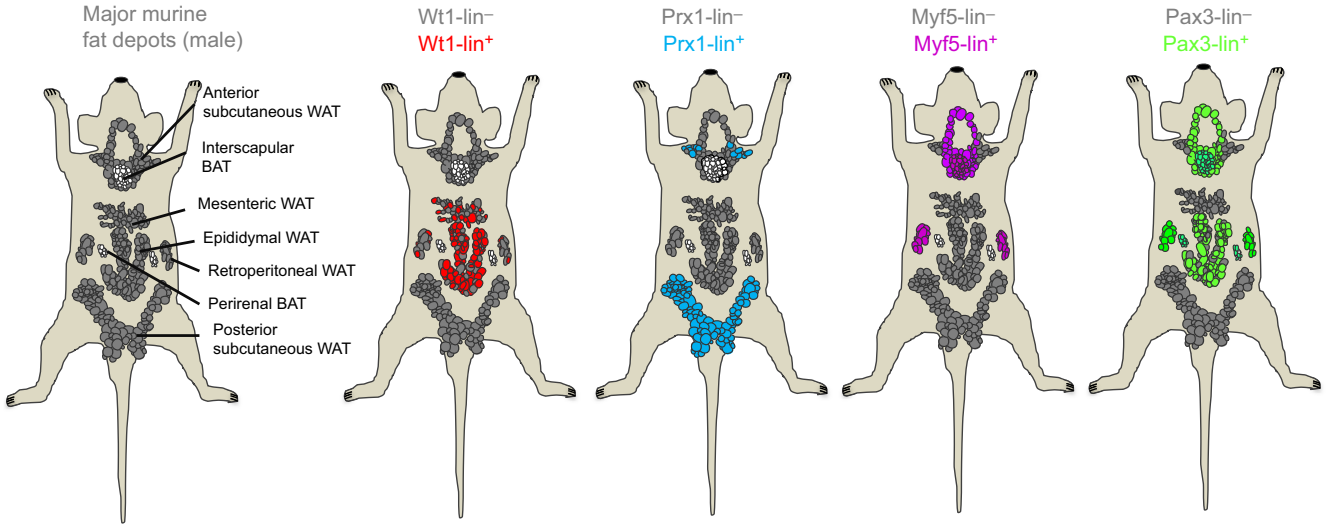

Fig. 3. Different precursor populations for adipose tissue development. White adipocytes located in the visceral cavity derive from cells expressing Wt1 during embryonic development. Heterogeneity also exists within visceral white fat, with different proportions of Wt1-lin$^+$ cells in distinct visceral fat depots. Subcutaneous adipocytes are Wt1-lin$^-$. Adipocytes of subcutaneous but not visceral white fat depots largely derive from progenitors expressing Prx1. Myf5-lin$^+$ adipocytes are located in both brown adipose tissue (BAT) and white adipose tissue (WAT) depots of the dorsal–anterior body region. Similar to Myf5, Pax3-lin$^+$ adipocytes are located in BAT and WAT depots of the dorsal–anterior body region. Myf5, myogenic factor 5; Pax3, paired box 3; Prx1, paired related homeobox 1; Wt1, Wilms tumor 1. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

Table 1. Expression and secretion pattern of extracellular matrix components in human and rat subcutaneous (SAT) and/or visceral white adipose tissue (VAT)

Name	Condition	Type	Species	References
Higher expression/secretion in SAT				
Collagen I (*COL1*)	Lean tissue	Expression	Rat	Mori et al., 2014
Collagen III (*COL3*)	Lean tissue	Expression	Rat	Mori et al., 2014
Collagen V (*COL5*)	Lean tissue	Expression	Rat	Mori et al., 2014
Collagen type I α2 chain (*COL1A2*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Collagen VI subunit α3 (*COL6A3*)	Lean and obese tissue	Expression	Human	McCulloch et al., 2015
Higher expression/secretion in VAT				
Laminin β-subunit 1 (*Lamb1*)	Lean tissue	Expression	Rat	Mori et al., 2014
Laminin γ-subunit 1 (*Lamc1*)	Lean tissue	Expression	Rat	Mori et al., 2014
Fibronectin 1 (*Fn1*)	Lean tissue	Expression	Rat	Mori et al., 2014
Basement membrane-specific heparin sulfate proteoglycan core protein (*HSPG2*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Laminin subunit α4 (*LAMA4*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Collagen α3 (VI) chain (*COL6A3*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Versican core protein (*VCAN*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Collagen α2 (VI) chain (*COL6A2*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Collagen α1 (VI) chain (*COL6A1*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Laminin subunit γ1 (*LAMC1*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Exclusive expression/secretion in VAT				
Collagen α1 (XVIII) chain (*COL18A1*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015
Collagen α2 (IV) chain (*COL4A2*)	Obese tissue	Secretion	Human	Roca-Rivada et al., 2015

Biopsies from adipose tissues of obese human subjects have revealed specific expression of all subunits of collagen VI (α1, α2, α3) from VAT, whereas collagen I and metalloproteinase inhibitor 1 (TIMP1) were specifically expressed in SAT (Roca-Rivada et al., 2015) (Table 1).

Nevertheless, most of these studies focused on the expression patterns of individual ECM components without studying their function in adipose tissue. Grandl and colleagues demonstrated that adipose depot-specific differences in the ECM regulate the differentiation of adipocyte precursor populations (Grandl et al., 2016). They showed that the reduced differentiation capacity of stromal vascular cells from VAT (Lee et al., 2013c) is primarily mediated by the composition of the ECM because de-cellularized ECM from subcutaneous preadipocytes restored the differentiation capacity of visceral preadipocytes (Grandl et al., 2016). Furthermore, these findings suggest that adipogenesis *in vivo* could be regulated by local rearrangements of the ECM that allow specific precursor populations to differentiate while inhibiting others. Moreover, collagen-related proteins such as endotrophin or lysyl oxidase (LOX) are tightly connected to adipose tissue fibrosis and dysfunction. Endotrophin is the cleaved C5 product of collagen VI and XVIII (Sun et al., 2013) and its overexpression is associated with upregulation of proinflammatory and profibrotic genetic programs, as well as with the downregulation of lipolysis, fueling hypertrophy and hyperplasia in the pgWAT of diet-induced obese mice (Sun et al., 2014; Zhao et al., 2016). LOX is a known hypoxia-inducible factor 1α (HIF1α) transcriptional target and contributes to the extracellular tensile strength by cross-linking collagens and elastins (Halberg et al., 2009). LOX is upregulated in hypertrophic, obese states of the adipose tissue where it increases fibrillar collagen accumulation; pharmacological inhibition significantly restores local tissue insulin sensitivity (Halberg et al., 2009). These are just two examples of how collagen-related proteins contribute to adipose tissue dysfunction; see Mariman and Wang (2010) for a more detailed overview.

Even though these initial studies suggest that the ECM has important functions within different adipose tissues, most of the conclusions are correlative and definitive proof is still lacking. Moreover, little to nothing is known about the role of adhesion receptors in adipose tissue. Of relevance, Saltiel and colleagues reported that a switch from α5- to α6-containing integrins is important for adipocyte differentiation in 3T3L1 cells (Liu et al., 2005). We previously described the cell surface proteoglycan glypican-4 (Gpc4) as differentially expressed between adipose tissue depots and to be highly regulated by obesity. Moreover, we have demonstrated that Gpc4, at least *in vitro*, binds to the unliganded insulin receptor and modulates its affinity toward insulin (Ussar et al., 2012). These data highlight the potential role of the ECM and adhesion receptors in directly influencing adipocyte signal transduction and function. This is further supported by a study performed by Khan and colleagues showing that obesity is associated with increased collagen VI expression, and that ablation of collagen VI increases adipocyte expansion while reducing macrophage infiltration into adipose tissue of obese mice, followed by improved systemic metabolic health (Khan et al., 2009). Thus, depot-specific differences in the ECM could contribute to the differential inflammatory state of VAT versus SAT upon excessive weight gain.

Adipose depot differences in inflammation and adipokine secretion

Local inflammation and insulin resistance in adipose tissue, together with deregulated release of FFA to the liver and skeletal muscle are the events initiating obesity-associated insulin resistance and metabolic syndrome. Adipose tissue expansion triggers hypoxia, which results in HIF1-α-mediated upregulation of pro-inflammatory cytokines such as interleukin 6 (IL-6) and macrophage inflammation factor (MIF1) (Sun et al., 2013). However, this initial low-grade inflammation appears to be important to orchestrate immune cell-mediated ECM remodeling, allowing blood vessel density to increase and restore normoxia (Sun et al., 2011; Wernstedt Asterholm et al., 2014) (Fig. 2). Yet, if this inflammatory response is not tightly controlled, or combined with other inflammatory stimuli such as adipocyte death, this results in a detrimental metabolic outcome. VAT exhibits a more pro-inflammatory cytokine expression profile compared with SAT in both humans and mice, including IL-6, interleukin 8 (IL-8),

monocyte chemotactic protein1 (MCP-1), RANTES, macrophage inflammatory protein 1 alpha (MIP-1a) and plasminogen activator inhibitor 1 (PAI-1) (Alvehus et al., 2010; Kwok et al., 2016; Lee et al., 2013c), which is further enhanced in obese subjects (Ouchi et al., 2011) (Fig. 2). This is accompanied by elevated numbers of proinflammatory type 1 macrophages (M1 macrophages), $CD8^+$ T cells and natural killer cell populations in VAT, further supporting the notion that VAT inflammation contributes to the detrimental metabolic effects of central obesity (Fig. 2).

In addition to the secretion of cytokines, adipocytes secrete a number of hormones that regulate not only immune functions within adipose tissue but also key metabolic functions in other organs. The most notable members of this family are leptin and adiponectin. There is a vast number of reviews describing the individual adipokines, their regulation and functions (Blüher and Mantzoros, 2015; Fasshauer and Blüher, 2015; Ouchi et al., 2011). However, in the context of this review, most adipokines are not secreted in equal amounts from individual adipose tissue depots of different genders. Most leptin is secreted by females in SAT (Geer and Shen, 2009; Hube et al., 1996; Lefebvre et al., 1998), whereas, adiponectin expression and secretion is threefold higher in VAT than in SAT, both in mice and humans, with higher expression in females than in men (Fain et al., 2004; Geer and Shen, 2009; Perrini et al., 2008; Skurk et al., 2007) (Fig. 2). Importantly, plasma adiponectin levels negatively correlate with VAT mass (Ryo et al., 2004), suggesting that the obesity-associated decline in adiponectin could contribute to the detrimental effects of excessive VAT accumulation on whole body metabolism. The majority of the gender differences can be attributed to differential sex hormone secretion: for example, the absence or decrease in estrogen is tightly associated with the onset of insulin resistance in men and women (Geer and Shen, 2009). However, androgens such as testosterone also have gender-specific effects on AT metabolism and insulin resistance, which are discussed in detail elsewhere (Geer and Shen, 2009).

Expression of developmental genes differs between adipose tissue depots

Cellular physiology, metabolic properties and metabolic risks vary between subcutaneous and visceral fat, as well as among some visceral fat depots (Edens et al., 1993; Fried et al., 1993; Tchkonia et al., 2007). However, the molecular and genetic basis for these differences remains poorly understood. Many studies on human and rodent adipose tissues have revealed differences in gene expression between anatomically distinct fat depots (Linder et al., 2004; Montague et al., 1998; Palou et al., 2009; Perrini et al., 2008; Tchkonia et al., 2007; van Beek et al., 2007; Vohl et al., 2004; Wu et al., 2008). However, determining whether these differences in gene expression are the cause or consequence of the functional differences has been a challenge. To overcome this limitation, we and others have focused on the differential expression of genes with important roles during embryonic development and preserved expression between preadipocytes and adipocytes (Gesta et al., 2006). The underlying hypothesis is that this set of genes could provide novel insights into the developmental differences between WAT depots. Table 2 provides an overview of developmental genes that are differentially expressed among adipose tissue depots in rodents and humans.

Table 2. Different expression of developmental/pattern genes in subcutaneous versus visceral/epicardial white adipose tissue or isolated adipocytes

Gene symbol	Gene name	Fold change	*P*-value	Species	References
Higher expression in subcutaneous versus visceral adipocytes/adipose tissue					
Tbx15	T-box 15	12.26	0.0012	Mouse	Gesta et al., 2006
Shox2	Short stature homeobox 2	8.71	0.0004	Mouse	Gesta et al., 2006
En1	Engrailed 1	7.96	0.0128	Mouse	Gesta et al., 2006
Sfrp2	Secreted frizzled-related sequence protein 2	5.37	0.0405	Mouse	Gesta et al., 2006
Hoxc9	Homeobox C9	1.76	0.0042	Mouse	Gesta et al., 2006
HOXC10	Homeobox C10	12.50	<0.001	Human	Brune et al., 2016
HOXC9	Homeobox C9	7.1	<0.001	Human	Brune et al., 2016
PAX3	Paired box 3	3.30	<0.0001*	Human	Lau et al., 2011
IRX5	Iroquois homeobox 5	2.97	<0.0001*	Human	Lau et al., 2011
EMX2	Empty spiracles homeobox 2	2.65	<0.0001*	Human	Lau et al., 2011
HOXC9	Homeobox C9	2.56	<0.0001*	Human	Lau et al., 2011
HOXA10	Homeobox A10	2.50	<0.0001*	Human	Lau et al., 2011
IRX3	Iroquois homeobox 3	2.49	0.0003*	Human	Lau et al., 2011
IRX1	Iroquois homeobox 1	2.48	0.0007*	Human	Lau et al., 2011
HOXB7	Homeobox B7	2.45	0.0001*	Human	Lau et al., 2011
HOXA9	Homeobox A9	2.39	0.0001*	Human	Lau et al., 2011
HOXC6	Homeobox C6	2.30	0.0011*	Human	Lau et al., 2011
SHOX2	Short stature homeobox 2	1.97	0.0260*	Human	Lau et al., 2011
HOXC10	Homeobox C10	1.79	0.0150*	Human	Lau et al., 2011
Higher expression in visceral versus subcutaneous adipocytes					
Nr2f1	Nuclear receptor subfamily 2, group F, member 1	5.45	0.0057	Mouse	Gesta et al., 2006
Thbd	Thrombomodulin	2.60	0.0094	Mouse	Gesta et al., 2006
Hoxa5	Homeobox A5	1.92	0.0305	Mouse	Gesta et al., 2006
Hrmt1l2	Heterogeneous nuclear ribonucleoproteins methyltransferase-like 2	1.64	0.0287	Mouse	Gesta et al., 2006
Hoxc8	Homeobox C8	1.56	0.0374	Mouse	Gesta et al., 2006
Gpc4	Glypican 4	1.49	0.0102	Mouse	Gesta et al., 2006
Higher expression in epicardial versus subcutaneous adipocytes					
HOXA2	Homeobox A2	2.53	0.0001*	Human	Lau et al., 2011
SATB1	SATB homeobox 1	1.84	0.0149*	Human	Lau et al., 2011

*Rounded values.

