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REVIEW

Heterogeneity of white adipose tissue: molecular basis and clinical implications

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Adipose tissue is a highly heterogeneous endocrine organ. The heterogeneity among different anatomical depots stems from their intrinsic differences in cellular and physiological properties, including developmental origin, adipogenic and proliferative capacity, glucose and lipid metabolism, insulin sensitivity, hormonal control, thermogenic ability and vascularization. Additional factors that influence adipose tissue heterogeneity are genetic predisposition, environment, gender and age. Under obese condition, these depot-specific differences translate into specific fat distribution patterns, which are closely associated with differential cardiometabolic risks. For instance, individuals with central obesity are more susceptible to developing diabetes and cardiovascular complications, whereas those with peripheral obesity are more metabolically healthy. This review summarizes the clinical and mechanistic evidence for the depot-specific differences that give rise to different metabolic consequences, and provides therapeutic insights for targeted treatment of obesity.
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INTRODUCTION

Owing to the modern sedentary lifestyle in both developed and rapidly developing countries, the prevalence of obesity has reached an alarming level and has become a worldwide epidemic, affecting over 500 million adults and 40 million children. Morbidly obese individuals with body mass index above 35 kg m^{-2} are associated with significantly higher all-cause mortality,[1,2] most of which are related to cardiovascular diseases, diabetes and cancers.[2]

Obesity is characterized by an abnormal and excess accumulation of adipose tissue in the body, and this has stimulated immense research interest on the pathophysiological role of adipose tissue in the development of obesity and its related medical complications. Over the past two decades, the adipose tissue has gradually transformed from merely an inert store for excess lipids into a metabolically active endocrine organ[3] involved in the regulation of glucose and lipid metabolism,[4] insulin sensitivity,[4] inflammatory response,[5] non-shivering thermogenesis[6] and vascular endothelial function.[7] Furthermore, adipose tissue is highly heterogeneous. Each anatomical depot differs in metabolic and hormonal profiles and has different physiological roles. The differential accumulation of specific depots therefore translates into different clinical outcomes. It is well established that abdominal or central obesity is more associated with cardiometabolic diseases compared with peripheral obesity.[8,9] By providing clinical and basic research evidence, this review aims to discuss the fundamental properties of adipose tissue, which give rise to its heterogeneity, and how these contribute to the resulting clinical outcomes.

BASIC FEATURES OF ADIPOSE TISSUE

Anatomy

White adipose tissue (WAT) is widely dispersed in humans. Major depots reside in subcutaneous region in the upper (deep and superficial abdominal) and lower body (gluteal–femoral), as well as in the visceral region (omental, mesenteric, mediastinal and epicardial; Figure 1a). Subcutaneous WAT is located under the skin where it acts as a barrier against dermal infection, an insulator to prevent heat loss, and a cushion for protection against external mechanical stress. Visceral WAT in the body trunk is buried around vital organs within the peritoneum and rib cage. With current technological advances, the distribution and accumulation of specific adipose depot can be accurately and quantitatively assessed by regional magnetic

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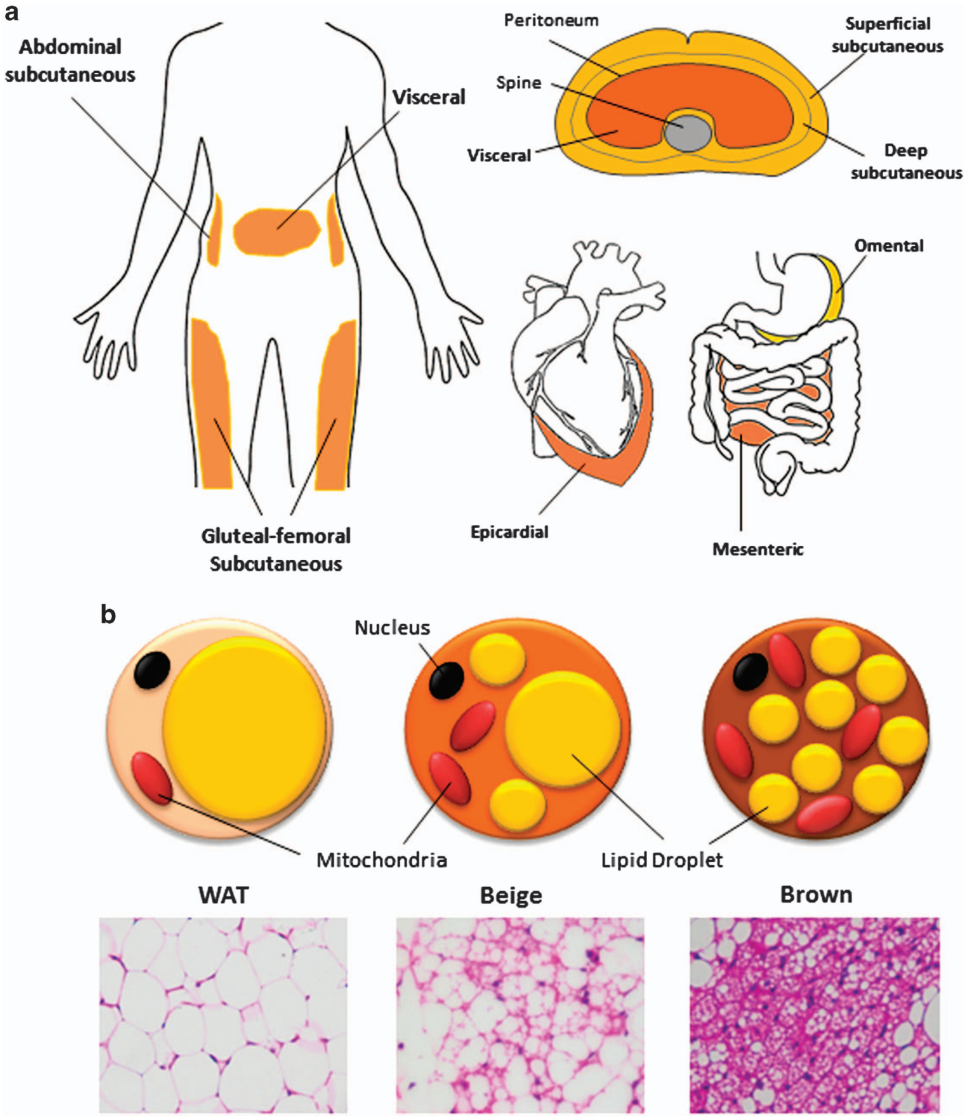

