REVIEW

Dysmetabolic adipose tissue in obesity: morphological and functional characteristics of adipose stem cells and mature adipocytes in healthy and unhealthy obese subjects

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Abstract

The way by which subcutaneous adipose tissue (SAT) expands and undergoes remodeling by storing excess lipids through expansion of adipocytes (hypertrophy) or recruitment of new precursor cells (hyperplasia) impacts the risk of developing cardiometabolic and respiratory diseases. In unhealthy obese subjects, insulin resistance, type 2 diabetes, hypertension, and obstructive sleep apnoea are typically associated with pathologic SAT remodeling characterized by adipocyte hypertrophy, as well as chronic inflammation, hypoxia, increased visceral adipose tissue (VAT), and fatty liver. In contrast, metabolically healthy obese individuals are generally associated with SAT development characterized by the presence of smaller and numerous mature adipocytes, and a lower degree of VAT inflammation and ectopic fat accumulation. The remodeling of SAT and VAT is under genetic regulation and influenced by inherent depot-specific differences of adipose tissue-derived stem cells (ASCs). ASCs have multiple functions such as cell renewal, adipogenic capacity, and angiogenic properties, and secrete a variety of bioactive molecules involved in vascular and extracellular matrix remodeling. Understanding the mechanisms regulating the proliferative and adipogenic capacity of ASCs from SAT and VAT in response to excess calorie intake has become a focus of interest over recent decades. Here, we summarize current knowledge about the biological mechanisms able to foster or impair the recruitment and adipogenic differentiation of ASCs during SAT and VAT development, which regulate body fat distribution and favorable or unfavorable metabolic responses.

Keywords ASCs · Adipose tissue · Healthy obese · WNT · BMP · Sirtuins · Insulin signaling · FAM13A · microRNAs

Introduction

White adipose tissue (WAT), traditionally regarded as the primary storage organ of energy in the form of triglycerides, is now considered as an important endocrine tissue that secretes biologically active molecules, known as adipokines, involved in whole-body energy metabolism and systemic inflammation [1]. Although chiefly composed of adipocytes, adipose tissue is also an abundant source of mesenchymal stem cells (adipose tissue-derived stem cells, ASCs), which

provide for the physiological cell turnover and are utilized in cell-based therapy for regenerative medicine purposes [2].

The generation of new adipocytes from ASCs is one of the major factors that fosters the growth of adipose tissue during lifetime. In a lifestyle characterized by a persistent positive energy balance, WAT expands by an increase in size of pre-existing adipocytes (hypertrophy) and by activating progenitors to generate new adipocytes (hyperplasia). Indeed, WAT is characterized by a continuous turnover of the adipocytes with ~ 10% of annual renewal, although the number of new adipocytes added is about twice as high in obese as compared to non-obese subjects [3, 4]. Adipose tissue undergoes a continuous remodeling process that is pathologically accelerated in the obese state and featured by coordinate increases of adipocyte size and number, with impaired angiogenic remodeling and subsequent increased immune cell infiltration and extracellular matrix (ECM) overproduction [5]. When obesity is sustained by a positive energy balance, adipose tissue becomes dysfunctional

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and is characterized by impaired secretion of adipokines and abnormal lipid storage, with an increased risk of developing obesity complications such as insulin resistance (IR), type 2 diabetes (T2D), obstructive sleep apnea, steatohepatitis, and cardiovascular and cerebrovascular diseases [6, 7].

Obesity-related cardiometabolic diseases are known to be associated with the expansion of visceral adipose tissue (VAT), whereas the increased amount of subcutaneous adipose tissue (SAT) does not raise these risks and may be even protective [8, 9]. Recently, several studies indicate that the SAT expansion capacity is limited and genetically determined in every individual. Once this capacity is exceeded, any excess circulating triacylglycerol will be stored in an undesirable fat depot, fostering the expansion of VAT, or in non-adipose tissues (e.g., liver, muscle) leading to liver steatosis and increased intramyocellular lipid content and thus promoting the cardiometabolic complications of obesity [10, 11] (Fig. 1). In contrast, the ability of SAT to store the excess of fat rather than allowing it to accumulate in ectopic depots is a major determinant for a healthy obesity status. Therefore, it has been suggested that the disease risk associated with obesity is not uniform, since obese individuals with higher SAT expansion and characterized by the absence of metabolic abnormalities may be referred as metabolically healthy (metabolically healthy obesity, MHO) (Fig. 1) [12]. The "subcutaneous adipose tissue expandability hypothesis"

argues that there are molecular mechanisms governing adipose tissue expansion that act through the promotion of adipogenesis by resident ASCs. The ability to recruit and differentiate ASCs into mature adipocytes in SAT, fostering its expansion, is under genetic regulation and is influenced by the environment (i.e., changes in nutrient load and energy expenditure). Here, we summarize the inherent depot-specific differences of subcutaneous and visceral ASCs and discuss the major biological mechanisms underlying the impaired adipogenesis and expansion of SAT under conditions requiring the need to store excess lipids.