Most notably, in humans, a set of homeobox-type transcription factors was identified to be differentially regulated between SAT and VAT (HOXC9, HOXC10, HOXA9, HOXA10, HOXC6, HOXB7 and PAX3) (Brune et al., 2016; Lau et al., 2011; Vohl et al., 2004), as well as between different SAT depots. HOXA2, HOXA3, HOXA4, HOXA5, HOXA9, HOXB7, HOXB8, HOXC8 and IRX2 are downregulated in both adipocytes and stromal vascular cells of the gluteal subcutaneous compared with the abdominal subcutaneous depot. Conversely, HOXA10 is upregulated in gluteal subcutaneous fat, and HOXC13 can only exclusively be detected in this type of SAT (Karastergiou et al., 2013).

Similar to humans, the differential expression of genes involved in development and pattern specification has been reported for murine abdominal fat versus subcutaneous fat. Among these genes, higher levels of Nr2f1, Gpc4, Thbd, HoxA5 and HoxC8 are expressed in intra-abdominal eWAT versus subcutaneous flank/psWAT, whereas higher levels of Tbx15, Shox2, En1, Sfrp2 and HoxC9 are expressed in cells of flank/psWAT (Candille et al., 2004; Deschamps and van Nes, 2005; Gehring, 1987; Gesta et al., 2006; Ussar et al., 2012; Yu et al., 2005). T-box protein 15 (Tbx15) regulates adipocyte differentiation and triglyceride accumulation (Gesta et al., 2011) and Gpc4 enhances both insulin receptor signaling and adipocyte differentiation (Ussar et al., 2012). Recently, Lee and colleagues have shown that Tbx15 regulates a switch from oxidative to glycolytic metabolism and marks a glycolytic subpopulation of adipocytes (Lee et al., 2017a). Moreover, Shox2 is highly expressed in cells of flank/psWAT compared with pgWAT, and controls lipolysis rates, orchestrating at least some of the differences between subcutaneous and visceral adipocytes (Lee et al., 2013b). However, additional studies are needed to fully explore the role of these genes in adipose tissue identity.

Interestingly, Shox, Hox and Tbx genes play an important role in anterior–posterior and dorsal–ventral axial formation during embryonic development (Candille et al., 2004; Deschamps and van Nes, 2005; Gehring, 1987; Gesta et al., 2006; Yu et al., 2005). This suggests that some or most of these genes could resemble remnants of embryonic development, pointing to their developmental origin rather than directly mediating functional differences between depots. Indeed, a gradient of Tbx15 and En1 expression has been observed, with high expression in flank, interscapular and perirenal depots and low expression in ventrally located mWAT and pgWAT (Yamamoto et al., 2010). Thus, the differential expression of developmental genes could suggest distinct developmental origins of individual fat depots (Gesta et al., 2006).

Spatiotemporal differences in adipocyte commitment and differentiation

An understanding of adipose tissue/adipocyte heterogeneity is key to understanding the developmental origin of adipose tissues (Sanchez-Gurmaches and Guertin, 2014a). In humans, both visceral and subcutaneous adipose depots are well developed at birth (Feng et al., 2013; Poissonnet et al., 1984). Serial paraffin sections of human fetuses aborted between gestational week 1 and 42 revealed that WAT first appears in week 14.5 in specific parts of the head and neck and that adipocytes differentiate within the abdominal cavity. This is followed by adipogenesis in the mammary and perirenal regions in week 15 and in the lower and upper limbs thereafter. At the end of gestational week 23, SAT development is completed; at approximately gestational week 28, adipocytes are present in the main adipose tissue deposit areas (head, neck, thorax, abdomen, upper limb and lower limb) (Poissonnet et al., 1984).

In rodents, WAT is undetectable macroscopically during embryogenesis, obstructing research on this topic until recently (Billon et al., 2007). Genetic lineage tracing in transgenic mice finally uncovered the spatiotemporal control of the commitment and differentiation of adipocytes in murine adipose depots (Birsoy et al., 2011, 2008; Sanchez-Gurmaches and Guertin, 2014a; Wang et al., 2013). The first adipocyte precursors are detectable at embryonic day 10.5 in a defined region of the mesenteric dorsal edge, which later develops into SAT (Hudak et al., 2014), with major lineage commitment between embryonic day 14 and embryonic day 18 (Birsoy et al., 2011; Wang et al., 2013). However, unlike in humans, SAT development proceeds after birth and fully matured adipose tissue depots are only present on day 56 postpartum.

In contrast to SAT, VAT formation is primarily initiated after birth (Berry et al., 2014; Han et al., 2011; Wang et al., 2013). First adipocytes within the abdominal cavity appear around postnatal day 1 (P1) in rpWAT, although the cells are smaller than those of completely differentiated, mature adipocytes (Han et al., 2011). Aside from this, undifferentiated adipocyte progenitors are located in a primitive, membrane-like structure around the gonads until P4, with the first lipid-filled adipocytes appearing on P7 (Han et al., 2011) and continued development until P20 and thereafter (Wang et al., 2013).

Together, the timing of adipocyte commitment and differentiation during development varies between distinct adipose depots and species. The process of adipose tissue formation in the human fetus starts at the beginning of the second trimester in the head and neck region, progresses to the trunk, and then progresses to the upper and lower limbs (Poissonnet et al., 1984). Although body fat is already well developed in human newborns, murine adipocytes finish differentiation postnatally and, hence, the developmental stage at birth in mice corresponds to the end of the second trimester in humans.

Subcutaneous and visceral adipocytes have different developmental routes

Adipose tissues are generally believed to arise from the mesoderm, which during embryonic development spreads along the anterio-posterior and dorso-ventral axes to form the axial, paraxial, intermediate and lateral plate mesoderm (Gesta et al., 2007; WJ, 2002). Recent analyses have revealed differences, similarities and overlaps in the developmental origin of individual adipose tissue depots.

One of the first markers identified for populations of visceral adipocytes was Wilms tumor 1 (Wt1) (Chau et al., 2014). Wt1 expression is restricted to the intermediate and lateral plate mesoderm during development, structures that give rise to the omentum, mesentery and the mesothelium lining the organs of the visceral cavity. Consequently, these studies showed that the mesothelium provides a source of adipocyte progenitors for VAT but not SAT (Asahina et al., 2011; Cano et al., 2013; Chau et al., 2014; Rinkevich et al., 2012). These studies also showed that Wt1 positive progenitors are not the sole source of adipocytes because, depending on the individual VAT depot, only 30–80% of adipocytes in adult mice derive from cells that express Wt1 during embryonic development (Chau et al., 2014) (Fig. 3). Although the paraxial mesoderm is commonly accepted as the primary source of brown adipocytes (Atit et al., 2006), the developmental origin(s) of subcutaneous white adipocytes are complex and are still incompletely understood.

Rare forms of human WAT dystrophies, such as Köbberling–Dunnigan syndrome (Anderson et al., 1999; Jackson et al., 1998;

Peters et al., 1998) and congenital infiltrating lipomatosis of the face (D'Souza et al., 2014; Padwa and Mulliken, 2001; Urs et al., 2013), suggest different origins for subcutaneous adipocytes in the lower versus upper body part (Lemos et al., 2012; Sanchez-Gurmaches and Guertin, 2014a). To this end, lineage tracing by expressing the Cre protein (which causes DNA recombination) under the control of the neural crest marker Sox10 (SRY-related HMG-box 10) showed that adipocytes in the head and neck, but not trunk pgWAT and SAT, develop from the neuroectoderm (Billon et al., 2007). Similarly, lineage tracing using Wnt family member 1 (Wnt1)-Cre identified that adipocytes in subcutaneous craniofacial WAT primarily arise from neural crest cells. However, in adults, these adipocytes are progressively replaced by adipocytes of mesodermal origin (Lemos et al., 2012). Thus, both a mesodermal and a neuroectodermal source of progenitors exist for subcutaneous adipocytes in the head region, with mesodermal precursor cells dominating these depots in adults (Billon et al., 2007; Lemos et al., 2012; Sanchez-Gurmaches and Guertin, 2014a). However, the embryonic origin of adipocytes in trunk and limb subcutaneous WAT still needs to be elucidated.

Adipocytes of SAT largely derive from progenitors expressing paired related homeobox 1 (Prx1), a non-clustered homeobox gene with mesenchyme-specific expression during embryogenesis and adulthood (Bergwerff et al., 2000; Calo et al., 2010; Kuratani et al., 1994; Leussink et al., 1995; Shimozaki et al., 2013) (Fig. 3). Mice carrying a Cre-responsive enhanced yellow fluorescent protein (EYFP) allele under the control of the Prx1-promotor (Prx1-Cre; ROSA 26 promotor-driven reporter-EYFP mice) revealed that Prx1-Cre activity is restricted to precursors of subcutaneous iWAT, with little recombination in pgWAT or BAT (Krueger et al., 2014). Studies using Prx1-Cre mice in combination with conditional fluorescence reporters showed little or no Prx1 expression in precursors of VAT (mWAT, pgWAT and rpWAT) and BAT (sBAT and iBAT), whereas axial patterning was observed throughout SAT. Prx1-Cre labeled the vast majority of precursors in psWAT. In contrast, Prx1-Cre labeled some adipocytes in the ventral asWAT but not in the dorsal part, resulting in the labeling of approximately 12–20% of the adipocytes in this fat pad (Sanchez-Gurmaches et al., 2015).

Together, these data point toward an anterior–posterior/dorsal–ventral gradient within subcutaneous adipocytes, with $Prx1^+$-progenitor-derived adipocytes dominating the ventral–posterior body region. Furthermore, these results indicate a major ontogenetic difference between adipocytes of visceral versus subcutaneous WAT (Chau et al., 2014; Sanchez-Gurmaches et al., 2015). It will be interesting to determine to what extent these different adipocyte populations differ functionally. However, this will require a more complete understanding of the developmental origin of all adipocytes present in these depots.

Heterogeneity within adult subcutaneous and visceral fat depots

White adipocytes were thought to arise from a unique, yet poorly characterized myogenic factor 5 negative ($Myf5^-$) mesenchymal lineage, whereas brown adipocytes share a $Myf5^+$ precursor with skeletal muscle cells (Seale et al., 2008). However, this classical model has recently been challenged because Myf5-Cre-driven depletion of the phosphatase and tensin homolog (Pten) caused highly specific overgrowth of some WAT depots, indicating Myf5 promoter activity in WAT. Myf5-regulated Pten expression increased the mass of interscapular, vertebral and retroperitoneal fat depots, which was accompanied by the loss of iWAT, mWAT and pgWAT (Peirce et al., 2014; Sanchez-Gurmaches et al., 2012). Furthermore, lineage tracing in Myf5-Cre;mTmG reporter mice, revealed that almost all adipocytes in asWAT and rpWAT are descendants of $Myf5^+$ precursors, whereas Myf5-Cre does not label any mature adipocytes in pgWAT, mWAT or psWAT (Sanchez-Gurmaches and Guertin, 2014b; Shan et al., 2013) (Fig. 3).

Thus, in addition to brown adipocytes, Myf5 expression has been observed in some visceral and subcutaneous precursor populations. Aside from expression of Myf5, the identification of the individual precursor cells appears even more complex given that Pax3 (paired box 3), which is also a classical myogenic transcription factor, has an overlapping but not identical lineage tracing pattern to that of Myf5. Pax3-Cre labels adipocytes in asWAT and rpWAT, with additional activity in many adipocytes in male, but not female pgWAT (Sanchez-Gurmaches and Guertin, 2014b) (Fig. 3), further pointing toward progenitor heterogeneity among different fat depots, genders and ages. As a result, the existing dogma that white adipocytes arise from $Myf5^-$ progenitors needs to be revised. SAT and VAT are composed of a mixture of Myf5-lin^+ and Myf5-lin^- adipocytes (and/or Pax3-lin^+ and Pax3-lin^- adipocytes, respectively), with Myf5-lin^+ (and/or Pax3-lin^+) adipocytes dominating the dorsal–anterior (asWAT, iBAT, sBAT and rpWAT) depots, and Myf5-lin^- (and/or Pax3-lin^-) adipocytes being more abundant in the ventral-posterior tissues (periaortic BAT, pgWAT, psWAT and mWAT).

Efforts to elucidate the embryonic origin of white adipocyte lineages (Macotela et al., 2012) have led to the identification of developmental differences between adipocytes of anatomically distinct fat depots. Major ontogenetic differences in adipocytes not only exist between SAT, VAT and BAT but also within individual fat depots of the two major types of WAT, pointing toward the existence of different adipocyte lineages. Finally, both gene expression studies and lineage tracing provide evidence for axial patterning of different types of adipocytes during development. Thus, as suggested already by Chau and colleagues, anatomically distinct fat depots should be regarded as 'separate mini organs' (Chau et al., 2014) with heterogeneous adipocyte populations derived from distinct embryonic precursors. However, it remains to be determined whether these different precursor cells indeed give rise to functionally different adipocytes.