Figure 1 Heterogeneous adipose organ in humans. (**a**) White adipose tissue (WAT) depots in humans are shown in orange. Major subcutaneous WAT includes superficial and deep abdominal depots and gluteal-femoral depot. Major visceral WAT includes epicardial, omental and mesenteric. (**b**) Morphological differences between WAT, beige and brown adipose tissue (BAT) adipocytes as shown by cartoon and hematoxylin/eosin staining (×40 magnification).

resonance imaging,[10] computed tomography[11] and, less frequently, echocardiography[12] and ultrasonography.[13]

Brown adipose tissue (BAT) is a distinct type of adipose tissue present predominantly in rodents and infant humans located around the scapular. BAT is specialized in utilizing and dissipating the energy derived from lipids to produce heat via the action of uncoupled protein 1 (UCP-1) located in the inner membrane of the mitochondria.[6] BAT appears brown because of the high mitochondrion content and dense vascularization compared with WAT. In between WAT and BAT is Brite (brown and white) or Beige adipose tissue, which is a subpopulation of WAT that has adopted features of BAT, including increased UCP-1 expression, adipocyte locularity, mitochondrion density and vascularization in a process known as adaptive thermogenesis, or 'browning', upon the stimulation by low temperatures (Figure 1b).[14] Recently, metabolically active adipose tissue depots with Beige-like characteristics were also found in healthy adult humans at the cervical, supraclavicular, axillary and paravertebral regions.[15]

Energy maintenance in WAT

The primary function of WAT is to maintain an energy homeostasis. At times of positive energy balance, it uptakes excess lipid and stores it in the form of triglycerides (TGs) to prevent ectopic lipid deposition and adverse metabolic complications as observed in lipodystrophic individuals[16] and mouse models.[17] On the other hand, it acts as an energy source and releases lipids in the form of non-esterified fatty acids (NEFAs) when there is a demand for energy. These processes act in concert and respond promptly to different energy states and hormonal cues.

The uptake of dietary TGs in WAT is mediated by lipoprotein lipase.[18] These enzymes are attached to the vascular endothelium via highly charged heparin sulphate proteoglycans, with their active sites located at the luminal surface of blood vessels, allowing them to hydrolyze TGs carried by chylomicrons and very-low-density lipoprotein in the bloodstream. With these, NEFA and monoacylglycerol are mobilized and taken up for cellular utilization and storage.[18] Uptake of hydrolyzed products is mediated by passive diffusion or by fatty-acid translocase/CD36,[19] caveolin-1,[20] fatty-acid-binding protein (FABP)[21] and fatty-acid transport protein.[22] Finally, these products are trapped via acylation by acyl coenzyme A synthase,[23] which lowers intracellular NEFA concentrations and favors further uptake, as well as by diacylglycerol acetyltransferase, which performs the committed step in TG synthesis.[24]

Signals for low nutritional state, including catecholamines and sympathetic nervous system, trigger lipolysis in WAT to release NEFA into the circulation. These signals transduce via cyclic AMP and activate protein kinase A, which in turn phosphorylates perilipin A and hormone-sensitive lipase on lipid droplets in adipocytes and promotes lipolysis. Insulin, a signal for high-energy state, inhibits the actions of hormone-sensitive lipase by reducing cyclic AMP levels and protein kinase A activity.[25]

The maintenance of energy homeostasis depends not only on the balance between lipid uptake and release, but also on the sensitivity to stimulatory and inhibitory signals mediated by the sympathetic nervous system and hormonal system.

Pathophysiology in obese WAT

In obesity, WAT depots experience abnormal and excess expansion, either by increase in adipocyte number or adipocyte size.[26] They become inflamed and release an increased amount of pro-inflammatory cytokines, including monocyte attractant protein 1, tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) and adipocyte FABP (A-FABP), and a reduced amount of anti-inflammatory adiponectin into the circulation.[27] Increased levels of monocyte attractant protein 1 lead to macrophage infiltration into inflamed WAT[28] and further exacerbate the low-grade, 'sterile' inflammatory response. Driven by the actions of TNF-α and IL-6, inflamed WAT switches from storing to releasing excess NEFAs into the circulation,[29] causing ectopic lipid deposition and lipotoxicity in vital metabolic organs including the liver and skeletal muscles.[30] Increased levels of NEFA activate the c-Jun N-terminal kinase pathway via membrane partitioning of c-Src,[31] which in turn cause insulin resistance through Ser-307 phosphorylation on insulin receptor substrate 1,[32] or inhibition of insulin-stimulated Akt phosphorylation.[32]