Depot-specific characteristics of adipose tissue

Adipose tissue plays a fundamental role in controlling the flow of circulating fatty acids in the post-prandial period. Insulin acts on adipose tissue by stimulating storage of triglycerides through multiple mechanisms, including increasing the uptake of glucose and fatty acids derived from circulating lipoproteins and inhibiting lipolysis in adipocytes, and, in the long term, promoting lipogenesis in mature adipocytes as well as differentiation of preadipocytes [13]. Moreover, adipose tissue has been recognized as an active endocrine organ that express and secrete a variety of
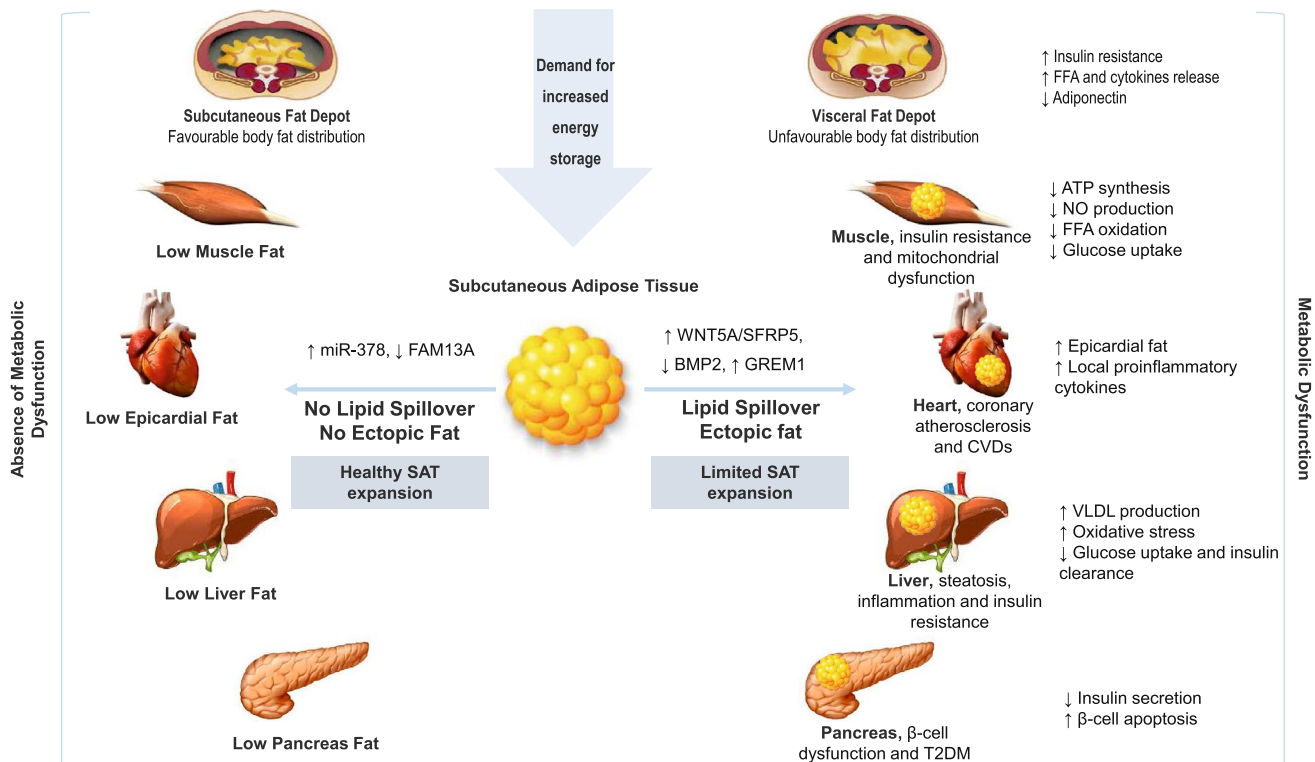

Fig. 1 Subcutaneous adipose tissue remodeling in response to demand for increased energy storage in healthy and unhealthy obesity

bioactive peptides, known as adipokines, which act at both the local (autocrine/paracrine) and systemic (endocrine) levels [14, 15].

The key role of the endocrine function of adipose tissue is emphasized by the adverse metabolic consequences derived by both adipose tissue excess and deficiency. Excess accumulation of WAT, particularly in the visceral compartment, is associated with increased pro-inflammatory adipokines that bring about several cardiometabolic abnormalities such as IR, T2D, dyslipidemia, non-alcoholic fatty liver disease, and hypertension [16]. Interestingly, lipodystrophy, characterized by loss of WAT, is also associated with dysregulation of adipokines and severe metabolic complications [17]. Thus, adipose tissue is a complex and highly active metabolic and endocrine organ, the excess or deficiency of which both lead to harmful metabolic consequences.