Origins of adult adipose tissue progenitor cells

Understanding the developmental origin of adipose tissue is important to understand the principal differences in fat depot function. However, adipose tissue is highly dynamic with a constant turnover of adipocytes and *de novo* differentiation of adipocytes from precursor populations (Lee et al., 2010a). Thus, to explore therapeutic avenues to target individual adipose depots or even adipocyte populations, the source of adipocytes in adult tissues needs to be understood and considered.

Most adipocytes arise from proliferating progenitors that have already been committed into the adipocyte lineage(s) prenatally or in early postnatal life (Tang et al., 2008). In murine abdominal fat, up to 0.6% of the total adipocyte population are replaced by new adipocytes every day, and 4.8% of preadipocytes are constantly replicating (Rigamonti et al., 2011). At the cellular level, adipogenesis occurs in two major phases: first, precursors are committed to preadipocytes, and second, preadipocytes terminally differentiate into mature lipid-laden adipocytes (Rosen and MacDougald, 2006; Rosen and Spiegelman, 2014). Although the latter process has been studied in detail, the origin and identity of the adult white adipocyte progenitor niche remain a subject of debate (Vishvanath et al., 2016).

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Mesenchymal stem cells

Adipocytes have long been thought to derive from tissue-resident mesenchymal stem cells. In 1895, Hammar posited that adipocytes arise from undifferentiated stem cells of a primitive mesenchymal type (Hammar, 1895). Since then, the ability of mesenchymal stem cells to differentiate into adipocytes in culture has been shown repeatedly (Kim et al., 2010; Lim et al., 2011; Qian et al., 2010; Scott et al., 2011; Styner et al., 2010; Valorani et al., 2010; Welter et al., 2013) because sets of mesenchymal markers can be used to enrich adipogenic precursors from the stromal vascular fraction (Berry et al., 2014; Rodeheffer et al., 2008). This was further corroborated by the identification of Pref-1 (preadipocyte factor 1) as a marker for early adipocyte precursor cells (Hudak et al., 2014). However, the niche and exact identity of adipose tissue mesenchymal stem cells is still not completely understood. Moreover, the long-standing dogma that all adipocytes differentiate from mesenchymal progenitors has been challenged over the past years, and additional adipose tissue-resident and non-resident cell types have been suggested as adipocyte progenitors.

During embryonic development, blood vessels are the first structures to form within the developing fat pads (Rosen and Spiegelman, 2014; Wassermann, 1965). This, together with a reported interaction between adipogenesis and angiogenesis (Brakenhielm et al., 2004; Fukumura et al., 2003; Han et al., 2011; Neels et al., 2004; Rupnick et al., 2002), led to the hypothesis that adipocytes could derive from blood vessel-associated cells (Rosen and Spiegelman, 2014).

Pericytes

Light and electron microscopy studies performed in the 1970s suggested that pericytes [perivascular cells enveloping blood capillaries, also referred to as vascular smooth muscle cells, mural cells or Rouget cells (Bergers and Song, 2005; Hirschi and D'Amore, 1996)] of capillaries in rat eWAT could differentiate into preadipocytes (Iyama et al., 1979), which was subsequently confirmed by others (Cinti et al., 1984). This is in line with the above discussed data given that mesenchymal stem cells have been suggested to be a subset of pericytes (Caplan, 2008; Cawthorn et al., 2012; Crisan et al., 2008; da Silva Meirelles et al., 2008; Traktuev et al., 2008) (Fig. 4). Transgenic mice expressing GFP under the control of the α-SMA (α-smooth muscle actin) promoter, a common marker for pericytes, showed that α-SMA^{+}, but not α-SMA^{-} cells, are multipotent *in vitro*, and represent a stem cell population in adipose tissue (Cai et al., 2011; Cawthorn et al., 2012). PdgfRβ (platelet-derived growth factor receptor β), NG2 (neural/glial antigen 2) and α-SMA are known markers for mural cells (Olson and Soriano, 2011; Tang et al., 2008) that were used to show that mural cells express Pparγ (peroxisome proliferator-activated receptor γ) (Tang et al., 2008). A PdgfRβ reporter mouse strain confirmed that adipocyte progenitors indeed derive from mural cells and that these cells are exclusively present in adipose tissue depots (Tang et al., 2008). Further, conditional knock-in mice with activating mutations in the PdgfRβ locus showed that increased PdgfRβ signaling in pericytes affects WAT development during the early days of postnatal life (P3–P13). Despite initial differentiation of some adipocytes, WAT failed to fully develop in PdgfRβ overexpressing mice, with SAT showing reduced lipids but a higher cell density and only a rudimentary mWAT depot (Olson and Soriano, 2011). Therefore, it has been assumed that pericytes represent WAT progenitors, and proper PdgfRβ signaling is required to guarantee progenitor potential (Olson and Soriano, 2011; Tang et al., 2008). However, the importance of PdgfRβ was recently challenged in a report by Onogi and colleagues that indicated that Pdgf signaling is only indirectly involved in adipose tissue expansion via the formation of blood vessels (Onogi et al., 2017).

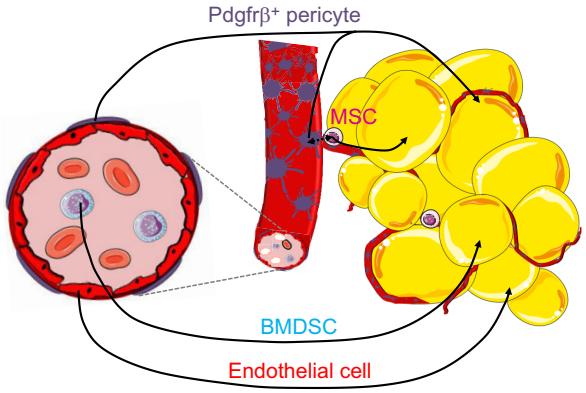

Fig. 4. Adult adipose tissue precursor populations. Tissue-resident mesenchymal stem cells are commonly accepted as adipocyte progenitors. Several alternative adipose tissue-resident and non-tissue-resident progenitors have been described. Adipocytes may also arise from endothelial cells and PdgfRβ^{+} pericytes associated with the adipose vasculature. Pericytes have been suggested as a subset of mesenchymal stem cells (dashed line). Apart from tissue-resident progenitors, bone marrow-derived stem cells, both hematopoietic and mesenchymal, may represent an alternative adipocyte progenitor niche. BMDSC, bone marrow-derived stem cell; MSC, mesenchymal stem cell; PdgfRβ, platelet-derived growth factor receptor-β. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

Nevertheless, additional evidence supporting the differentiation of preadipocytes from pericytes was provided by transgenic mice expressing GFP under the control of the zinc-finger protein 423 (Zfp423) promoter (Gupta et al., 2010, 2012), revealing GFP expression in mural cells. Interestingly, PdgfRβ expression only partially overlapped with GFP expression, indicating that preadipocytes derive from a subset of specialized PdgfRβ^{+} pericytes (Gupta et al., 2012). Recent studies suggest that differentiation from Zfp423^{+} precursors occurs predominantly in pgWAT, a depot where adipocyte hyperplasia substantially contributes to increased tissue mass (Jeffery et al., 2015; Vishvanath et al., 2016; Wang et al., 2013). As indicated above, these data are in line with mesenchymal stem cells giving rise to adipocytes given that similar to mesenchymal stem cells, fluorescence-activated cell scanning (FACS)-sorted pericytes are multipotent, even after long-term passaging in culture (Crisan et al., 2008).

Endothelial cells

Besides mesenchymal stem cells and pericytes, endothelial cells have also been described as white adipocyte progenitors (Fig. 4). Zfp423 expression occurs not only in mural cells but also in a subset of capillary endothelial cells in WAT and BAT, both in the developing and adult state, but not in endothelial cells of other tissues (Gupta et al., 2012). Additional lineage tracing experiments, crossing vascular endothelial cadherin (VE-cadherin)-Cre mice with reporter lines expressing cytoplasmic GFP or β-galactosidase demonstrated that VE-cadherin-Cre marks mature adipocytes. Given that VE-cadherin is a classical endothelial cell marker, this suggests that white adipocytes can also derive from endothelial cells (Tran et al., 2012).

Although an endothelial origin for adipocytes could contradict the work of Seale and colleagues and Sanchez-Gurmaches and

colleagues, describing adipocytes arising from Myf5$^+$ myogenic precursors (Sanchez-Gurmaches and Guertin, 2014b; Seale et al., 2008; Tran et al., 2012), De Angelis and colleagues reported the existence of progenitor cells from embryonic dorsal aorta, expressing both endothelial (including VE-cadherin) and myogenic markers (i.e. Myf5) (De Angelis et al., 1999). Therefore, some adipocytes may arise from a population of VE-cadherin- and Myf5-expressing progenitors (Tran et al., 2012). By contrast, a study involving reporter mice using a switch from membrane-targeted Tomato to GFP expression upon Cre activity excluded the possibility that adipocyte precursors are of endothelial (VE-cadherin-Cre, Tie2-Cre) origin, either during WAT development or obesity (Berry and Rodeheffer, 2013; Hudak et al., 2014). However, the high-vessel density of WAT and the tight association of capillaries with mature adipocytes could complicate the interpretation of these imaging studies because adipocytes completely surrounded by GFP$^+$ vasculature could be misinterpreted as GFP$^+$ themselves (Berry and Rodeheffer, 2013). Hence, based on the available experimental data, the contribution of endothelial cells to the adipogenic cell niche can currently neither be confirmed, nor excluded.

Non-resident progenitors – a hematopoietic origin of adipocytes?

In addition to tissue-resident precursor cells, non-tissue-resident cells are also thought to serve as adipocyte precursors (Fig. 4). The strongest evidence for the contribution of hematopoietic cells to adipogenesis comes from human studies after bone marrow transplantation. These studies identified adipocytes derived from bone marrow progenitors after hematopoietic stem cell transplantation (Gavin et al., 2016) contributing to <5–35% of the subcutaneous adipocyte population (Gavin et al., 2016; Rydén et al., 2015). The amount of bone marrow-derived adipocytes was independent of gender and age, but strongly associated with fat mass, with up to 2.5-fold increased donor cell contribution in obese individuals. Thus, in humans, bone marrow appears to serve as a reservoir for adipocyte progenitors, particularly in obese subjects (Rydén et al., 2015). However, little is known regarding the extent to which therapy prior to bone marrow transplantation affects tissue-resident precursors in the recipients.

The contribution of bone marrow-derived progenitor cells to adipogenesis in mice is a controversial subject. Several studies using transgenic transplantation models have indicated that bone marrow cells can significantly contribute to the adipocyte population in adipose tissues (Crossno et al., 2006; Gavin et al., 2016; Sera et al., 2009). Moreover, bone marrow hematopoietic progenitors have been shown to give rise to adipocytes via myeloid intermediates in a non-bone marrow transplant model, suggesting that adipocytes arising from hematopoietic progenitors are not an artifact of myeloablative injury (Majka et al., 2010). Finally, both myeloablative and non-myeloablative fate-mapping models have indicated that bone-marrow-derived adipocytes occur primarily in gonadal adipose tissue, with lower numbers in SAT (Gavin et al., 2016; Majka et al., 2010). Thus, these studies suggest heterogeneity between sexes, with adipocytes of hematopoietic origin primarily in female mice contributing up to 25% of adipocytes in pgWAT (Majka et al., 2010). Interestingly, bone marrow-derived adipocytes differed greatly to adipocytes differentiated from tissue-resident progenitors, with increased inflammatory gene expression and lower expression of genes involved in mitochondrial biogenesis and lipid oxidation (Majka et al., 2010). Therefore, bone-marrow-derived adipocytes may account in part for the detrimental changes in adipose metabolism and inflammation with aging and obesity (Majka et al., 2010). However, the significance of these findings has been questioned by others given that several studies did not observe a significant contribution of bone-marrow-derived progenitors to adipogenesis (Berry and Rodeheffer, 2013; Koh et al., 2007; Tomiyama et al., 2008).

Together, data on the contribution of bone marrow-derived stem cells to the adipocyte progenitor population are conflicting. For mice, there is no consensus about whether hematopoietic stem cells contribute to adipogenesis, and if so, to what extent, neither in the setting of transplantation, nor under standard physiological conditions. However, for humans, transplantation studies indicate that a significant proportion of adipocytes in adipose tissue depots may arise from bone-marrow-derived progenitors. Thus, further studies are needed to determine the contribution of circulating versus tissue-resident progenitor cells in adipose tissue expansion and turnover.

The central question in understanding adipocyte heterogeneity relates to the origin and development of adipocytes. However, we still do not have a clear understanding of these issues. Different lineage tracing technologies are currently widely applied to uncover the progenitor population in transgenic mice (Sanchez-Gurmaches and Guertin, 2014a), albeit not without controversy (Ye et al., 2015). Although mesenchymal stem cells are generally well accepted as adipocyte progenitors, there is still a lively debate and divergent opinion on the contribution of pericytes, endothelial cells and bone-marrow-derived hematopoietic and mesenchymal stem cells to the adipocyte progenitor niche(s) (Fig. 4). There may be multiple reasons for data divergence; however, the use of different transgenic models exhibiting different expression patterns may partly account for the contradictory results (Gavin et al., 2016; Jeffery et al., 2014; Lee et al., 2013a). However, it seems safe to conclude that adipocytes arise from a heterogeneous population of progenitors. Furthermore, there is evidence that adipocyte progenitors vary between individual adipose depots, and vary depending on the age, sex and body weight of the individual.