CLINICAL EVIDENCE FOR WAT HETEROGENEITY

Differential predisposition to cardiometabolic diseases

Several independent studies have reported the paradoxical existence of lean but metabolically obese individuals,[33] as well as those who are metabolically healthy but obese.[34] A recent study further showed that obese individuals who were metabolically healthy, as categorized according to hepatic TG content and insulin sensitivity, were resistant to adverse cardiometabolic effects following a moderate (~6%) weight gain, whereas those who were obese and metabolically abnormal were predisposed.[35] Increased expression of genes involved in glucose uptake and lipogenesis in subcutaneous depots from metabolically healthy obese individuals was proposed to be one possible explanation for protective lipid diversion and thus different clinical outcomes.[35] However, the impact of fat distribution on metabolism and the relative contribution of different adipose tissue depots were not further explored in this study. In 1956, Vague reported that central or 'android' obesity was more closely associated with cardio-metabolic diseases compared with peripheral or 'gynaecoid' obesity.[8] In line with this, later cross-sectional and longitudinal studies showed that the waist-to-hip ratio and waist circumference, both indicative of central obesity, were even stronger independent risk factors for insulin resistance, type 2 diabetes, hyperlipidemia and atherosclerosis, as compared with body mass index.[9,36]

Portal Theory

The 'Portal Theory' proposed that these differential outcomes can be attributed to the unique anatomical locations of particular adipose depots.[37] In humans, the visceral depots (omental and mesenteric WAT) are drained by the hepatic portal vein into the liver, whereas subcutaneous depots are drained systemically by inferior or superior venae cava.[37] Whereas NEFAs and pro-inflammatory cytokines released by subcutaneous depots are diluted in the circulation, those released by portal drained visceral depots directly flow to the liver. Thus, individuals with abnormal accumulation of visceral depots are particularly prone to developing hepatic insulin resistance.[37] A fat transplantation study revealed that mice receiving transplantation of portal drained wild-type WAT developed hepatic insulin resistance, but not when receiving IL-6-deficient WAT transplant,[38] suggesting a significant role of IL-6-mediated NEFA release in this model. Nevertheless, the contribution of NEFAs by visceral WAT depots appears to be insufficient in causing systemic insulin resistance, which is a hallmark of overall metabolic dysfunction.[39] This is because the upper body subcutaneous depots, but not visceral depots, were shown to be the major source of NEFAs, accounting for up to 70% of the circulating level in obesity.[40] Therefore, anatomical location and portal drainage may only represent one of the many factors that give rise to the different association with cardiometabolic risk.

Different origins of WAT depots in human

Several studies on patients with inherited lipodystrophy have suggested a depot-specific developmental program for adipocytes in humans.[16] For instance, patients with type 1 congenital generalized lipodystrophy, also known as Berardinelli–Seip lipodystrophy, completely lack adipose tissue, except the mechanical depots in their palms and soles, scalp,

retro-orbital and peri-articular regions.[41] Patients with the more common Dunnigan type familial partial lipodystrophy, on the other hand, experience continuous loss of subcutaneous depots in extremities and anterior truncal region, but an excessive fat accumulation in spared locations including the visceral region.[42] Using microarray and quantitative PCR analysis, Gesta *et al.*[43] reported major differences in the expression of a cluster of developmental and patterning genes between subcutaneous and visceral WAT depots taken from humans of both genders. Whereas subcutaneous depot expressed higher levels of Shox2, En1, HoxC9 and Gpc4, visceral depots were more abundant with the transcripts of Sfrp2, Nr2f1, Thbd, HoxA5, HoxC8 and Tbx15.[43] In particular, *Tbx15*, a mesodermal developmental gene, not only showed different expression levels between depots, but also a strong exponential negative relationship between its expression in visceral WAT and waist-to-hip ratio as well as body mass index.[43] In another study, intrinsic differences between human subcutaneous and visceral WAT depots in the expression of early developmental genes, including homeotic genes, were traced back to yet undifferentiated preadipocytes.[44] After virtually immortalizing single isolated primary preadipocytes from different depots by stably expressing the telomerase subunit, human telomerase reverse transcriptase, the depot-specific expression profile partially sustained for at least 40 population doublings,[44] supporting the notion that subcutaneous and visceral WAT depots are intrinsically different. Interestingly, the expression of developmental genes also differed between subcutaneous depots in the abdomen and gluteal region in both lean and obese adults, and that these differences correlated with the extent of DNA methylation of these genes.[45] Further studies are required to investigate whether the depot-specific developmental programs between subcutaneous and visceral depots are epigenetically regulated and how.

WAT expansion

Association studies have shown that adipocyte enlargement, but not increase in adipocyte number, is associated with elevated cardiometabolic risks.[46] Preadipocytes in the stromal vascular fraction from WAT, the largest pool of progenitor cells in humans, are the major progenitor cells that give rise to new adipocytes. In colonies raised from single preadipocyte clones, two subtypes of preadipocytes have been identified, with one being more proliferative, replicative, adipogenic and resistant to TNF-α-induced apoptosis, and the other subtype being less so.[47] Clonal preadipocytes from subcutaneous WAT depots behaved like the former subtype, whereas those from visceral depots displayed characteristics of the latter, in a cell-autonomous and inheritable manner,[48] which is consistent with the findings that peripheral obese individuals are more metabolically healthy.[8] With overfeeding, abdominal subcutaneous WAT experienced mainly adipocyte enlargement, whereas a massive increase in adipocyte number was observed in the femoral subcutaneous depot.[26] Adipocyte hypertrophy in omental, but not subcutaneous depot, in obese women was independently associated with dyslipidemia.[49] On the contrary, Arner *et al.*[50] recently showed that adipocyte number rather than cell size strongly correlated with the weight of greater omentum in obese humans, and proposed that omental depot accumulation is mainly determined by the adipocyte proliferation rate.