Body fat tissue is traditionally distributed into two main compartments with different metabolic characteristics: SAT, located under the skin and VAT, located around the digestive organs (mainly mesenteric and omental) [18]. SAT constitutes most of the body's adipose mass (about 80%) and is uniformly distributed at birth; subsequently, it tends to localize differently in the various body regions, mainly as a consequence of sexual development and progression of age [17]. The distinct adipose tissue depots exhibit differences in cellular composition, microvasculature, innervation, and ECM composition, and have a specific metabolic profile and endocrine activity. VAT compared to SAT is more innervated and vascularized, contains a larger number of inflammatory and immune cells [19], and has a large percentage of hypertrophic adipocytes, but few preadipocytes per gram of tissue [20] with a lower differentiation capability compared to SAT [21–24].

Triglyceride turnover in WAT is determined by the balance between lipid storage and lipid removal [3], and different turnover rates between SAT and VAT may cause metabolic complications in obesity [25]. Lipids are stored in adipocytes by esterification of free fatty acids (FFAs) and glycerol to form triglycerides, and FFAs are released from fat cells into the bloodstream through triglyceride hydrolysis (lipolysis). The adverse metabolic impact of visceral fat has been attributed to greater mobilization of FFAs into the portal circulation, impairing liver metabolism and leading to systemic IR [26]. Particularly, higher triglyceride turnover and lipolytic rates in response to catecholamines have been shown in VAT versus SAT in humans. Moreover, visceral adipocytes show lower sensitivity to the anti-lipolytic effect of α2-adrenergic receptor (AR) agonists and insulin compared with subcutaneous adipocytes [27]. With respect to metabolic differences, Virtanen et al. demonstrated that, in the absence of significant differences in tissue blood flow, insulin-stimulated glucose uptake was higher in VAT compared to SAT both in normal-weight and obese subjects [28].

On the other hand, Lundgren et al. showed that glucocorticoids exerted a marked suppression of glucose uptake and expression of insulin signaling proteins in visceral but not in subcutaneous adipocytes [29]. Studies on gene and protein expression have further highlighted the broad differences between SAT and VAT, showing that visceral ASCs and mature adipocytes from non-obese subjects produce more eotaxin, VEGF, IL-6, IL-8, and MCP-1 compared to subcutaneous cells [30–32]. Thus, VAT has a propensity to promote inflammation, which explains the link between VAT expansion, increased secretion of pro-inflammatory cytokines, and impaired insulin signaling and action in central obesity.

Adipose tissue-derived stem cells (ASCs) in adipose tissue

In 2001, multipotent stem cells were identified in the vascular fraction of adipose tissue (stromal vascular fraction, SVF) [33]. ASCs differentiate in vitro towards different lineages belonging to different embryonic origin also different from the mesoderm, such as the neurogenic lineage, thus showing multipotency [34]. The SVF was obtained after digestion of adipose tissue fragments with collagenase followed by differential centrifugations [35, 36], and consists of a heterogeneous cell population, including circulating blood cells, fibroblasts, pericytes, endothelial cells, macrophages, and preadipocytes in different stage of differentiation. In 2004, the International Fat Applied Technology Society (IFATS) adopted the term "adipose tissue-derived stem cells" (ASCs), to define plastic-adherent cells with multilineage capacity isolated from the SVF of adipose tissue. However, during the procedure of SVF isolation, two additional fibroblast-like cell populations can be isolated from the floating layer, exhibiting the potential of unlimited self-renewing proliferation, as well as of multiple differentiation potential along the mesenchymal lineage [2, 31].

In culture, ASCs express cell-surface markers similar to those expressed by mesenchymal stem cells (MSCs), including CD105, CD90, and CD44. Although an exact phenotypic characterization of ASCs is still in development, ASCs can be identified by the expression of CD49d that is otherwise absent in non-adipose MSCs cultures [31]. However, there are still significant gaps in understanding the origin and in vivo location of ASCs, since there is no marker that is uniquely associated with undifferentiated ASCs [26]. From studies in rodents, it has been suggested that adipocytes descend from a pool of proliferating progenitors that are already committed, either prenatally or early in postnatal life [37]. In a recent study, perilipin-positive or adiponectin-positive preadipocytes were found at embryonic day 16.5 in adipose tissue, and these cells underwent active proliferation