Considerations for identifying adipocyte precursor populations

In 2005, the adipogenic capacity of the CD31$^-$:CD34$^+$ subpopulation of human WAT stromal vascular cells was identified (Sengenès et al., 2005). Major progress in the characterization of adipocyte progenitors was achieved by Rodeheffer and Berry and colleagues who sorted murine adipose tissue stromal vascular cells based on their expression of stem cell markers, followed by a noninvasive assay for analyzing fat mass reconstitution *in vivo*, which led to the identification of Lin$^-$:CD29$^+$:CD34$^+$:Sca-1$^+$:CD24$^+$ cells as early adipocyte progenitor cells in WAT (Rodeheffer et al., 2008). PdgfRα-Cre was identified tracing adipogenesis *in vivo* (Lee et al., 2012). Analysis of adipose tissue from PdgfRα-Cre-GFP reporter mice revealed CD24$^+$ and Lin$^-$:CD29$^+$:CD34$^+$:Sca-1$^+$:CD24$^-$ cells as adipocyte precursors (Berry and Rodeheffer, 2013). As a result of these studies, great progress was made in the identification of a class of adipocyte progenitors and, consequently, numerous studies on adipose tissue precursors operate on the basis of the markers mentioned. However, regarding the ongoing discussion about endothelial cells and bone marrow-derived stem cells as adipocyte progenitors, one could argue that sorting adipocytes according to these markers may be simplified. Despite being positive for mesenchymal and stem-cell markers (Sca-1, CD29, CD34), the lineage-negative (Lin$^-$) population excludes endothelial and hematopoietic cells on the

basis of the staining of CD31, CD45 and Ter119 (Rodeheffer et al., 2008). Furthermore, the Sca-1$^+$ population is negative for CD105 and CD117, distinguishing these cells from bone-marrow-derived mesenchymal and hematopoietic stem cells (Li and Johnson, 1995; Rodeheffer et al., 2008; Sun et al., 2003). FACS analysis of eWAT stromal vascular cells demonstrated that PdgfRα$^+$ cells represent only a subpopulation of cells expressing the stem cell markers CD34 and Sca-1, suggesting that CD34$^+$:Sca-1$^+$ cells also arise from an alternative linage (Lee et al., 2012). Thus, caution may be required extrapolating results obtained from a subset of adipocyte progenitors that have been sorted by the markers mentioned (Lin$^-$:CD29$^+$:CD34$^+$:Sca-1$^+$:CD24$^-$/CD24$^+$), or by lineage tracing using specific Cre lines, to the entirety of adipocyte precursors or adipocytes.

Conclusion

For a long time, adipose tissue was considered to be a relatively 'boring' tissue, storing and releasing energy when needed. However, over the past decades we have developed a much better understanding of the central role that adipose tissue plays in maintaining whole body energy homeostasis. A deep understanding of this regulation is becoming increasingly important given that obesity and its associated diseases are rapidly on the rise and adipose tissue dysfunction is a central initiating event for developing these diseases. As a consequence of the increased attention, our understanding of the complexity of adipose tissue is slowly increasing. Central to the development of therapeutic strategies preventing the pandemic of obesity, type 2 diabetes and other diseases of metabolic syndrome is the understanding that individual adipose tissue depots are independent organs, developing from different precursor populations, and serving different metabolic functions. Thus, this Review highlights the complexity between and within adipose tissues and underlines the need for very targeted strategies enhancing the function of metabolically beneficial adipocytes while at the same time inhibiting metabolically unfavorable adipocytes. Importantly, this distinction should not be based solely on whole adipose tissue depots because there is growing support for the idea that even within visceral fat, subpopulations of adipocytes could exist that prevent metabolic deterioration upon continued weight gain.

Competing interests

The authors declare no competing or financial interests.

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References

Abella, E., Feliu, E., Granada, I., Millá, F., Oriol, A., Ribera, J. M., Sánchez-Planell, L., Berga, L., Reverter, J. C. and Rozman, C. (2002). Bone marrow changes in anorexia nervosa are correlated with the amount of weight loss and not with other clinical findings. *Am. J. Clin. Pathol.* **118**, 582-588.

Addison, O., Marcus, R. L., Lastayo, P. C. and Ryan, A. S. (2014). Intermuscular fat: a review of the consequences and causes. *Int. J. Endocrinol.* **2014**, 309570.

Alexander, C. M., Kasza, I., Yen, C.-L. E., Reeder, S. B., Hernando, D., Gallo, R. L., Jahoda, C. A. B., Horsley, V. and MacDougald, O. A. (2015). Dermal white adipose tissue: a new component of the thermogenic response. *J. Lipid Res.* **56**, 2061-2069.

Alvehus, M., Burén, J., Sjöström, M., Goedecke, J. and Olsson, T. (2010). The human visceral fat depot has a unique inflammatory profile. *Obesity* **18**, 879-883.

Ambrosi, T. H., Scialdone, A., Graja, A., Gohlke, S., Jank, A. M., Bocian, C., Woelk, L., Fan, H., Logan, D. W., Schurmann, A. et al. (2017). Adipocyte accumulation in the bone marrow during obesity and aging impairs stem cell-based hematopoietic and bone regeneration. *Cell Stem Cell* **20**, 771-784.e6.

Anderson, J. L., Khan, M., David, W. S., Mahdavi, Z., Nuttall, F. Q., Krech, E., West, S. G., Vance, J. M., Pericak-Vance, M. A. Nance, M. A. (1999). Confirmation of linkage of hereditary partial lipodystrophy to chromosome 1q21-22. *Am. J. Med. Genet.* **82**, 161-165.

Andersson, D. P., Thorell, A., Löfgren, P., Wirén, M., Toft, E., Qvisth, V., Riserus, U., Berglund, L., Näslund, E., Bringman, S. et al. (2014). Omentectomy in addition to gastric bypass surgery and influence on insulin sensitivity: A randomized double blind controlled trial. *Clin. Nutr.* **33**, 991-996.

Andersson, D. P., Arner, E., Hogling, D. E., Ryden, M. and Arner, P. (2017). Abdominal subcutaneous adipose tissue cellularity in men and women. *Int. J. Obes.*

Arner, P. (1995). Differences in lipolysis between human subcutaneous and omental adipose tissues. *Ann. Med.* **27**, 435-438.

Asahina, K., Zhou, B., Pu, W. T. and Tsukamoto, H. (2011). Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. *Hepatology* **53**, 983-995.

Atit, R., Sgaier, S. K., Mohamed, O. A., Taketo, M. M., Dufort, D., Joyner, A. L., Niswander, L. and Conlon, R. A. (2006). Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. *Dev. Biol.* **296**, 164-176.

Barrera, C., Gatica, A. and Morgan, C. (2012). Obese visceral adipose tissue grafted in lean mice can alter glucose homeostasis and energy efficiency. *J. Biol. Regul. Homeost. Agents* **26**, 411-417.

Beasley, L. E., Koster, A., Newman, A. B., Javaid, M. K., Ferrucci, L., Kritchevsky, S. B., Kuller, L. H., Pahor, M., Schaap, L. A., Visser, M. et al. (2009). Inflammation and race and gender differences in computerized tomography-measured adipose depots. *Obesity* **17**, 1062-1069.

Bergers, G. and Song, S. (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol.* **7**, 452-464.

Bergwerff, M., Gittenberger-de Groot, A. C., Wisse, L. J., DeRuiter, M. C., Wessels, A., Martin, J. F., Olson, E. N. and Kern, M. J. (2000). Loss of function of the Prx1 and Prx2 homeobox genes alters architecture of the great elastic arteries and ductus arteriosus. *Virchows Arch.* **436**, 12-19.

Berry, R. and Rodeheffer, M. S. (2013). Characterization of the adipocyte cellular lineage in vivo. *Nat. Cell Biol.* **15**, 302-308.

Berry, R., Jeffery, E. and Rodeheffer, M. S. (2014). Weighing in on adipocyte precursors. *Cell Metab.* **19**, 8-20.

Billon, N., Iannarelli, P., Monteiro, M. C., Glavieux-Pardanaud, C., Richardson, W. D., Kessaris, N., Dani, C. and Dupin, E. (2007). The generation of adipocytes by the neural crest. *Development* **134**, 2283-2292.

Birsoy, K., Soukas, A., Torrens, J., Ceccarini, G., Montez, J., Maffei, M., Cohen, P., Fayzikhodjaeva, G., Viale, A., Socci, N. D. et al. (2008). Cellular program controlling the recovery of adipose tissue mass: an in vivo imaging approach. *Proc. Natl. Acad. Sci. USA* **105**, 12985-12990.

Birsoy, K., Berry, R., Wang, T., Ceyhan, O., Tavazoie, S., Friedman, J. M. and Rodeheffer, M. S. (2011). Analysis of gene networks in white adipose tissue development reveals a role for ETS2 in adipogenesis. *Development* **138**, 4709-4719.

Bjørndal, B., Burri, L., Staalesen, V., Skorve, J. and Berge, R. K. (2011). Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *J. Obes.* **2011**, 490650.

Björntorp, P. (1990). "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arterioscler. Thromb. Vasc. Biol.* **10**, 493-496.

Blüher, M. and Mantzoros, C. S. (2015). From leptin to other adipokines in health and disease: Facts and expectations at the beginning of the 21st century. *Metabolism* **64**, 131-145.

Boettcher, M., Machann, J., Stefan, N., Thamer, C., Häring, H.-U., Claussen, C. D., Fritsche, A. and Schick, F. (2009). Intermuscular adipose tissue (IMAT): association with other adipose tissue compartments and insulin sensitivity. *J. Magn. Reson. Imaging* **29**, 1340-1345.

Boivin, A., Brochu, G., Marceau, S., Marceau, P., Hould, F.-S. and Tchernof, A. (2007). Regional differences in adipose tissue metabolism in obese men. *Metabolism* **56**, 533-540.

Brakenhielm, E., Cao, R., Gao, B., Angelin, B., Cannon, B., Parini, P. and Cao, Y. (2004). Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice. *Circ. Res.* **94**, 1579-1588.

Bredella, M. A., Fazeli, P. K., Miller, K. K., Misra, M., Torriani, M., Thomas, B. J., Ghomi, R. H., Rosen, C. J. and Klibanski, A. (2009). Increased bone marrow fat in anorexia nervosa. *J. Clin. Endocrinol. Metab.* **94**, 2129-2136.

Bredella, M. A., Torriani, M., Ghomi, R. H., Thomas, B. J., Brick, D. J., Gerweck, A. V., Rosen, C. J., Klibanski, A. and Miller, K. K. (2011). Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. *Obesity* **19**, 49-53.

Brune, J. E., Kern, M., Kunath, A., Flehmig, G., Schön, M. R., Lohmann, T., Dressler, M., Dietrich, A., Fasshauer, M., Kovacs, P. et al. (2016). Fat depot-specific expression of HOXC9 and HOXC10 may contribute to adverse fat distribution and related metabolic traits. *Obesity* **24**, 51-59.

Cai, X., Lin, Y., Hauschka, P. V. and Grottkau, B. E. (2011). Adipose stem cells originate from perivascular cells. *Biol. Cell* **103**, 435-447.