Lipid metabolism

Another important factor controlling the rate and extent of expansion of WAT is the regulation of lipid flux in adipocytes. As gender appears to be an important factor in the manifestation of central (android) or peripheral (gynaecoid) obesity, a majority of human studies on lipid metabolism in WAT explored both gender and interdepot differences. The uptake of NEFA in men was increased in abdominal subcutaneous depot compared with femoral depot, although the opposite was observed in women.[51] In both lean and obese individuals, women were shown to be more efficient in storing lipids in femoral depot than men, likely mediated by higher post-prandial activities of local lipoprotein lipase, although the efficiency in abdominal subcutaneous depot was similar.[52] In support of these findings, the expression of NEFA-transporting proteins, including CD36 and A-FABP, was higher in the femoral depot in women.[51] Moreover, northern blotting results showed that the gene transcript of lipoprotein lipase was more abundant in subcutaneous depot than in visceral omental depot in obese women, which was opposite to that found in obese men.[53] Collectively, these findings suggest that women are in general more efficient in partitioning fat in the periphery compared with men, partly explaining the stereotypical gender-specific fat distribution pattern and hence the typically better metabolic health in women. Interestingly, whereas the insulin responsiveness is higher in omental depot than in subcutaneous depot in lean individuals, as evidenced by intensified and more rapid insulin-induced Akt phosphorylation in the former depot,[54] the increase in lipoprotein lipase expression in response to insulin in morbidly obese individuals is much greater in subcutaneous depot compared with omental depot without a significant gender effect,[53] suggesting that there may be a switch in storage pattern and blunted insulin response as obesity develops.

The effects of catecholamines on lipolysis were much greater in omental depot than in subcutaneous depot[55] because of an increase in pro-lipolytic β-adrenergic receptor activity[56] and reduction in anti-lipolytic α-adrenergic receptor activity in the former.[56] These differences were also noted in obese individuals.[57] Another study reported that the higher basal and adrenaline-stimulated lipolytic rate in abdominal subcutaneous and visceral depots in women with central obesity were less responsive to the suppressive effects of insulin when compared with those with peripheral obesity.[58] One possible explanation for this is the increased protein–tyrosine phosphatase 1B activity in visceral depot compared with subcutaneous depot.[59] A recent lipidomic study that focused on subcutaneous and omental depots in obese human adults revealed depot-specific differences in glycerophospholipid

levels, degree of saturation of lipid chains and oxysterol accumulation, which likely reflect the differential capacity in adipogenesis, lipolysis and endoplasmic reticulum stress reponse, respectively.[60] These differences were much smaller in lean individuals,[60] suggesting a depot-specific lipidomic response to excess WAT accumulation. The effects of sex hormones on WAT heterogeneity in lipid handling have been well documented,[61] but relatively few studies looked at the effects of ethnicity.[58]

Inflammation

The depot-specific expression of pro-inflammatory and anti-inflammatory cytokines in humans has been extensively reviewed by Lee *et al.*[62] Compared with subcutaneous WAT depots, visceral WAT depots in general display a more pro-inflammatory profile[62] with greater secretory capacity.[63] Although obesity led to increase in expression of pro-inflammatory cytokines in both depots,[64] visceral depot retained its dominance in secreting these cytokines, including IL-6,[53] TNF-α,[65] IL-8,[65] C-reactive protein,[65] complement C3,[65] macrophage migration inhibitory factor[66] and CC chemokine receptor 2.[66] Conversely, subcutaneous WAT was shown to express more adiponectin,[66] an adipocyte-derived adipokine that exerts anti-inflammatory and multiple beneficial effects on metabolism,[67,68] in both lean and obese states, although at a much lower level in the latter. In line with this, several studies showed increased macrophage infiltration in obese visceral depots compared with subcutaneous depots,[69] whereas others demonstrated elevated mitogen-activated protein kinase- and inhibitory-κB kinase-mediated inflammatory response predominantly in the visceral depot upon TNF-α stimulation.[70,71]

MECHANISTIC EVIDENCE FOR WAT HETEROGENEITY

In addition to the mounting evidence from human association studies, accumulating findings from *in vitro* and *in vivo* studies in animals provide further mechanistic insights in WAT heterogeneity, which can potentially be translated into therapeutic applications.

Developmental origin and adipogenesis

Similar to humans, WAT in mice have depot-specific developmental origins, suggesting a possibly conserved developmental program. This is evidenced by the high resemblance in the depot-specific expression profiles of developmental genes between genetically obese *ob/ob* mice and humans.[43,72] In a recent study, which applied delicate cell-sorting strategies, varying proportions of adipocytes specifically from visceral depots in mice were found to arise from Wilm's tumor (Wt1)-positive mesothelial progenitors during embryogenesis.[73] Adipocytes from subcutaneous depot probably originate from other sources of progenitor via different mechanisms. Indeed, low-density lipoprotein-related protein (LRP)-5 of the WNT signaling cascade appears to be a major regulator for fat distribution among subcutaneous depots. In humans, gain of function mutation in the LRP5 allele was associated with fat accumulation in the lower body, better metabolic health and reduced WAT inflammation.[74] In line with this clinical report, the knockdown of LRP5 by short hairpin RNA severely impaired adipogenesis and increased inflammation through the canonical β-catenin pathway in SVF isolated from gluteal WAT depot but not from abdominal subcutaneous depot,[74] partially explaining the predisposition to different fat distribution patterns and cardiometabolic risks. Unlike in mice,[75] manipulation of LRP5/WNT in these human preadipocytes did not affect insulin signaling.[74]

Another piece of evidence for depot-specific developmental program came from a study on 14-3-3ζ, an adaptor protein involved in metabolic regulation, autophagy and apoptosis.[76] Mice deficient in 14-3-3ζ displayed marked reduction only in visceral depot, which contributed to reduced body weight and fat mass, as well as improved glucose tolerance and insulin sensitivity, whereas overexpression resulted in the opposite effect.[77] 14-3-3ζ was necessary for adipogenesis in visceral WAT, and loss of 14-3-3ζ led to autophagy-dependent degradation of adipogenic factors and disruption of cell cycle progression.[77] However, there was no evidence on the temporal control of adipogenesis, and the metabolic consequences in these mice were likely altered by the catch-up growth since the age of ~3 weeks.[77] Besides, it is still unclear how subcutaneous depots were spared from the effects of global 14-3-3ζ deficiency.