until birth. Moreover, these preadipocytes resided as clusters and were distributed along growing adipose vasculatures [38]. Importantly, the embryonic preadipocytes exhibited considerable co-expression of stem cell markers, such as CD24, CD29, and PDGFRA, while a small portion of preadipocytes were derived from PDGFRB-positive mural cells of the adipose vasculature, as a subset of both pericytes and capillary endothelial cells, distinct from the adult preadipocytes present in the SVF. Thus, the adipose vasculature appears to function as a progenitor niche and may provide signals for adipocyte development [38]. In adipose tissue, perivascular stromal cells are organized in two discrete layers, the innermost consisting of pericytes positive for CD146 and α-SMA and negative for CD34, and outermost represented by supra-adventitial ASCs negative for CD146 and α-SMA and positive for CD34, both showing adipogenic potential in vitro. Pericytes exhibit a stronger adipogenic potential, followed by the more numerous supra-adventitial ASCs. Moreover, only the α-SMA-positive cells seem to show multilineage differentiation ability, while the α-SMA-negative cells can differentiate only into adipocytes [39].

In addition, evidence suggests that SAT and VAT have distinct metabolic properties probably due to inherent characteristics of the ASCs that are resident in these fat depots [26]. Indeed, the distinct features of these cells are also maintained in vitro, since ASCs retain the memory of the depot of origin [21, 31, 36] and even the effect of drug treatment in vivo, as shown in an animal model of metabolic syndrome [40].

Noteworthy, the multipotency attributable to ASCs is also referred to their ability to acquire a brown phenotype, thus giving rise to brown adipose tissue (BAT). The presence of BAT is essential to warrant energy balance in terms of heat production through fat burning, a critical event named thermogenesis that is typically mediated by mitochondrial uncoupling protein-1 (UCP-1) activation [41]. However, this property has a trend to weaken with obesity [42] and aging, and in the latter case, it achieves only 1% in adulthood [43]. The source of brown fat cells changes according to anatomical locations; for instance, interscapular and perirenal BAT appear to be derived from myf5-expressing myogenic precursors; meanwhile, BAT within WAT appears to derive from myf5-negative cells and to have different features, such as greater sensitivity to β3-adrenergic stimulation and cold exposure [44–46]. In addition, it has been reported that in specific adult human WAT depots, such as the periadrenal fat, two distinct brown and white adipose lineages coexist and that upon the local hormone condition as in the case of catecholamine-secreting pheochromocytoma, the equilibrium among the two lineages will lead toward the expansion of BAT or WAT, respectively [47].

As previously mentioned, BAT is a metabolically active tissue whose activity is associated with improved glycemic status in healthy individuals regardless of age, sex, and adiposity. The ability of BAT to regulate glucose homeostasis is maintained in the basal state (i.e., fasting and thermoneutrality) and is accelerated by insulin and cold exposure that are known stimuli able to enhance glucose disposal [45]. In light of this evidence, findings from cross-sectional investigations observed that BAT functions correlate with clinical indexes of cardiometabolic health [41], thus considering this tissue as a potential target for the treatment of obesity and metabolic diseases. Therefore, it has been hypothesized that a metabolically healthy condition could depend on whole-body activity of BAT. However, to date, results obtained from mice and human studies regarding BAT distribution in both SAT and VAT are controversial. In particular, it has been observed that the expression of browning genes in obese mice is greater in SAT rather than VAT, while an opposite pattern of browning mediators (i.e., UCP-1, PPARG1A, etc.), with VAT having higher expression than SAT, was observed in humans with severe obesity [48, 49]. Consistently with previous results obtained from obese women [50], recent data from severe obese subjects reported a simple correlation between resting energy expenditure (REE) and waist, a measure of VAT, and identified a BAT-related or 'brite' signature in visceral depot in association with increased mitochondrial biogenesis markers [51]. These data suggest that increased REE and browning in metabolically complicated severe obesity could represent an effort to counteract further weight gain. Conversely, Lim J. et al. have recently observed that UCP-1 mRNA levels in VAT were significantly higher than in SAT in both non-diabetic and diabetic obese patients [52]. Moreover, in agreement with the previous report [53], a negative correlation between UCP-1 mRNA expression in SAT and several obesity-related metabolic parameters (i.e., BMI, HOMA-IR, visceral fat area, insulin, etc.) was noted in obese individuals [52]. In VAT from obese subjects, mRNA levels of UCP-1 were found to be inversely correlated with the VAT/SAT ratio, fasting glucose, and triglycerides, thus suggesting that visceral obesity may be aggravated when UCP-1 is down-regulated in VAT [52]. Finally, these results suggest that depot-specific patterns of UCP-1 expression in human WAT could be a pathognomonic feature of human obesity and obesity-related metabolic diseases. Nevertheless, the extent of 'brite' signature in both SAT and VAT needs to be still elucidated in human obesity.