Calo, E., Quintero-Estades, J. A., Danielian, P. S., Nedelcu, S., Berman, S. D. and Lees, J. A. (2010). Rb regulates fate choice and lineage commitment in vivo. *Nature* **466**, 1110-1114.
Candille, S. I., Van Raamsdonk, C. D., Chen, C., Kuijper, S., Chen-Tsai, Y., Russ, A., Meijlink, F. and Barsh, G. S. (2004). Dorsoventral patterning of the mouse coat by Tbx15. *PLoS Biol.* **2**, E3.
Cannon, B. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **84**, 277-359.
Cano, E., Carmona, R. and Munoz-Chapuli, R. (2013). Wt1-expressing progenitors contribute to multiple tissues in the developing lung. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **305**, L322-L332.
Cao, Y. (2007). Angiogenesis modulates adipogenesis and obesity. *J. Clin. Invest.* **117**, 2362-2368.
Cao, Y. (2013). Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. *Cell Metab.* **18**, 478-489.
Caplan, A. I. (2008). All MSCs are pericytes? *Cell Stem Cell* **3**, 229-230.
Catanzaro, R., Cuffari, B., Italia, A. and Marotta, F. (2016). Exploring the metabolic syndrome: Nonalcoholic fatty pancreas disease. *World J. Gastroenterol.* **22**, 7660-7675.
Cawthorn, W. P., Scheller, E. L. and MacDougald, O. A. (2012). Adipose tissue stem cells meet preadipocyte commitment: going back to the future. *J. Lipid Res.* **53**, 227-246.
Cawthorn, W. P., Scheller, E. L., Parlee, S. D., Pham, H. A., Learman, B. S., Redshaw, C. M. H., Sulston, R. J., Burr, A. A., Das, A. K., Simon, B. R. et al. (2016). Expansion of bone marrow adipose tissue during caloric restriction is associated with increased circulating glucocorticoids and not with hypoleptinemia. *Endocrinology* **157**, 508-521.
Chau, Y.-Y., Bandiera, R., Serrels, A., Martínez-Estrada, O. M., Qing, W., Lee, M., Slight, J., Thornburn, A., Berry, R., McHaffie, S. et al. (2014). Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. *Nat. Cell Biol.* **16**, 367-375.
Cinti, S. (2007). The adipose organ. In *Nutrition and Health: Adipose Tissue and Adipokines in Health and Disease* (ed. G. Fantuzzi, T. Mazzone), pp. 3-19. New York: Humana Press.
Cinti, S., Cigolini, M., Bosello, O. and Bjorntorp, P. (1984). A morphological study of the adipocyte precursor. *J. Submicrosc. Cytol.* **16**, 243-251.
Corvera, S. and Gealekman, O. (2014). Adipose tissue angiogenesis: impact on obesity and type-2 diabetes. *Biochim. Biophys. Acta* **1842**, 463-472.
Crisan, M., Yap, S., Casteilla, L., Chen, C.-W., Corselli, M., Park, T. S., Andriolo, G., Sun, B., Zheng, B., Zhang, L. et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**, 301-313.
Crossno, J. T., Jr, Majka, S. M., Grazia, T., Gill, R. G. and Klemm, D. J. (2006). Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. *J. Clin. Invest.* **116**, 3220-3228.
Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y.-H., Doria, A. et al. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* **360**, 1509-1517.
da Silva Meirelles, L., Caplan, A. I. and Nardi, N. B. (2008). In search of the in vivo identity of mesenchymal stem cells. *Stem Cells* **26**, 2287-2299.
De Angelis, L., Berghella, L., Coletta, M., Lattanzi, L., Zanchi, M., Gabriella, M., Ponzetto, C. and Cossu, G. (1999). Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration. *J. Cell Biol.* **147**, 869-878.
de Jong, J. M. A., Larsson, O., Cannon, B. and Nedergaard, J. (2015). A stringent validation of mouse adipose tissue identity markers. *Am. J. Physiol. Endocrinol. Metab.* **308**, E1085-E1105.
Deschamps, J. and van Nes, J. (2005). Developmental regulation of the Hox genes during axial morphogenesis in the mouse. *Development* **132**, 2931-2942.
Devlin, M. J., Cloutier, A. M., Thomas, N. A., Panus, D. A., Lotinun, S., Pinz, I., Baron, R., Rosen, C. J. and Bouxsein, M. L. (2010). Caloric restriction leads to high marrow adiposity and low bone mass in growing mice. *J. Bone Miner. Res.* **25**, 2078-2088.
Di Iorgi, N., Rosol, M., Mittelman, S. D. and Gilsanz, V. (2008). Reciprocal relation between marrow adiposity and the amount of bone in the axial and appendicular skeleton of young adults. *J. Clin. Endocrinol. Metab.* **93**, 2281-2286.
Driskell, R. R., Jahoda, C. A. B., Chuong, C.-M., Watt, F. and Horsley, V. (2014). Defining dermal adipose tissue. *Exp. Dermatol.* **23**, 629-631.
D'Souza, D., Babu, G. S., Shetty, S. R. and Rasquinha, V. (2014). Congenital infiltrating lipomatosis of the face: A case report with review of literature. *Indian Dermatol. Online J.* **5**, 303-305.
Dvorak, R. V., DeNino, W. F., Ades, P. A. and Poehlman, E. T. (1999). Phenotypic characteristics associated with insulin resistance in metabolically obese but normal-weight young women. *Diabetes* **48**, 2210-2214.
Ecklund, K., Vajapeyam, S., Feldman, H. A., Buzney, C. D., Mulkern, R. V., Kleinman, P. K., Rosen, C. J. and Gordon, C. M. (2010). Bone marrow changes in adolescent girls with anorexia nervosa. *J. Bone Miner. Res.* **25**, 298-304.
Edens, N. K., Fried, S. K., Kral, J. G., Hirsch, J. and Leibel, R. L. (1993). In vitro lipid synthesis in human adipose tissue from three abdominal sites. *Am. J. Physiol.* **265**, E374-E379.
Elder, C. P., Apple, D. F., Bickel, C. S., Meyer, R. A. and Dudley, G. A. (2004). Intramuscular fat and glucose tolerance after spinal cord injury–a cross-sectional study. *Spinal Cord* **42**, 711-716.
Engfeldt, P. and Amer, P. (1988). Lipolysis in human adipocytes, effects of cell size, age and of regional differences. *Horm. Metab. Res. Suppl.* **19**, 26-29.
Fain, J. N., Madan, A. K., Hiler, M. L., Cheema, P. and Bahouth, S. W. (2004). Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* **145**, 2273-2282.
Fasshauer, M. and Blüher, M. (2015). Adipokines in health and disease. *Trends Pharmacol. Sci.* **36**, 461-470.
Feng, B., Zhang, T. and Xu, H. (2013). Human adipose dynamics and metabolic health. *Ann. N. Y. Acad. Sci.* **1281**, 160-177.
Festa, E., Fretz, J., Berry, R., Schmidt, B., Rodeheffer, M., Horowitz, M. and Horsley, V. (2011). Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. *Cell* **146**, 761-771.
Festuccia, W. T., Blanchard, P.-G., Turcotte, V., Laplante, M., Sariahmetoglu, M., Brindley, D. N. and Deshaies, Y. (2009). Depot-specific effects of the PPARgamma agonist rosiglitazone on adipose tissue glucose uptake and metabolism. *J. Lipid Res.* **50**, 1185-1194.
Fischer, I. P., Irmler, M., Meyer, C. W., Sachs, S. J., Neff, F., Beckers, J., Hrabè de Angelis, M., Tschöp, M. H., Hofmann, S. M. and Ussar, S. (2017). A history of obesity leaves an inflammatory fingerprint in liver and adipose tissue. *Int. J. Obes.*
Foster, M. T., Softic, S., Caldwell, J., Kohli, R., de Kloet, A. D. and Seeley, R. J. (2013). Subcutaneous adipose tissue transplantation in diet-induced obese mice attenuates metabolic dysregulation while removal exacerbates it. *Physiol. Rep.* **1**.
Fox, C. S., Massaro, J. M., Hoffmann, U., Pou, K. M., Maurovich-Horvat, P., Liu, C.-Y., Vasan, R. S., Murabito, J. M., Meigs, J. B., Cupples, L. A. et al. (2007). Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation* **116**, 39-48.
Frayn, K. N., Karpe, F., Fielding, B. A., Macdonald, I. A. and Coppack, S. W. (2003). Integrative physiology of human adipose tissue. *Int. J. Obes. Relat. Metab. Disord.* **27**, 875-888.
Fried, S. K., Leibel, R. L., Edens, N. K. and Kral, J. G. (1993). Lipolysis in intraabdominal adipose tissues of obese women and men. *Obes. Res.* **1**, 443-448.
Fukumura, D., Ushiyama, A., Duda, D. G., Xu, L., Tam, J., Krishna, V., Chatterjee, K., Garkavtsev, I. and Jain, R. K. (2003). Paracrine regulation of angiogenesis and adipocyte differentiation during in vivo adipogenesis. *Circ. Res.* **93**, e88-e97.
Gallagher, D., Kuznia, P., Heshka, S., Albu, J., Heymsfield, S. B., Goodpaster, B., Visser, M. and Harris, T. B. (2005). Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. *Am. J. Clin. Nutr.* **81**, 903-910.
Gastaldelli, A., Miyazaki, Y., Pettiti, M., Matsuda, M., Mahankali, S., Santini, E., DeFronzo, R. A. and Ferrannini, E. (2002). Metabolic effects of visceral fat accumulation in type 2 diabetes. *J. Clin. Endocrinol. Metab.* **87**, 5098-5103.
Gavin, K. M., Gutman, J. A., Kohrt, W. M., Wei, Q., Shea, K. L., Miller, H. L., Sullivan, T. M., Erickson, P. F., Helm, K. M., Acosta, A. S. et al. (2016). De novo generation of adipocytes from circulating progenitor cells in mouse and human adipose tissue. *FASEB J.* **30**, 1096-1108.
GBD 2015 Obesity Collaborators, **Afshin, A., Forouzanfar, M. H., Reitsma, M. B., Sur, P., Estep, K., Lee, A., Marczak, L., Mokdad, A. H., Moradi-Lakeh, M. et al.** (2017). Health effects of overweight and obesity in 195 countries over 25 years. *N. Engl. J. Med.* **377**, 13-27.
Geer, E. B. and Shen, W. (2009). Gender differences in insulin resistance, body composition, and energy balance. *Gend Med.* **6** Suppl. 1, 60-75.
Gehring, W. J. (1987). Homeo boxes in the study of development. *Science* **236**, 1245-1252.
Gesta, S., Bluher, M., Yamamoto, Y., Norris, A. W., Berndt, J., Kralisch, S., Boucher, J., Lewis, C. and Kahn, C. R. (2006). Evidence for a role of developmental genes in the origin of obesity and body fat distribution. *Proc. Natl. Acad. Sci. USA* **103**, 6676-6681.
Gesta, S., Tseng, Y.-H. and Kahn, C. R. (2007). Developmental origin of fat: tracking obesity to its source. *Cell* **131**, 242-256.
Gesta, S., Bezy, O., Mori, M. A., Macotela, Y., Lee, K. Y. and Kahn, C. R. (2011). Mesodermal developmental gene Tbx15 impairs adipocyte differentiation and mitochondrial respiration. *Proc. Natl. Acad. Sci. USA* **108**, 2771-2776.
Giordano, A., Frontini, A. and Cinti, S. (2016). Convertible visceral fat as a therapeutic target to curb obesity. *Nat. Rev. Drug Discov.* **15**, 405-424.
Goodpaster, B. H., Thaete, F. L., Simoneau, J.-A. and Kelley, D. E. (1997). Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes* **46**, 1579-1585.
Goodpaster, B. H., Thaete, F. L. and Kelley, D. E. (2000). Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *Am. J. Clin. Nutr.* **71**, 885-892.
Goodpaster, B. H., Krishnaswami, S., Resnick, H., Kelley, D. E., Haggerty, C., Harris, T. B., Schwartz, A. V., Kritchevsky, S. and Newman, A. B. (2003). Association between regional adipose tissue distribution and both type 2 diabetes