The role of microRNA (miRNA) in WAT development and adipogenesis is well recognized in multiple species including humans,[78] mice[78] and bovine.[79] The depot-specific expression pattern of miRNA has also been implicated in obese[80] or diabetic[81] humans. The processing and maturation of miRNA requires the RNA-processing enzyme Dicer.[82] The expression of Dicer was previously found to be constantly declining over time in *Caenorhabditis elegans* as well as in subcutaneous WAT of mice, likely because of calorie intake and aging.[83] A follow-up study using mice with adipose-specific deficiency in Dicer clearly showed depot-specific effects of miRNA dysregulation on the development and metabolism of WAT, which were exacerbated when challenged by high-fat diet.[84] The authors successfully identified miRNAs that were responsible for the drastic phenotypes globally (miR346 and miR362) and in BAT (miR-365).[84] It is reasonable to speculate the existence of depot-specific expression profile and even secretion pattern of miRNAs, which orchestrate the complex development and endocrine function of the adipose organ.

Although it was shown in obese humans that hypertrophy occurred in visceral WAT while hyperplasia was predominant in subcutaneous WAT,[85] two recent independent studies showed a different scenario in mice.[86,87] Wang *et al.*[86] developed a triple transgenic, 'Adipochaser' mouse model that utilizes adipose-specific Cre-loxP- and LacZ-doxycycline-inducible systems to monitor the development and adipogenesis of WAT at a cellular level. The authors found that adipocytes in visceral depot started the differentiation process postnatally, whereas those in subcutaneous depot differentiated during prenatal stage at a slower rate and completed well after

birth.[86] The expansion of WAT initially occurred through hypertrophy in both depots in response to high-fat diet, but switched to hyperplasia after ~1 month only in the visceral depot.[86] This seemed counterintuitive as hyperplasia is linked to more healthy expansion of WAT, although visceral depot expansion is metabolically detrimental. Similarly, a tamoxifen-inducible adipocyte-tracking mouse model showed a rapid but transient induction of adipocyte precursor proliferation and Akt2-dependent differentiation only in visceral WAT depot from diet-induced obese or *ob/ob* mice, which resulted in an increased adipocyte number not observed in subcutaneous depot.[87] On the contrary, Macotela *et al.*[88] found that despite there are more adipocyte precursor cells in the SVF from visceral depot, those in subcutaneous depot seemed to have a greater potential to differentiate in response to classical induction cocktail than in visceral depot, which required bone morphogenetic protein (BMP)-2 or BMP-4 supplementation to achieve similar effects. However, the pathological relevance in these *in vitro*-based findings remained to be validated. Currently, the precise mechanism of depot-specific activation of adipocyte precursor cells is still poorly understood, and the effects of hyperplasia or hypertrophy on metabolic parameters such as insulin sensitivity remain unclear. More studies are needed to reveal the significance of cellular heterogeneity in WAT in these aspects.

Metabolism

The 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1)-deficient mouse is a classic model to demonstrate a healthier fat accumulation pattern and improved metabolism driven by depot-specific mechanisms.[89] Upon feeding with high-fat diet, subcutaneous WAT of 11β-HSD1-deficient mice displayed increased expansion, better insulin sensitivity and reduced adipocyte hypertrophy, whereas visceral depots had increased AMPK activity and decreased infiltration of macrophage and T lymphocyte, but not dramatic changes in volume.[89]

Whole-adipose-tissue transplantation in mice allows the assessment of metabolic properties of isolated WAT depots (Figure 2). Konrad *et al.*[90] reported that intraperitoneal transplantation of lean visceral depot into C57B6/J mice improved, rather than impaired, glucose tolerance and insulin sensitivity, suggesting that the functionality of WAT, rather than anatomical location or fat mass, is the major determinant for the association with different metabolic risks. In contrast, other groups have shown that the intraperitoneal transplantation of subcutaneous depot instead of visceral depot conferred metabolic benefits in the recipient mice.[91–93] The transplantation of subcutaneous depot into the visceral cavity of obese mice also improved hepatic insulin sensitivity.[94] Another transplantation study showed that the removal of visceral depot or intraperitoneal implantation of visceral depot protected rats from aging-related glucose intolerance.[95] This was achieved through enhanced insulin secretion and transient induction of fasting leptin level to improve insulin sensitivity.[95] It would be interesting to see whether transplanting visceral depot will have any effects on aging mice. The underlying mechanisms of transplantation-mediated metabolic changes are still incompletely understood. All of the above studies noted changes in glucose metabolism or insulin sensitivity, but none reported alterations in lipid profile or metabolism, suggesting that fat graft did not simply act as an extra lipid reservoir. Although leptin was shown to be essential in rescuing the metabolic dysfunction in lipoatrophic A-ZIP/F-1 mice that received fat transplantation,[96] evidence for its contribution in the above depot-specific metabolic effects is limited and inconsistent.[92,95] Collectively, these transplantation studies generally support a cell-autonomous beneficial role of subcutaneous depots in the overall metabolism, but the molecular basis remains poorly understood.