Adipose tissue expandability

The World Health Organization (WHO) defines obesity as a complex metabolic disorder with multifactorial etiology, characterized by adipose tissue expansion in response to excess caloric intake and defined by a body mass index (BMI) > 30 kg/m^2 [54]. However, the risk for obesity-related comorbidities is not uniform, since cardiometabolic

abnormalities develop with a different extent in obese individuals with the same degree of BMI [55]. IR, T2D, and cardiovascular disease (CVD) are less likely to develop when fat accumulation occurs in the lower body (gluteo-femoral depots). In contrast, upper body adipose tissue expansion (abdominal depot) is characterized by high risk to develop cardiometabolic diseases [8, 9]. Several studies showed that the determinant of increased cardiometabolic risk is not the degree of obesity per se, but the distribution of fat and, in particular, its accumulation in visceral compartment and insulin-sensitive organs or tissues [56]. In WAT, the capacity to store and mobilize triglycerides is subjected to broad region-specific differences. SAT has a greater storage capacity as compared to VAT and is the first depot responding to lipid excess. However, in human subjects, the capacity of SAT to store lipids is limited, such that when this limit is exceeded, storage of lipids in VAT occurs [57, 58] (Fig. 1). Thus, although the triglyceride storage capacity of SAT is higher than VAT, in subjects with expansion of central or visceral adipose tissue and classified as metabolic unhealthy, the lipid turnover is higher in VAT than SAT [59]. In addition, using radiocarbon dating methods to investigate triglyceride storage, Spalding et al. provided evidence that the impact of progressively higher BMI is selective only for VAT and that lipid turnover in SAT is not influenced by different degrees of BMI. Indeed, excessively obese individuals had a visceral triglyceride age in VAT higher than healthy subjects, due to a lower lipid removal capacity, while no difference was observed in SAT between healthy and unhealthy subjects [58]. These findings support the importance of the adipose tissue expandability, highlighting that ectopic fat deposition is a result of an insufficient increase in the number and size of SAT adipocytes (Fig. 1). Indeed, differences in depot-specific lipid accumulation depend on hyperplasia and hypertrophy or the combination of both [60]. In obesity-prone C57BL/6 mice under high-fat diet, during the initial phase of obesity, SAT expands through hyperplasia, while hypertrophy preferentially occurs in VAT [61]. However, in AdipoChaser mice, an inducible labeling model of adipogenesis in vivo where the formation of new adipocytes is tracked by the expression of β-galactosidase, a high capacity for adipogenesis at more prolonged stages of high-fat diet was apparent in VAT, whereas SAT maintained an extremely low rate of adipogenesis [60]. These results have been confirmed in humans, supporting the concept that morbid obesity results from impaired adipogenic capability of SAT, with a limited hypertrophy of VAT cells displaying a pathological expansion through adipogenesis [62, 63].

Interestingly, regional differences in lipid storage ability in obesity could also be related to a specific genetic background. It is well known that both SAT and VAT express a similar set of genes whose expression levels change according to local distribution and BMI [23, 64]. As recently demonstrated, VAT from obese adolescent females shows an aberrant methylation profile of genes involved in the insulin signaling pathway (i.e., PI3K/Akt) and mitochondrial function (i.e., TFAM) as compared to VAT from lean controls, an event that promotes adipose tissue dysfunction [64]. In addition, specific single-nucleotide polymorphisms (SNPs) may affect VAT oxidative capacity, particularly during fat expansion. In particular, obese carriers of specific SNPs in antioxidant defense genes, such as superoxide dismutase and catalase, show an enhanced body fat distribution as well as a wide visceral area [65]. Accordingly, a recent large-scale genome-wide association study (GWAS) performed on VAT from individuals with different grades of BMI found a new polymorphism in hydroxymethylbilane synthase (*HMBS*, rs1799993), a gene involved in adipogenesis via modulation of mitochondrial respiratory activity [66], which may stimulate adipocyte differentiation and VAT expansion [67]. Similarly, Wang et al. found significant associations of VAT and VAT/SAT ratio with genetic variations in rs671 of aldehyde dehydrogenase-2 (ALDH2) and rs4846567 near lysophospholipase-like 1 (LYPLAL1), genes typically involved in mitochondrial function and lipid metabolism, as well as in rs17782313 near melanocortin 4 receptor (MC4R), a gene known to predict the individual susceptibility to obesity [68]. Furthermore, data derived from a large genetic study support the hypothesis that VAT biology is indeed linked to a detrimental metabolic profile [69], since 202-variant and 144-variant polygenic scores were associated with higher VAT/SAT ratio and an unfavorable cardiometabolic risk [70]. Among all these candidate genes, new 49 loci were recently discovered to be associated with waist-to-hip ratio (i.e., FAM13A rs9991328; FGFR4 rs6556301; BMP2 rs979012, etc.), many of which are implicated in adipogenesis, angiogenesis, transcriptional regulation, and IR, as observed in a recent analysis of genetic architecture performed in 224,459 individuals [71]. Moreover, some genetic variants linked to obesity could also be affected by sex dimorphism as exemplified by a genome-wide analysis in which a significant association between rs1659258 variant at chromosome 2 and higher VAT and altered lipid profile (i.e., lower HDL) was observed in women but not in men [72]. Therefore, these data support the concept that metabolic abnormalities during obesity may derive not only from a limited expandability of SAT, but also from a genetic susceptibility of VAT to respond to energy overload with a compromised insulin sensitivity [73], alterations of fatty acids metabolism [68, 73], impaired oxidant species clearance [65], and enhanced adipogenesis [67, 71]. Other heritable traits could be involved in VAT-related changes in adiposity and metabolic derangement, including the fat mass and obesity-associated (*FTO*) gene, whose rs9939609 variant was recently found to correlate with body fat distribution and specific expansion of visceral fat [74].