and impaired glucose tolerance in elderly men and women. *Diabetes Care* **26**, 372-379.
Gorgey, A. S. and Dudley, G. A. (2007). Skeletal muscle atrophy and increased intramuscular fat after incomplete spinal cord injury. *Spinal Cord* **45**, 304-309.
Grandl, G., Müller, S., Moest, H., Moser, C., Wollscheid, B. and Wolfrum, C. (2016). Depot specific differences in the adipogenic potential of precursors are mediated by collagenous extracellular matrix and Flotillin 2 dependent signaling. *Mol. Metab.* **5**, 937-947.
Griffith, J. F., Yeung, D. K. W., Ma, H. T., Leung, J. C. S., Kwok, T. C. Y. and Leung, P. C. (2012). Bone marrow fat content in the elderly: a reversal of sex difference seen in younger subjects. *J. Magn. Reson. Imaging* **36**, 225-230.
Gupta, R. K., Arany, Z., Seale, P., Mepani, R. J., Ye, L., Conroe, H. M., Roby, Y. A., Kulaga, H., Reed, R. R. and Spiegelman, B. M. (2010). Transcriptional control of preadipocyte determination by Zfp423. *Nature* **464**, 619-623.
Gupta, R. K., Mepani, R. J., Kleiner, S., Lo, J. C., Khandekar, M. J., Cohen, P., Frontini, A., Bhowmick, D. C., Ye, L., Cinti, S. et al. (2012). Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. *Cell Metab.* **15**, 230-239.
Halberg, N., Khan, T., Trujillo, M. E., Wernstedt-Asterholm, I., Attie, A. D., Sherwani, S., Wang, Z. V., Landskroner-Eiger, S., Dineen, S., Magalang, U. J. et al. (2009). Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol. Cell. Biol.* **29**, 4467-4483.
Hammar, J. A. (1895). Zur Kenntniss des Fettgewebes. *Arch. Mikrosk. Anat.* **45**, 512-574.
Han, J., Lee, J.-E., Jin, J., Lim, J. S., Oh, N., Kim, K., Chang, S.-I., Shibuya, M., Kim, H. and Koh, G. Y. (2011). The spatiotemporal development of adipose tissue. *Development* **138**, 5027-5037.
Hardouin, P., Rharass, T. and Lucas, S. (2016). Bone marrow adipose tissue: to be or not to be a typical adipose tissue? *Front. Endocrinol.* **7**, 85.
Harms, M. and Seale, P. (2013). Brown and beige fat: development, function and therapeutic potential. *Nat. Med.* **19**, 1252-1263.
Heid, I. M., Jackson, A. U., Randall, J. C., Winkler, T. W., Qi, L., Steinthorsdottir, V., Thorleifsson, G., Zillikens, M. C., Speliotes, E. K., Mägi, R. et al. (2010). Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat. Genet.* **42**, 949-960.
Hellmér, J., Marcus, C., Sonnenfeld, T. and Arner, P. (1992). Mechanisms for differences in lipolysis between human subcutaneous and omental fat cells. *J. Clin. Endocrinol. Metab.* **75**, 15-20.
Hirschi, K. K. and D'Amore, P. A. (1996). Pericytes in the microvasculature. *Cardiovasc. Res.* **32**, 687-698.
Hocking, S. L., Stewart, R. L., Brandon, A. E., Suryana, E., Stuart, E., Baldwin, E. M., Kolumam, G. A., Modrusan, Z., Junutula, J. R., Gunton, J. E. et al. (2015). Subcutaneous fat transplantation alleviates diet-induced glucose intolerance and inflammation in mice. *Diabetologia* **58**, 1587-1600.
Hoffstedt, J., Arner, P., Hellers, G. and Lönnqvist, F. (1997). Variation in adrenergic regulation of lipolysis between omental and subcutaneous adipocytes from obese and non-obese men. *J. Lipid Res.* **38**, 795-804.
Hoffstedt, J., Arner, E., Wahrenberg, H., Andersson, D. P., Qvisth, V., Löfgren, P., Rydén, M., Thörne, A., Wirén, M., Palmér, M. et al. (2010). Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. *Diabetologia* **53**, 2496-2503.
Hube, F., Lietz, U., Igel, M., Jensen, P. B., Tornqvist, H., Joost, H.-G. and Hauner, H. (1996). Difference in Leptin mRNA levels between omental and subcutaneous abdominal adipose tissue from obese humans. *Horm. Metab. Res.* **28**, 690-693.
Hudak, C. S., Gulyaeva, O., Wang, Y., Park, S.-M., Lee, L., Kang, C. and Sul, H. S. (2014). Pref-1 marks very early mesenchymal precursors required for adipose tissue development and expansion. *Cell Rep* **8**, 678-687.
Iyama, K., Ohzono, K. and Usuku, G. (1979). Electron microscopical studies on the genesis of white adipocytes: differentiation of immature pericytes into adipocytes in transplanted preadipose tissue. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **31**, 143-155.
Jackson, S. N. J., Pinkney, J., Bargiotta, A., Veal, C. D., Howlett, T. A., McNally, P. G., Corral, R., Johnson, A. and Trembath, R. C. (1998). A defect in the regional deposition of adipose tissue (partial lipodystrophy) is encoded by a gene at chromosome 1q. *Am. J. Hum. Genet.* **63**, 534-540.
Jeffery, E., Berry, R., Church, C. D., Yu, S., Shook, B. A., Horsley, V., Rosen, E. D. and Rodeheffer, M. S. (2014). Characterization of Cre recombinase models for the study of adipose tissue. *Adipocyte* **3**, 206-211.
Jeffery, E., Church, C. D., Holtrup, B., Colman, L. and Rodeheffer, M. S. (2015). Rapid depot-specific activation of adipocyte precursor cells at the onset of obesity. *Nat. Cell Biol.* **17**, 376-385.
Justesen, J., Stenderup, K., Ebbesen, E. N., Mosekilde, L., Steiniche, T. and Kassem, M. (2001). Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. *Biogerontology* **2**, 165-171.
Kahn, B. B. (1996). Lilly lecture 1995: glucose transport: pivotal step in insulin action. *Diabetes* **45**, 1644-1654.
Kajimura, S., Spiegelman, B. M. and Seale, P. (2015). Brown and beige fat: physiological roles beyond heat generation. *Cell Metab.* **22**, 546-559.
Karampinos, D. C., Baum, T., Nardo, L., Alizai, H., Yu, H., Carballido-Gamio, J., Yap, S. P., Shimakawa, A., Link, T. M. and Majumdar, S. (2012). Characterization of the regional distribution of skeletal muscle adipose tissue in type 2 diabetes using chemical shift-based water/fat separation. *J. Magn. Reson. Imaging* **35**, 899-907.
Karastergiou, K., Fried, S. K., Xie, H., Lee, M.-J., Divoux, A., Rosencrantz, M. A., Chang, R. J. and Smith, S. R. (2013). Distinct developmental signatures of human abdominal and gluteal subcutaneous adipose tissue depots. *J. Clin. Endocrinol. Metab.* **98**, 362-371.
Kasza, I., Suh, Y., Wollny, D., Clark, R. J., Roopra, A., Colman, R. J., MacDougald, O. A., Shedd, T. A., Nelson, D. W., Yen, M.-I. et al. (2014). Syndecan-1 is required to maintain intradermal fat and prevent cold stress. *PLoS Genet.* **10**, e1004514.
Kershaw, E. E. and Flier, J. S. (2004). Adipose tissue as an endocrine organ. *J. Clin. Endocrinol. Metab.* **89**, 2548-2556.
Khan, T., Muise, E. S., Iyengar, P., Wang, Z. V., Chandalia, M., Abate, N., Zhang, B. B., Bonaldo, P., Chua, S. and Scherer, P. E. (2009). Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. *Mol. Cell. Biol.* **29**, 1575-1591.
Kim, M.-H., Park, J.-S., Seo, M.-S., Jung, J.-W., Lee, Y.-S. and Kang, K.-S. (2010). Genistein and daidzein repress adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells via Wnt/beta-catenin signalling or lipolysis. *Cell Prolif.* **43**, 594-605.
Kim, S. M., Lun, M., Wang, M., Senyo, S. E., Guillermier, C., Patwari, P. and Steinhauser, M. L. (2014). Loss of white adipose hyperplastic potential is associated with enhanced susceptibility to insulin resistance. *Cell Metab.* **20**, 1049-1058.
Kim, J. Y., Tfayli, H., Michaliszyn, S. F., Lee, S. and Arslanian, S. (2016). Distinguishing characteristics of metabolically healthy versus metabolically unhealthy obese adolescent girls with polycystic ovary syndrome. *Fertil. Steril.* **105**, 1603-1611.
Klingenspor, M., Fromme, T., Hughes, D. A., Jr, Manzke, L., Polymeropoulos, E., Riemann, T., Trzcionka, M., Hirschberg, V. and Jastroch, M. (2008). An ancient look at UCP1. *Biochim. Biophys. Acta* **1777**, 637-641.
Koh, Y. J., Kang, S., Lee, H. J., Choi, T.-S., Lee, H. S., Cho, C.-H. and Koh, G. Y. (2007). Bone marrow-derived circulating progenitor cells fail to transdifferentiate into adipocytes in adult adipose tissues in mice. *J. Clin. Invest.* **117**, 3684-3695.
Krueger, K. C., Costa, M. J., Du, H. and Feldman, B. J. (2014). Characterization of Cre recombinase activity for in vivo targeting of adipocyte precursor cells. *Stem Cell Rep.* **3**, 1147-1158.
Kubo, Y., Kaidzu, S., Nakajima, I., Takenouchi, K. and Nakamura, F. (2000). Organization of extracellular matrix components during differentiation of adipocytes in long-term culture. *In Vitro Cell. Dev. Biol. Anim.* **36**, 38-44.
Kugel, H., Jung, C., Schulte, O. and Heindel, W. (2001). Age- and sex-specific differences in the 1H-spectrum of vertebral bone marrow. *J. Magn. Reson. Imaging* **13**, 263-268.
Kuratani, S., Martin, J. F., Wawersik, S., Lilly, B., Eichele, G. and Olson, E. N. (1994). The expression pattern of the chick homeobox gene gMHox suggests a role in patterning of the limbs and face and in compartmentalization of somites. *Dev. Biol.* **161**, 357-369.
Kwok, K. H. M., Lam, K. S. L. and Xu, A. (2016). Heterogeneity of white adipose tissue: molecular basis and clinical implications. *Exp. Mol. Med.* **48**, e215.
Lau, F. H., Deo, R. C., Mowrer, G., Caplin, J., Ahfeldt, T., Kaplan, A., Ptaszek, L., Walker, J. D., Rosengard, B. R. and Cowan, C. A. (2011). Pattern specification and immune response transcriptional signatures of pericardial and subcutaneous adipose tissue. *PLoS ONE* **6**, e26092.
Lean, M. E. J. (1989). Brown adipose tissue in humans. *Proc. Nut. Soc.* **48**, 243-257.
Lee, M.-J., Wu, Y. and Fried, S. K. (2010a). Adipose tissue remodeling in pathophysiology of obesity. *Curr. Opin Clin. Nutr. Metab. Care* **13**, 371-376.
Lee, Y., Lingvay, I., Szczepaniak, L. S., Ravazzola, M., Orci, L. and Unger, R. H. (2010b). Pancreatic steatosis: harbinger of type 2 diabetes in obese rodents. *Int. J. Obes.* **34**, 396-400.
Lee, P., Greenfield, J. R., Ho, K. K. Y. and Fulham, M. J. (2010). A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* **299**, E601-E606.
Lee, Y.-H., Petkova, A. P., Mottillo, E. P. and Granneman, J. G. (2012). In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. *Cell Metab.* **15**, 480-491.
Lee, K. Y., Russell, S. J., Ussar, S., Boucher, J., Vernochet, C., Mori, M. A., Smyth, G., Rourk, M., Cederquist, C., Rosen, E. D. et al. (2013a). Lessons on conditional gene targeting in mouse adipose tissue. *Diabetes* **62**, 864-874.
Lee, K. Y., Yamamoto, Y., Boucher, J., Winnay, J. N., Gesta, S., Cobb, J., Bluher, M. and Kahn, C. R. (2013b). Shox2 is a molecular determinant of depot-specific adipocyte function. *Proc. Natl. Acad. Sci. USA* **110**, 11409-11414.
Lee, M.-J., Wu, Y. and Fried, S. K. (2013c). Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. *Mol. Aspects Med.* **34**, 1-11.