Inflammation

WAT macrophages are subjected to phenotypic polarization in obesity, resulting in an increase in classically activated, pro-inflammatory M1 macrophages and reduction in alternatively activated, anti-inflammatory M2 macrophages.[97] However, this process appears to occur in different extents and through different mechanisms in various WAT depots.

Fyn is a Src family non-tyrosine kinase with a wide range of biological functions, participating in immunological[98] and metabolic processes.[99] Mice deficient in Fyn were not protected against diet-induced obesity. Instead, they exhibited preferential fat partitioning into the subcutaneous depots and, intriguingly, improved lipid profile, glucose tolerance, insulin sensitivity and reduced systemic inflammation.[100] There was less macrophage infiltration and increased M2/M1 ratio in visceral WAT, which were accompanied by reduced infiltration of T lymphocytes.[100] This finding demonstrates a possible crosstalk between WAT depots, where, in the absence of Fyn, excess lipids were redirected to subcutaneous depots, which experienced a healthier expansion, sparing the less healthy visceral depots from developing inflammation due to lipid accumulation.

Interferon regulatory factor 5 (IRF5), another major factor in the immune system, has been implicated T_H17 response[101], which was observed in inflamed WAT from obese humans and mice.[102] Interestingly, IRF5 expression was selectively induced in macrophages in obese visceral depots and continued to rise over time.[103] Knocking out IRF5 both globally and selectively in macrophages promoted M2 polarization and anti-inflammatory response selectively in visceral WAT, and on the other hand stimulated adiposity in subcutaneous WAT.[103] Concomitantly, glucose tolerance and insulin sensitivity were restored in IRF5-deficient mice.[103] The metabolic actions of Fyn and IRF5 may share similar pathways as the deficiency in either protein led to similar fat-partitioning and metabolic improvement through macrophage polarization. The adaptor protein 14-3-3ζ is known to regulate monocyte migration upon redox metabolic priming[104] and macropinocytosis for the modulation of macrophage-related inflammatory response.[105] It would be interesting to investigate whether 14-3-3ζ modulates metabolism by having a role in macrophage infiltration and polarization in WAT, in addition to its contribution in WAT development.[77] MitoNEET, a membrane

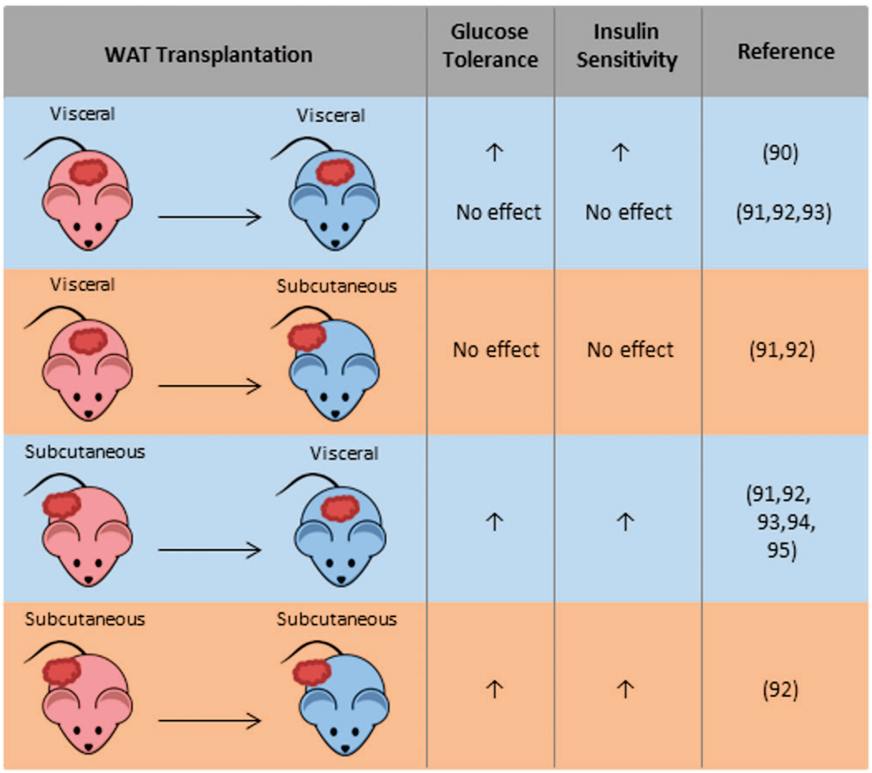

Figure 2 Impacts of fat transplantation on metabolism and insulin sensitivity in mice. Subcutaneous or visceral depots from donor mice were transplanted subcutaneously or intraperitoneally into recipient mice, which were then assessed for glucose tolerance and insulin sensitivity.

protein located in the outer mitochondrial membrane, was also shown to modulate the polarization of macrophages in WAT with depot specificity.[106] When overexpressed in WAT, it reduced macrophage infiltration and stimulated M2 polarization selectively in subcutaneous depot,[106] where it also improved insulin response and promoted lipid uptake.[106,107]

The importance of T lymphocytes in the development of obesity is being increasingly recognized. Recently, adipocyte class II major histocompatibility complex-dependent activation of CD4+ T cell was reported to drive adipose tissue inflammation upon high-fat diet treatment, which surprisingly preceded the actions of macrophages.[108] Signal transducer and activator of transcription 3 in T cells was responsible for the high T helper 1/Foxp3$^+$ T regulatory ratio and the resultant pro-inflammatory response in obese adipose tissue, at least in the visceral depot.[109] The accumulation of Foxp3$^+$ T regulatory cells in visceral depots was further explored in lean aging mice.[110] However, evidence for these processes in subcutaneous depot is relatively scarce; thus, it is unknown whether the reduced inflammatory state in this depot is due to an inversely skewed T-cell response.