A recent study has also highlighted the critical role of inherent features of adipogenic precursor cells in determining the rate of in vivo adipogenesis and differences in tissue expandability of various fat depots. Indeed, in obese adolescents with high VAT/SAT ratio, the lack of expandability of SAT, as assessed by measuring the preadipocyte adipogenic rate in vitro, correlated with down-regulation of key lipogenic/adipogenic genes and increased VAT accumulation [75]. Therefore, based on the fat depot expandability, two obesogenic phenotypes have been identified: "healthy obesity" (metabolic healthy obese, MHO), i.e., obese subjects without metabolic disease, and "unhealthy obesity" (metabolic unhealthy obese, MUHO), i.e., obese subjects with expansion of VAT who develop metabolic alterations [76]. Hence, the identification of both adiposity subgroups is necessary and useful to predict the development cardiometabolic diseases.

During the past decade, we and others have shown that ASCs possess intrinsic depot-specific characteristics in terms of gene expression patterns and biological features [31, 36, 77], yet how ASCs mediate the biological machinery that supports expandability of the adipose tissue is not fully understood. Indeed, understanding the mechanisms controlling ASCs differentiation into mature adipocytes may provide key insights into SAT/VAT expansion. Thus, MHO or MUHO may be characterized by different molecular pathways able to foster or impair adipogenic differentiation of ASCs from distinct fat depots. Knowledge of these pathways may have important implications for clinical interventions and drug development in obesity-related metabolic diseases.

Molecular pathways involved in recruitment and adipogenic differentiation of ASCs

Wingless-type (Wnt) signaling

Wnt proteins play key roles in embryonic development by regulating the staminal precursor's fate and critical steps of metabolic processes [78]. Research conducted over the past decade has established the Wnt/β-catenin signaling pathway as an important player in adipocyte differentiation both in vitro and in vivo. There are 19 proteins belonging to the Wnt family, and these can activate different signaling molecules through autocrine and paracrine mechanisms according to canonical (β-catenin-dependent) or non-canonical (β-catenin-independent) pathways. Particularly, WNT1, WNT6, WNT10A, and WNT10B act in a β-catenin-dependent fashion in adipose tissue homeostasis as potent inhibitors of adipogenesis through prevention of the induction of master adipogenic regulators, such as CEBPA and PPARG [79]. WNT10B is the best prototype for the endogenous inhibitory Wnt, whose expression is higher

in preadipocytes and stromovascular cells and then is rapidly suppressed following induction of adipogenesis [80]. In this regard, it has been shown that the adipose-specific expression of *Wnt10b* also protects against genetic obesity and counteracts adipose tissue inflammation, as observed in obese mice with leptin deficiency and ectopic *agouti* expression [81]. Consistent with this, the previous findings have provided evidence that non-synonymous variants of WNT10B (C256Y) exist in the human population, resulting in loss of protein function which in turn prevents the inhibition of adipogenesis and can induce early onset obesity [82]. In addition, two single-nucleotide polymorphisms (SNP) in WNT10B (rs4018511, rs10875902) were found to be associated with BMI and body weight in male individuals of a case–control Belgian population [83]. In keeping with these findings, lower expression of *Wnt10b* in skeletal muscle, SAT, and derived ASCs of obese Zucker rats could trigger the enhancement of in vitro adipogenesis of both muscular and adipocyte precursors, resulting in SAT expansion and intermuscular accumulation of adipose tissue in vivo [84] (Table 1). However, whether the unhealthy adipose tissue expansion is fostered by an impaired expression of *Wnt10b* in fat and/or skeletal muscle precursors or due to polymorphisms in *Wnt10b* with its loss of function is not entirely clear.