Lee, K. Y., Sharma, R., Gase, G., Ussar, S., Li, Y., Welch, L., Berryman, D. E., Kispert, A., Bluher, M. and Kahn, C. R. (2017a). Tbx15 defines a glycolytic subpopulation and white adipocyte heterogeneity. *Diabetes* **66**, 2822-2829.
Lee, Y.-H., Kim, S.-N., Kwon, H.-J. and Granneman, J. G. (2017b). Metabolic heterogeneity of activated beige/brite adipocytes in inguinal adipose tissue. *Sci. Rep.* **7**, 39794.
Lefebvre, A.-M., Laville, M., Vega, N., Riou, J. P., van Gaak, L., Auwerx, J. and Vidal, H. (1998). Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes* **47**, 98-103.
Leiss, M., Beckmann, K., Girós, A., Costell, M. and Fässler, R. (2008). The role of integrin binding sites in fibronectin matrix assembly in vivo. *Curr. Opin. Cell Biol.* **20**, 502-507.
Lemos, D. R., Paylor, B., Chang, C., Sampaio, A., Underhill, T. M. and Rossi, F. M. V. (2012). Functionally convergent white adipogenic progenitors of different lineages participate in a diffused system supporting tissue regeneration. *Stem Cells* **30**, 1152-1162.
Leskinen, T., Sipilä, S., Kaprio, J., Kainulainen, H., Alen, M. and Kujala, U. M. (2013). Physically active vs. inactive lifestyle, muscle properties, and glucose homeostasis in middle-aged and older twins. *Age* **35**, 1917-1926.
Leussink, B., Brouwer, A., el Khattabi, M., Poelmann, R. E., Gittenberger-de Groot, A. C. and Meijlink, F. (1995). Expression patterns of the paired-related homeobox genes MHox/Prx1 and S8/Prx2 suggest roles in development of the heart and the forebrain. *Mech. Dev.* **52**, 51-64.
Li, C. L. and Johnson, G. R. (1995). Murine hematopoietic stem and progenitor cells: I. Enrichment and biologic characterization. *Blood* **85**, 1472-1479.
Lim, S., Jang, H.-J., Kim, J. K., Kim, J.-M., Park, E. H., Yang, J. H., Kim, Y.-H., Yea, K., Ryu, S. H. and Suh, P.-G. (2011). Ochratoxin A inhibits adipogenesis through the extracellular signal-related kinases-peroxisome proliferator-activated receptor-gamma pathway in human adipose tissue-derived mesenchymal stem cells. *Stem Cells Dev.* **20**, 415-426.
Lima, M. M. O., Pareja, J. C., Alegre, S. M., Geloneze, S. R., Kahn, S. E., Astiarraga, B. D., Chaim, A., Baracat, J. and Geloneze, B. (2013). Visceral fat resection in humans: effect on insulin sensitivity, beta-cell function, adipokines, and inflammatory markers. *Obesity* **21**, E182-E189.
Linder, K., Arner, P., Flores-Morales, A., Tollet-Egnell, P. and Norstedt, G. (2004). Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. *J. Lipid Res.* **45**, 148-154.
Liu, J., DeYoung, S. M., Zhang, M., Zhang, M., Cheng, A. and Saltiel, A. R. (2005). Changes in integrin expression during adipocyte differentiation. *Cell Metab.* **2**, 165-177.
Macotela, Y., Emanuelli, B., Mori, M. A., Gesta, S., Schulz, T. J., Tseng, Y.-H. and Kahn, C. R. (2012). Intrinsic differences in adipocyte precursor cells from different white fat depots. *Diabetes* **61**, 1691-1699.
Majka, S. M., Fox, K. E., Psilas, J. C., Helm, K. M., Childs, C. R., Acosta, A. S., Janssen, R. C., Friedman, J. E., Woessner, B. T., Shade, T. R. et al. (2010). De novo generation of white adipocytes from the myeloid lineage via mesenchymal intermediates is age, adipose depot, and gender specific. *Proc. Natl. Acad. Sci. USA* **107**, 14781-14786.
Manini, T. M., Clark, B. C., Nalls, M. A., Goodpaster, B. H., Ploutz-Snyder, L. L. and Harris, T. B. (2007). Reduced physical activity increases intermuscular adipose tissue in healthy young adults. *Am. J. Clin. Nutr.* **85**, 377-384.
Marangoni, R. G., Korman, B. D., Wei, J., Wood, T. A., Graham, L. V., Whitfield, M. L., Scherer, P. E., Tourtellotte, W. G. and Varga, J. (2015). Myofibroblasts in murine cutaneous fibrosis originate from adiponectin-positive intradermal progenitors. *Arthritis Rheumatol.* **67**, 1062-1073.
Margoni, A., Fotis, L. and Papavassiliou, A. G. (2012). The transforming growth factor-beta/bone morphogenetic protein signalling pathway in adipogenesis. *Int. J. Biochem. Cell Biol.* **44**, 475-479.
Mariman, E. C. M. and Wang, P. (2010). Adipocyte extracellular matrix composition, dynamics and role in obesity. *Cell. Mol. Life Sci.* **67**, 1277-1292.
Martinsson, A. (1969). Hypertrophy and hyperplasia of human adipose tissue in obesity. *Pol. Arch. Med. Wewn.* **42**, 481-486.
McCulloch, L. J., Rawling, T. J., Sjöholm, K., Franck, N., Dankel, S. N., Price, E. J., Knight, B., Liversedge, N. H., Mellgren, G., Nystrom, F. et al. (2015). COL6A3 is regulated by leptin in human adipose tissue and reduced in obesity. *Endocrinology* **156**, 134-146.
Migliorini, R. H., Garofalo, M. A. and Kettelhut, I. C. (1997). Increased sympathetic activity in rat white adipose tissue during prolonged fasting. *Am. J. Physiol.* **272**, 656-661.
Mohammed, B. S., Cohen, S., Reeds, D., Young, V. L. and Klein, S. (2008). Long-term effects of large-volume liposuction on metabolic risk factors for coronary heart disease. *Obesity* **16**, 2648-2651.
Montague, C. T., Prins, J. B., Sanders, L., Zhang, J., Sewter, C. P., Digby, J., Byrne, C. D. and O'Rahilly, S. (1998). Depot-related gene expression in human subcutaneous and omental adipocytes. *Diabetes* **47**, 1384-1391.
Mori, S., Kiuchi, S., Ouchi, A., Hase, T. and Murase, T. (2014). Characteristic expression of extracellular matrix in subcutaneous adipose tissue development and adipogenesis; comparison with visceral adipose tissue. *Int. J. Biol. Sci.* **10**, 825-833.
Moyer-Mileur, L. J., Slater, H., Jordan, K. C. and Murray, M. A. (2008). IGF-1 and IGF-binding proteins and bone mass, geometry, and strength: relation to metabolic control in adolescent girls with type 1 diabetes. *J. Bone Miner. Res.* **23**, 1884-1891.
Muruganandan, S., Roman, A. A. and Sinal, C. J. (2009). Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: cross talk with the osteoblastogenic program. *Cell. Mol. Life Sci.* **66**, 236-253.
Neels, J. G., Thinnes, T. and Loskutoff, D. J. (2004). Angiogenesis in an in vivo model of adipose tissue development. *FASEB J.* **18**, 983-985.
Nielsen, S., Guo, Z. K., Johnson, M., Hensrud, D. D. and Jensen, M. D. (2004). Splanchnic lipolysis in human obesity. *J. Clin. Invest.* **113**, 1582-1588.
Ohta, H. and Itoh, N. (2014). Roles of FGFs as adipokines in adipose tissue development, remodeling, and metabolism. *Front. Endocrinol.* **5**, 18.
Olson, L. E. and Soriano, P. (2011). PDGFRbeta signaling regulates mural cell plasticity and inhibits fat development. *Dev. Cell* **20**, 815-826.
Onogi, Y., Wada, T., Kamiya, C., Inata, K., Matsuzawa, T., Inaba, Y., Kimura, K., Inoue, H., Yamamoto, S., Ishii, Y. et al. (2017). PDGFRbeta regulates adipose tissue expansion and glucose metabolism via vascular remodeling in diet-induced obesity. *Diabetes* **66**, 1008-1021.
Ouchi, N., Parker, J. L., Lugus, J. J. and Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **11**, 85-97.
Padwa, B. L. and Mulliken, J. B. (2001). Facial infiltrating lipomatosis. *Plast. Reconstr. Surg.* **108**, 1544-1554.
Palou, M., Priego, T., Sánchez, J., Rodríguez, A. M., Palou, A. and Picó, C. (2009). Gene expression patterns in visceral and subcutaneous adipose depots in rats are linked to their morphologic features. *Cell. Physiol. Biochem.* **24**, 547-556.
Peirce, V., Carobbio, S. and Vidal-Puig, A. (2014). The different shades of fat. *Nature* **510**, 76-83.
Pellegrinelli, V., Carobbio, S. and Vidal-Puig, A. (2016). Adipose tissue plasticity: how fat depots respond differently to pathophysiological cues. *Diabetologia* **59**, 1075-1088.
Perrini, S., Laviola, L., Cignarelli, A., Melchiorre, M., De Stefano, F., Caccioppoli, C., Natalicchio, A., Orlando, M. R., Garruti, G., De Fazio, M. et al. (2008). Fat depot-related differences in gene expression, adiponectin secretion, and insulin action and signalling in human adipocytes differentiated in vitro from precursor stromal cells. *Diabetologia* **51**, 155-164.
Peters, J. M., Barnes, R., Bennett, L., Gitomer, W. M., Bowcock, A. M. and Garg, A. (1998). Localization of the gene for familial partial lipodystrophy (Dunnigan variety) to chromosome 1q21-22. *Nat. Genet.* **18**, 292-295.
Pinnick, K. E., Collins, S. C., Londos, C., Gauguier, D., Clark, A. and Fielding, B. A. (2008). Pancreatic ectopic fat is characterized by adipocyte infiltration and altered lipid composition. *Obesity* **16**, 522-530.
Poissonnet, C. M., Burdi, A. R. and Garn, S. M. (1984). The chronology of adipose tissue appearance and distribution in the human fetus. *Early Hum. Dev.* **10**, 1-11.
Pond, C. M. (1992). An evolutionary and functional view of mammalian adipose tissue. *Proc. Nut. Soc.* **51**, 367-377.
Pramme-Steinwachs, I., Jastroch, M. and Ussar, S. (2017). Extracellular calcium modulates brown adipocyte differentiation and identity. *Sci. Rep.* **7**, 8888.
Primeau, V., Coderre, L., Karelis, A. D., Brochu, M., Lavoie, M.-E., Messier, V., Sladek, R. and Rabasa-Lhoret, R. (2011). Characterizing the profile of obese patients who are metabolically healthy. *Int. J. Obes.* **35**, 971-981.
Qian, S.-W., Li, X., Zhang, Y.-Y., Huang, H.-Y., Liu, Y., Sun, X. and Tang, Q.-Q. (2010). Characterization of adipocyte differentiation from human mesenchymal stem cells in bone marrow. *BMC Dev. Biol.* **10**, 47.
Ramis, J. M., Salinas, R., Garcia-Sanz, J. M., Moreiro, J., Proenza, A. M. and Lladó, I. (2006). Depot-gender-related differences in the lipolytic pathway of adipose tissue from severely obese patients. *Cell Physiol. Biochem.* **17**, 173-180.
Randall, J. C., Winkler, T. W., Kutalik, Z., Berndt, S. I., Jackson, A. U., Monda, K. L., Kilpeläinen, T. O., Esko, T., Mägi, R., Li, S. et al. (2013). Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet.* **9**, e1003500.
Reynisdottir, S., Dauzats, M., Thörne, A. and Langin, D. (1997). Comparison of hormone-sensitive lipase activity in visceral and subcutaneous human adipose tissue. *J. Clin. Endocrinol. Metab.* **82**, 4162-4166.
Rigamonti, A., Brennand, K., Lau, F. and Cowan, C. A. (2011). Rapid cellular turnover in adipose tissue. *PLoS ONE* **6**, e17637.
Rinkevich, Y., Mori, T., Sahoo, D., Xu, P.-X., Bermingham, J. R., Jr. and Weissman, I. L. (2012). Identification and prospective isolation of a mesothelial precursor lineage giving rise to smooth muscle cells and fibroblasts for mammalian internal organs, and their vasculature. *Nat. Cell Biol.* **14**, 1251-1260.
Roca-Rivada, A., Bravo, S. B., Pérez-Sotelo, D., Alonso, J., Castro, A. I., Baamonde, I., Baltar, J., Casanueva, F. F. and Pardo, M. (2015). CILAIR-based secretome analysis of obese visceral and subcutaneous adipose tissues reveals distinctive ECM remodeling and inflammation mediators. *Sci. Rep.* **5**, 12214.
Rodeheffer, M. S., Birsoy, K. and Friedman, J. M. (2008). Identification of white adipocyte progenitor cells in vivo. *Cell* **135**, 240-249.
Rosen, E. D. and MacDougald, O. A. (2006). Adipocyte differentiation from the inside out. *Nat. Rev. Mol. Cell Biol.* **7**, 885-896.
Rosen, E. D. and Spiegelman, B. M. (2006). Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* **444**, 847-853.

Rosen, E. D. and Spiegelman, B. M. (2014). What we talk about when we talk about fat. *Cell* **156**, 20-44.
Ruderman, N. B., Schneider, S. H. and Berchtold, P. (1981). The "metabolically-obese," normal-weight individual. *Am. J. Clin. Nutr.* **34**, 1617-1621.
Ruderman, N., Chisholm, D., Pi-Sunyer, X. and Schneider, S. (1998). The metabolically obese, normal-weight individual revisited. *Diabetes* **47**, 699-713.
Rupnick, M. A., Panigrahy, D., Zhang, C.-Y., Dallabrida, S. M., Lowell, B. B., Langer, R. and Folkman, M. J. (2002). Adipose tissue mass can be regulated through the vasculature. *Proc. Natl. Acad. Sci. USA* **99**, 10730-10735.
Ryan, A. S. and Nicklas, B. J. (1999). Age-related changes in fat deposition in mid-thigh muscle in women: relationships with metabolic cardiovascular disease risk factors. *Int. J. Obes. Relat. Metab. Disord.* **23**, 126-132.
Ryan, A. S., Dobrovolny, C. L., Smith, G. V., Silver, K. H. and Macko, R. F. (2002). Hemiparetic muscle atrophy and increased intramuscular fat in stroke patients. *Arch. Phys. Med. Rehabil.* **83**, 1703-1707.
Ryan, A. S., Buscemi, A., Forrester, L., Hafer-Macko, C. E. and Ivey, F. M. (2011). Atrophy and intramuscular fat in specific muscles of the thigh: associated weakness and hyperinsulinemia in stroke survivors. *Neurorehabil. Neural Repair* **25**, 865-872.
Rydén, M., Uzunel, M., Hård, J. L., Borgström, E., Mold, J. E., Arner, E., Mejhert, N., Andersson, D. P., Widlund, Y., Hassan, M. et al. (2015). Transplanted bone marrow-derived cells contribute to human adipogenesis. *Cell Metab.* **22**, 408-417.
Ryo, M., Nakamura, T., Kihara, S., Kumada, M., Shibazaki, S., Takahashi, M., Nagai, M., Matsuzawa, Y. and Funahashi, T. (2004). Adiponectin as a biomarker of the metabolic syndrome. *Circ. J.* **68**, 975-981.
Sackmann-Sala, L., Berryman, D. E., Munn, R. D., Lubbers, E. R. and Kopchick, J. J. (2012). Heterogeneity among white adipose tissue depots in male C57BL/6J mice. *Obesity* **20**, 101-111.
Samad, F., Badeanlou, L., Shah, C. and Yang, G. (2011). Adipose tissue and ceramide biosynthesis in the pathogenesis of obesity. In *Sphingolipids and Metabolic Disease* (ed. L. A. Cowart), pp. 67-86. New York, NY: Springer New York.
Sanchez-Gurmaches, J., Hung, C.-M., Sparks, C. A., Tang, Y., Li, H. and Guertin, D. A. (2012). PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors. *Cell Metab.* **16**, 348-362.
Sanchez-Gurmaches, J. and Guertin, D. A. (2014a). Adipocyte lineages: tracing back the origins of fat. *Biochim. Biophys. Acta* **1842**, 340-351.
Sanchez-Gurmaches, J. and Guertin, D. A. (2014b). Adipocytes arise from multiple lineages that are heterogeneously and dynamically distributed. *Nat. Commun.* **5**, 4099.
Sanchez-Gurmaches, J., Hsiao, W.-Y. and Guertin, D. A. (2015). Highly selective in vivo labeling of subcutaneous white adipocyte precursors with Prx1-Cre. *Stem Cell Rep.* **4**, 541-550.
Satoor, S. N., Puranik, A. S., Kumar, S., Williams, M. D., Ghale, M., Rahalkar, A., Karandikar, M. S., Shouche, Y., Patole, M., Bhonde, R. et al. (2011). Location, location, location: Beneficial effects of autologous fat transplantation. *Sci. Rep.* **1**, 81.
Schiller, H. B. and Fässler, R. (2013). Mechanosensitivity and compositional dynamics of cell-matrix adhesions. *EMBO Rep.* **14**, 509-519.
Schmitz, J., Evers, N., Awazawa, M., Nicholls, H. T., Brönneke, H. S., Dietrich, A., Mauer, J., Blüher, M. and Brüning, J. C. (2016). Obesogenic memory can confer long-term increases in adipose tissue but not liver inflammation and insulin resistance after weight loss. *Mol. Metab.* **5**, 328-339.
Schöttl, T., Kappler, L., Braun, K., Fromme, T. and Klingenspor, M. (2015). Limited mitochondrial capacity of visceral versus subcutaneous white adipocytes in male C57BL/6N mice. *Endocrinology* **156**, 923-933.
Schulz, T. J. and Tseng, Y.-H. (2009). Emerging role of bone morphogenetic proteins in adipogenesis and energy metabolism. *Cytokine Growth Factor. Rev.* **20**, 523-531.
Scott, M. A., Nguyen, V. T., Levi, B. and James, A. W. (2011). Current methods of adipogenic differentiation of mesenchymal stem cells. *Stem Cells Dev.* **20**, 1793-1804.
Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scimè, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H. et al. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* **454**, 961-967.
Sell, H., Habich, C. and Eckel, J. (2012). Adaptive immunity in obesity and insulin resistance. *Nat. Rev. Endocrinol.* **8**, 709-716.
Sengenès, C., Lolmède, K., Zakaroff-Girard, A., Busse, R. and Bouloumié, A. (2005). Preadipocytes in the human subcutaneous adipose tissue display distinct features from the adult mesenchymal and hematopoietic stem cells. *J. Cell. Physiol.* **205**, 114-122.
Sera, Y., LaRue, A. C., Moussa, O., Mehrotra, M., Duncan, J. D., Williams, C. R., Nishimoto, E., Schulte, B. A., Watson, P. M., Watson, D. K. et al. (2009). Hematopoietic stem cell origin of adipocytes. *Exp. Hematol.* **37**, 1108-1120, 1120 e1-4.
Seretis, K., Goulis, D. G., Koliakos, G. and Demiri, E. (2015). Short- and long-term effects of abdominal lipectomy on weight and fat mass in females: a systematic review. *Obes. Surg.* **25**, 1950-1958.
Shan, T., Liang, X., Bi, P., Zhang, P., Liu, W. and Kuang, S. (2013). Distinct populations of adipogenic and myogenic Myf5-lineage progenitors in white adipose tissues. *J. Lipid Res.* **54**, 2214-2224.
Shen, W., Chen, J., Punyanitya, M., Shapses, S., Heshka, S. and Heymsfield, S. B. (2007). MRI-measured bone marrow adipose tissue is inversely related to DXA-measured bone mineral in Caucasian women. *Osteoporos. Int.* **18**, 641-647.
Shimozaki, K., Clemenson, G. D., Jr. and Gage, F. H. (2013). Paired related homeobox protein 1 is a regulator of stemness in adult neural stem/progenitor cells. *J. Neurosci.* **33**, 4066-4075.
Shungin, D. Winkler, T. W. Croteau-Chonka, D. C. Ferreira, T. Locke, A. E. Mägi, R. Strawbridge, R. J. Pers, T. H. Fischer, K. Justice, A. E. et al. (2015). New genetic loci link adipose and insulin biology to body fat distribution. *Nature* **518**, 187-196.
Skurk, T., Alberti-Huber, C., Herder, C. and Hauner, H. (2007). Relationship between adipocyte size and adipokine expression and secretion. *J. Clin. Endocrinol. Metab.* **92**, 1023-1033.
Song, M. Y., Ruts, E., Kim, J., Janumala, I., Heymsfield, S. and Gallagher, D. (2004). Sarcopenia and increased adipose tissue infiltration of muscle in elderly African American women. *Am. J. Clin. Nutr.* **79**, 874-880.
Stefan, N., Kantartzis, K., Machmann, J., Schick, F., Thamer, C., Rittig, K., Balletshofer, B., Machicao, F., Fritsche, A. and Häring, H.-U. (2008). Identification and characterization of metabolically benign obesity in humans. *Arch. Intern. Med.* **168**, 1609-1616.
Strissel, K. J., Stancheva, Z., Miyoshi, H., Perfield, J. W., II, DeFuria, J., Jick, Z., Greenberg, A. S. and Obin, M. S. (2007). Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes* **56**, 2910-2918.
Styner, M., Sen, B., Xie, Z., Case, N. and Rubin, J. (2010). Indomethacin promotes adipogenesis of mesenchymal stem cells through a cyclooxygenase independent mechanism. *J. Cell. Biochem.* **111**, 1042-1050.
Sun, S., Guo, Z., Xiao, X., Liu, B., Liu, X., Tang, P.-H. and Mao, N. (2003). Isolation of mouse marrow mesenchymal progenitors by a novel and reliable method. *Stem Cells* **21**, 527-535.
Sun, K., Kusminski, C. M. and Scherer, P. E. (2011). Adipose tissue remodeling and obesity. *J. Clin. Invest.* **121**, 2094-2101.
Sun, K., Tordjman, J., Clément, K. and Scherer, P. E. (2013). Fibrosis and adipose tissue dysfunction. *Cell Metab.* **18**, 470-477.
Sun, K., Park, J., Gupta, O. T., Holland, W. L., Auerbach, P., Zhang, N., Goncalves Marangoni, R., Nicoloro, S. M., Czech, M. P., Varga, J. et al. (2014). Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction. *Nat. Commun.* **5**, 3485.
Tang, W., Zeve, D., Suh, J. M., Bosnakovski, D., Kyba, M., Hammer, R. E., Tallquist, M. D. and Graff, J. M. (2008). White fat progenitor cells reside in the adipose vasculature. *Science* **322**, 583-586.
Tchkonia, T., Lenburg, M., Thomou, T., Giorgadze, N., Frampton, G., Pirtskhalava, T., Cartwright, A., Cartwright, M., Flanagan, J., Karagiannides, I. et al. (2007). Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. *Am. J. Physiol. Endocrinol. Metab.* **292**, E298-E307.
Tchkonia, T., Thomou, T., Zhu, Y., Karagiannides, I., Pothoulakis, C., Jensen, M. D. and Kirkland, J. L. (2013). Mechanisms and metabolic implications of regional differences among fat depots. *Cell Metab.* **17**, 644-656.
Tchoukalova, Y. D., Votruba, S. B., Tchkonia, T., Giorgadze, N., Kirkland, J. L. and Jensen, M. D. (2010). Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. *Proc. Natl. Acad. Sci. USA* **107**, 18226-18231.
Thörne, A., Lönnqvist, F., Apleman, J., Hellers, G. and Arner, P. (2002). A pilot study of long-term effects of a novel obesity treatment: omentectomy in connection with adjustable gastric banding. *Int. J. Obes.* **26**, 193-199.
Tomiyama, K., Murase, N., Stolz, D. B., Toyokawa, H., O'Donnell, D. R., Smith, D. M., Dudas, J. R., Rubin, J. P. and Marra, K. G. (2008). Characterization of transplanted green fluorescent protein(+) bone marrow cells into adipose tissue. *Stem Cells* **26**, 330-338.
Traktuev, D. O., Merfeld-Clauss, S., Li, J., Kolonin, M., Arap, W., Pasqualini, R., Johnstone, B. H. and March, K. L. (2008). A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ. Res.* **102**, 77-85.
Tran, T. T. and Kahn, C. R. (2010). Transplantation of adipose tissue and stem cells: role in metabolism and disease. *Nat. Rev. Endocrinol.* **6**, 195-213.
Tran, T. T., Yamamoto, Y., Gesta, S. and Kahn, C. R. (2008). Beneficial effects of subcutaneous fat transplantation on metabolism. *Cell Metab.* **7**, 410-420.
Tran, K.-V., Gealekman, O., Frontini, A., Zingaretti, M. C., Morroni, M., Giordano, A., Smorlesi, A., Perugini, J., De Matteis, R., Sbarbati, A. et al. (2012). The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. *Cell Metab.* **15**, 222-229.
Trujillo, M. E. and Scherer, P. E. (2006). Adipose tissue-derived factors: impact on health and disease. *Endocr. Rev.* **27**, 762-778.