Intriguingly, despite the mounting evidence demonstrating a deleterious role of adipose tissue inflammation in metabolic syndrome, this response seems to exert paradoxically beneficial effects on the expansion of adipose tissue challenged with high-fat diet, again with interdepot differences.[111] This raises the possibility that the magnitude and duration of inflammatory response rather than its nature is more important in obese adipose tissue.

Browning

Browning is the development of thermogenic Beige cells in WAT in response to environmental stimuli such as low temperatures, exercise and treatment with peroxisome proliferator-activated receptor γ (PPARγ) agonists.[112] WAT browning positively correlates with improved overall metabolism as a consequence of increased energy expenditure and insulin sensitivity, and weight loss.[112] It is widely accepted that browning occurs almost exclusively in the subcutaneous depots of WAT,[112] which supports the notion that subcutaneous WAT confers more metabolic benefits. PRDM16, a transcription co-regulator responsible for brown adipocyte development in classic BAT depot,[113] has unique roles that distinguish subcutaneous WAT from visceral WAT in browning capacity. Compared with visceral WAT, PRDM16 was highly expressed in subcutaneous WAT, where its overexpression markedly promoted browning in a cell-autonomous manner[114] likely through *de novo* adipogenesis.[86] Conversely, lack of PRDM16 sharply reduced the extent of browning in subcutaneous WAT.[114,115] The browning effects mediated by PPARγ agonist rosiglitazone also required PRDM16 specifically in subcutaneous WAT.[116] Further studies are required to unveil whether other factors involved in browning, including UCP-1 and PPARγ co-activator 1-α (PGC-1α), share similar

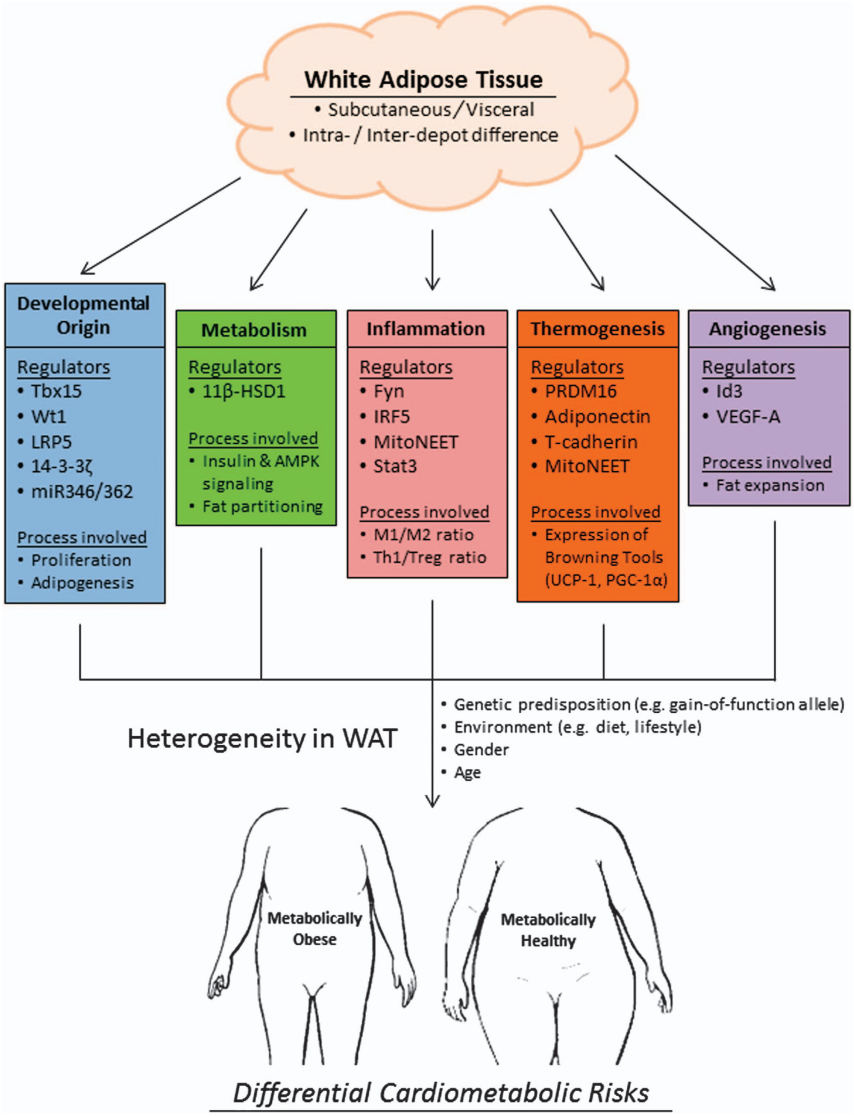

Figure 3 Currently identified regulators and processes that give rise to heterogeneity in white adipose tissue (WAT) which, together with environmental factors, in turn contribute to differential cardiometabolic risks.

depot-specific regulatory network. Notably, gene expression profiling and lineage studies using immortalized adipocytes showed that thermogenic genes not only display an interdepot variation, but also intradepot difference, giving rise to subpopulations of adipocytes with greater browning capacities within the same subcutaneous depot.[117]