A large body of evidence supports the anti-adipogenic role of canonical WNT proteins, but some studies reported controversial data regarding the implication of non-canonical WNT molecules in adipocyte function. In particular, both β-catenin-independent *Wnt5a* and *Wnt5b* are expressed in undifferentiated adipose cells and appear to be potent enhancers of adipogenesis by stimulation of PPARG and adipocyte Protein 2 (aP2) [85]. WNT5A has a stimulatory effect in the early phase of adipogenesis, since *Wnt5a* is down-regulated 12 h after induction of differentiation in 3T3-L1 preadipocytes, and its knockdown results in decreased adipogenesis and reduced expression of key regulators of adipogenesis, such as PPARG and CEBPA [85, 86]. WNT5B, a paralog of WNT5A, is up-regulated during adipocyte differentiation with the highest expression at day two; overexpression of *Wnt5b* significantly stimulated adipogenesis in murine preadipocytes [87, 88]. To date, data regarding the role of WNT5A in adipogenesis have yielded conflicting conclusions (Table 1). In vitro data ascribed to WNT5A an anti-adipogenic effect both when it was genetically depleted in 3T3-L1 cells [86] and when it was administered to rat ASCs, leading to suppression of lipid accumulation [89]. In contrast, other reports highlighted that any in vivo genetic manipulation of *Wnt5a*, in terms of abrogation or upregulation of gene expression, did not impair body weight, body fat mass, adipocyte size, and expression of adipogenic markers in visceral depots of diet-induced obese mice, but affected adipose tissue inflammation and metabolic dysfunction [90].

Table 1 Changes in main molecular determinants involved in recruitment and adipogenic differentiation of ASCs according to presence or absence of obesity and fat depot: in vivo/ex vivo and in vitro results

Markers	Species	In vivo/ex vivo *Obese vs lean*	In vivo/ex vivo *SAT vs VAT of obese*	In vitro *ASCs, adipocytes differentiated from ASCs(d-ASCs), Isolated adipocytes*
WNT signaling				
WNT10B				
mRNA	Mouse	↓ in SAT of obese vs lean [84]	–	↓ in ASCs from SAT of obese vs lean [84]
WNT5A				
mRNA	Human	↑ in VAT of obese vs lean [90, 91]	↓ in SAT vs VAT [90, 91]	↑ in ASCs from VAT vs VAT adipocytes of obese [91]
SFRP5				
mRNA	Human	↓ in VAT of obese vs lean [91]		
	Mouse	↓ in VAT of obese vs lean [97] ↑ in VAT of obese vs lean [98]	↓ in SAT vs VAT [98]	↓ in ASCs from SAT vs SAT adipocytes [98]
SFRP4				
mRNA	Human	↑ in VAT of obese vs lean [94]	-	
mRNA/protein				↑ in d-ASCs from VAT vs SAT [136]
BMP signaling				
BMP2				
mRNA	Human	↑ in SAT and VAT of obese vs lean [114]	↓ in SAT vs VAT [114]	↑ in ASCs from VAT vs SAT of obese [114] ↑ in adipocytes from VAT vs SAT of lean [114] ↑ in adipocytes from SAT vs VAT of obese [114] ↑ in ASCs vs d-ASCs [114]
BMP4				
protein	Human	-	-	↓ in ASCs vs d-ASCs from SAT of obese [116]
WISP2				
mRNA	Human	↑ in SAT of obese vs lean [115] ↑ in VAT of obese vs lean [121]	↑ in SAT vs VAT [115] ↑ in SAT vs VAT [121]	↑ in ASCs vs d-ASCs from SAT [115]
SIRT				
SIRT1				
mRNA	Human	↓ in SAT of obese vs lean [131, 132]	↔ in SAT vs VAT [132]	↓ in ASCs from VAT of obese vs lean [23]
mRNA/protein		↓ in VAT of obese vs lean [23, 131]		↓ in d-ASCs from VAT of obese vs lean [23]
SIRT2				
mRNA	Human	↓ in SAT of obese vs lean [132] ↔ in SAT and VAT of obese vs lean [131] ↓ in VAT of obese vs lean [23]	↔ in SAT vs VAT [131]	
mRNA/protein				↓ in ASCs from VAT of obese vs lean [23] ↓ in d-ASCs from VAT of obese vs lean [23]
SIRT3				
mRNA	Human	↓ in SAT of obese vs lean [132] ↔ in SAT and VAT of obese vs lean [131]	↔ in SAT vs VAT [131]	-
SIRT6				
mRNA		↔ in SAT and VAT of obese vs lean [131]	–	

Table 1 (continued)