Urs, A. B., Augustine, J., Kumar, P., Arora, S., Aggarwal, N. and Sultana, N. (2013). Infiltrating lipomatosis of the face: a case series. *J. Nat. Sci. Biol. Med.* **4**, 252-257.
Ussar, S., Bezy, O., Bluher, M. and Kahn, C. R. (2012). Glypican-4 enhances insulin signaling via interaction with the insulin receptor and serves as a novel adipokine. *Diabetes* **61**, 2289-2298.
Ussar, S., Lee, K. Y., Dankel, S. N., Boucher, J., Haering, M.-F., Kleinridders, A., Thomou, T., Xue, R., Macotela, Y., Cypess, A. M. et al. (2014). ASC-1, PAT2, and P2RX5 are cell surface markers for white, beige, and brown adipocytes. *Sci. Transl. Med.* **6**, 247ra103.
Valorani, M. G., Germani, A., Otto, W. R., Harper, L., Biddle, A., Khoo, C. P., Lin, W. R., Hawa, M. I., Tropel, P., Patrizi, M. P. et al. (2010). Hypoxia increases Sca-1/CD44 co-expression in murine mesenchymal stem cells and enhances their adipogenic differentiation potential. *Cell Tissue Res.* **341**, 111-120.
van Beek, E. A., Bakker, A. H., Kruyt, P. M., Hofker, M. H., Saris, W. H. and Keijer, J. (2007). Intra- and interindividual variation in gene expression in human adipose tissue. *Pflugers Arch.* **453**, 851-861.
van Beek, L., van Klinken, J. B., Pronk, A. C. M., van Dam, A. D., Dirven, E., Rensen, P. C. N., Koning, F., Willems van Dijk, K. and van Harmelen, V. (2015). The limited storage capacity of gonadal adipose tissue directs the development of metabolic disorders in male C57Bl/6J mice. *Diabetologia* **58**, 1601-1609.
Van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M. A. F. L., Kemerink, G. J., Bouvy, N. D., Schrauwen, P. and Teule, G. J. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* **360**, 1500-1508.
Veilleux, A., Blouin, K., Rhéaume, C., Daris, M., Marette, A. and Tchernof, A. (2009). Glucose transporter 4 and insulin receptor substrate-1 messenger RNA expression in omental and subcutaneous adipose tissue in women. *Metabolism* **58**, 624-631.
Virtanen, K. A., Lönnroth, P., Parkkola, R., Peltoniemi, P., Asola, M., Viljanen, T., Tolvanen, T., Knuuti, J., Rönnemaa, T., Huupponen, R. et al. (2002). Glucose uptake and perfusion in subcutaneous and visceral adipose tissue during insulin stimulation in nonobese and obese humans. *J. Clin. Endocrinol. Metab.* **87**.
Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N.-J., Enerbäck, S. et al. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* **360**, 1518-1525.
Vishvanath, L., MacPherson, K. A., Hepler, C., Wang, Q. A., Shao, M., Spurgin, S. B., Wang, M. Y., Kusminski, C. M., Morley, T. S. and Gupta, R. K. (2016). Pdgfrbeta+ mural preadipocytes contribute to adipocyte hyperplasia induced by high-fat-diet feeding and prolonged cold exposure in adult mice. *Cell Metab.* **23**, 350-359.
Vohl, M.-C., Sladek, R., Robitaille, J., Gurd, S., Marceau, P., Richard, D., Hudson, T. J. and Tchernof, A. (2004). A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men. *Obes. Res.* **12**, 1217-1222.
Votruba, S. B. and Jensen, M. D. (2007). Regional fat deposition as a factor in FFA metabolism. *Annu. Rev. Nutr.* **27**, 149-163.
Wajchenberg, B. L., Giannella-Neto, D., da Silva, M. E. R. and Santos, R. F. (2002). Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. *Horm. Metab. Res.* **34**, 616-621.
Waldén, T. B., Hansen, I. R., Timmons, J. A., Cannon, B. and Nedergaard, J. (2012). Recruited vs. nonrecruited molecular signatures of brown, “brite”, and white adipose tissues. *Am. J. Physiol. Endocrinol. Metab.* **302**, E19-E31.
Wang, Q. A., Tao, C., Gupta, R. K. and Scherer, P. E. (2013). Tracking adipogenesis during white adipose tissue development, expansion and regeneration. *Nat. Med.* **19**, 1338-1344.
Wang, Q., Zhang, M., Xu, M., Gu, W., Xi, Y., Qi, L., Li, B. and Wang, W. (2015). Brown adipose tissue activation is inversely related to central obesity and metabolic parameters in adult human. *PLoS ONE* **10**, e0123795.
Wassermann, F. (1965). The development of adipose tissue. In *Handbook of Physiology, Section V. Adipose tissue* (ed. A. E. Renold, G. F. Cahill Jr), pp. 87-100. Washington, DC: American Physiological Society.
Welter, J. F., Penick, K. J. and Solchaga, L. A. (2013). Assessing adipogenic potential of mesenchymal stem cells: a rapid three-dimensional culture screening technique. *Stem Cells Int.* **2013**, 806525.
Wernstedt Asterholm, I., Tao, C., Morley, T. S., Wang, Q. A., Delgado-Lopez, F., Wang, Z. V. and Scherer, P. E. (2014). Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. *Cell Metab.* **20**, 103-118.
WJ, L. (2002). *Human Embryology*, 3rd edn. Elsevier Health Sciences.
Wu, Y., Kim, J. Y., Zhou, S. and Smas, C. M. (2008). Differential screening identifies transcripts with depot-dependent expression in white adipose tissues. *BMC Genomics* **9**, 397.
Wu, J., Boström, P., Sparks, L. M., Ye, L., Choi, J. H., Giang, A.-H., Khandekar, M., Virtanen, K. A., Nuutila, P., Schaart, G. et al. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* **150**, 366-376.
Xue, B., Rim, J.-S., Hogan, J. C., Coulter, A. A., Koza, R. A. and Kozak, L. P. (2007). Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *J. Lipid Res.* **48**, 41-51.
Yamamoto, Y., Gesta, S., Lee, K. Y., Tran, T. T., Saadatirad, P. and Kahn, C. R. (2010). Adipose depots possess unique developmental gene signatures. *Obesity* **18**, 872-878.
Ye, R., Wang, Q. A., Tao, C., Vishvanath, L., Shao, M., McDonald, J. G., Gupta, R. K. and Scherer, P. E. (2015). Impact of tamoxifen on adipocyte lineage tracing: Inducer of adipogenesis and prolonged nuclear translocation of Cre recombinase. *Mol. Metab.* **4**, 771-778.
Yim, J.-E., Heshka, S., Albu, J., Heymsfield, S., Kuznia, P., Harris, T. and Gallagher, D. (2007). Intermuscular adipose tissue rivals visceral adipose tissue in independent associations with cardiovascular risk. *Int. J. Obes.* **31**, 1400-1405.
Yu, L., Gu, S., Alappat, S., Song, Y., Yan, M., Zhang, X., Zhang, G., Jiang, Y., Zhang, Z., Zhang, Y. et al. (2005). Shox2-deficient mice exhibit a rare type of incomplete clefting of the secondary palate. *Development* **132**, 4397-4406.
Zamarron, B. F., Mergian, T. A., Cho, K. W., Martinez-Santibanez, G., Luan, D., Singer, K., DelProposto, J. L., Geletka, L. M., Muir, L. A. and Lumeng, C. N. (2017). Macrophage proliferation sustains adipose tissue inflammation in formerly obese mice. *Diabetes* **66**, 392-406.
Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425-432.
Zhang, L.-J., Guerrero-Juarez, C. F., Hata, T., Bapat, S. P., Ramos, R., Plikus, M. V. and Gallo, R. L. (2015). Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. *Science* **347**, 67-71.
Zhao, Y., Gu, X., Zhang, N., Kolonin, M. G., An, Z. and Sun, K. (2016). Divergent functions of endotrophin on different cell populations in adipose tissue. *Am. J. Physiol. Endocrinol. Metab.* **311**, E952-E963.