M2 macrophages promote WAT browning in mice by inducing catecholamine production, and the expression of tyrosine hydroxylase and thermogenic machineries, including UCP-1 and PRDM16.[118–120] The depot-specific browning capacity may arise from the marked induction of adiponectin expression selectively in subcutaneous WAT, which in turn stimulated the local *de novo* proliferation of M2 macrophages.[120] The crucial actions of adiponectin were assisted by elevated local levels of T-cadherin,[120] a glycoprotein anchored on the plasma membrane that facilitates the intracellular trapping of adiponectin.[121,122] In line with these, overexpression of mitoNEET in WAT caused a selective upregulation of adiponectin expression in the subcutaneous depot, which was accompanied by M2 macrophage polarization and increased browning.[106,107] It is unclear how the expression of adiponectin is controlled in different WAT depots, but epigenetic regulation is a highly possible mechanism, as it was recently shown that obesity-induced cytokine production in visceral WAT suppressed the expression of adiponectin via DNA methylation at its promoter region.[123]

Angiogenesis

As with other organs, the expansion of WAT requires angiogenesis,[124] stimulated either through metabolic and developmental cues or by hypoxic signals due to adipocyte hyperplasia and hypertrophy. Angiogenesis is implicated in the pathogenic expansion of WAT in humans, which leads to cardiometabolic diseases.[125] Indeed, visceral adiposity was

associated with elevated serum levels of vascular endothelial growth factor in humans.[126] Inhibitor of differentiation 3 (Id3), a helix–loop–helix factor that regulates tumor and angiogenesis,[127] was found to have a role in high-fat-diet-induced angiogenesis, specifically in the visceral depots in mice,[128] through unknown mechanisms. Deficiency in Id3 abolished the upregulation of vascular endothelial growth factor-A expression in obese visceral WAT and resulted in reduced weight gain.[128]

THERAPEUTIC APPROACHES TARGETING WAT HETEROGENEITY

A majority of current antiobese strategies, including lifestyle intervention,[129] drug treatment,[130] exercise and metabolic surgery,[131] reduces overall obesity without targeting any particular depot. These approaches either have compliance problem or adverse side effects, or they fail to combat weight regain. In other cases, a direct physical removal of subcutaneous fat is performed by liposuction and cosmetic surgery. However, lipectomy of subcutaneous WAT may induce subsequent enlargement in the visceral depot,[132] which can be more damaging to the metabolism. Although intriguing, increased peripheral adiposity may not always be harmful to metabolism. In fact, an expansion of subcutaneous WAT, particularly in the lower body, can perhaps be beneficial to individuals with visceral adiposity, at least in terms of insulin sensitivity, as supported by the human and animal studies above. Thus, a region-specific healthy expansion of adipose tissue or fat distribution remodeling mediated by PPARγ agonists or 11β-HSD1 inhibitors might be the choice of therapy for obesity. These have been proven efficacious at least in rats.[133,134] However, it cannot be neglected that morbid peripheral obesity, despite metabolically healthy, still correlates with increased mortality.[1,2]

WAT browning is a promising therapeutic strategy in combating obesity owing to its efficacious outcomes as evidenced by a recent human study.[135] Pharmacological agents that promote WAT browning in animal or cell models include sympathetic activators, prostaglandins, natriuretic peptides, retinoids, thyroid hormones, AMPK activators, fibroblast growth factor 21 (FGF21) and BMPs.[136] Although their efficacy may appear encouraging in animal models, these agents were developed only recently and shall require further investigations to avoid any possible adverse effects in humans. The recent mechanistic evidence that explains the depot-specific origin and activation of Beige adipocytes forms a strong foundation for the development of potent targeted therapies with fewer side effects.

Adipose angiogenesis, on the other hand, has recently become a popular target for treating obesity.[125] It is generally believed that inhibition of angiogenesis in adipose tissue can limit its growth, and hence alleviate obesity and the related complications. However, this may selectively limit the protective buffering effect of the healthier subcutaneous adipose tissue because of its greater angiogenic ability[137] and hence a higher sensitivity to inhibition. Furthermore, it may cause hypoxia in adipose tissues, which will exacerbate the inflammatory response, leading to a futile cycle. Depot-specific manipulation of angiogenesis via targets such as Id3 may therefore be an alternative approach to achieve a healthy fat partitioning and improved metabolism.

CONCLUDING REMARKS

WAT is a highly heterogeneous organ. Depot-specific differences are present in rodents, rabbits, guinea pigs, humans and other mammals, indicating a strong evolutionary advantage. The apparent compartmentalization of adipose tissue in the body roots from the differential developmental origin of the precursor cells in different and within depots. This inter- and intradepot heterogeneity is gradually amplified during WAT expansion by cell-autonomous differences in the dynamic turnover of adipocytes, regulation of lipid flux and metabolism and also cytokine profile. All these factors are influenced by genetic predisposition, environment, gender and age. Together, they define the tendency and mode of the development of obesity, which in turn determine the susceptibility to a range of cardiometabolic diseases (Figure 3). Finally, it should be noted that the adipose organ has been grossly categorized into subcutaneous and visceral depots to fit the scope of this review, and does not entirely reflect the real situation in animals or humans. In particular, the unique anatomical and physiological roles of epicardial[138] and perivascular adipose tissue,[139] two subdepots of visceral fat, have further contributed to the complex concept of depot specificity of WAT.

Further studies to refine current knowledge on the heterogeneity of WAT are required before the clinical implementation of WAT depot-specific therapies to combat obesity-related cardiometabolic diseases. In particular, prospective studies that examine the association between each of the fat depot and cardiovascular risks will help to define the roles of each individual fat depot in the onset and/or progression of the diseases. In addition, further genetic and biochemical manipulation of the identified key factors that give rise to the heterogeneity in WAT may provide further mechanistic insights on the origin, development and physiological functions of each WAT depot.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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