Markers	*Species*	In vivo/ex vivo *Obese vs lean*	In vivo/ex vivo *SAT vs VAT of obese*	In vitro *ASCs, adipocytes differentiated from ASCs(d-ASCs), Isolated adipocytes*
mRNA/protein	Human	↓ in VAT of obese vs lean [140]		
protein	Mouse			↓ in ASCs vs d-ASCs [131]
Insulin signaling				
SLC2A4				
protein	Human		–	↓ in adipocytes of obese insulin-resistant vs lean [154]
mRNA/protein				↑ in d-ASCs from SAT vs VAT [23, 24]
WISP1				
mRNA	Human	↑ in VAT of obese vs lean [152]	↓ in SAT vs VAT [148, 152]	
mRNA/protein				↓ in ASCs vs d-ASCs from SAT [148]
FAM13A				
FAM13A				
mRNA/protein	Mouse	↓ in VAT of obese vs lean [165]		↓ in ASCs vs adipocytes from VAT [165]
mRNA/protein		↓ in VAT of obese vs lean [169]	↔ in SAT vs VAT [169]	
mRNA	Human	↓ in SAT of obese vs lean [169]		
Genetic variants rs3822072/ rs9991328				
mRNA	Human		↑ in SAT vs VAT [169]	↓ in ASCs vs d-ASCs [169] ↓ in ASCs vs adipocytes from SAT [169]
miRNAs				
miR17-5p	Human	↓ in VAT of obese vs lean [194]	↓ in SAT vs VAT [187]	–
miR-132		↓ in VAT of obese vs lean [194]	↓ in SAT vs VAT [194]	–
miR-378		–	-	↑ in d-ASCs from SAT vs VAT [191]
miR-181a-5p		↓ in VAT of obese vs lean [192]	↓ in SAT vs VAT [192]	–
miR-23a-3p		↓ in SAT and VAT of obese vs lean [192]	↓ in SAT vs VAT [192]	–

↑, increase; ↓, decrease; ↔, no difference;– not available

BMP bone morphogenetic proteins, *FAM13a* family with sequence similarity 13 member A, *miRNAs* microRNAs, *SAT* subcutaneous adipose tissue, *SFRP* secreted frizzled-related proteins, *SIRT* sirtuin proteins, *VAT* visceral adipose tissue, *WAT* white adipose tissue, *WNT* wingless-type proteins, *miRNAs* microRNAs

However, an adipose tissue comparative analysis obtained from obese individuals found that gene and protein levels of WNT5A were significantly elevated in VAT rather than SAT (Table 1) [90, 91] supporting the observations of an association between increased expression of *Wnt5a* levels and enhanced inflammation in visceral depot [90]. Indeed, obese subjects with unhealthy expansion of the adipose tissue exhibit a specific phenotype, with fat depots characterized by hypertrophic adipocytes, hypoxia, fibrosis, and accumulation of pro-inflammatory macrophages [92]. In this setting, impaired ASCs' differentiation into mature adipocytes may underly an unhealthy adipose tissue expansion. On the other hand, *WNT5A* expression was significantly higher in SAT macrophages of obese as compared to lean subjects, and the conditioned medium from a macrophage cell line inhibited in vitro adipogenesis of 3T3-L1 preadipocytes via WNT5A,

thus suggesting that this molecule could potentially concur to determine the limited expandability of SAT [93]. Inability to expand SAT is an important determinant of IR in obesity, as well as accumulation of VAT per se. In this scenario, a family of WNT antagonists, the secreted frizzled-related proteins (SFRPs) participate in adipose tissue homeostasis by sequestering WNT molecules, thus affecting adipogenesis and inflammation of the adipose tissue [94].

SFRP5 is considered as a novel adiposity indicator, since its protein levels vary according to BMI, waist–hip ratio, body fat proportion, and lipid profile [95, 96]. However, changes in SFRP5 occurring in obesity show conflicting data. Several studies have shown that while *WNT5A* gene expression levels in VAT were higher in obese patients, mRNA or circulating levels of SFRP5 were significantly reduced [91, 95, 97] (Table 1). However, most studies

indicate that *Sfrp5* expression is induced during adipogenesis, becoming higher in isolated adipocytes than in ASCs of WAT from lean animals, and increasing dramatically when obesity ensues [95, 98–101]. Moreover, a causal link between elevated expression of SFRP5 and fat mass expansion has been suggested, since, in rodents, differences in *Sfrp5* gene expression observed in biopsies of VAT at 7 week of age (before exposure to a high-fat diet) correlated with increased adiposity after 8 week on a high-fat diet [98]. However, expression of *Sfrp5* was high in the visceral depot, but was decreased under obesogenic diet, and its genetic abrogation appeared to exacerbate serious obesity-related metabolic dysfunction [97]. These discrepancies may be due to the different stages in which the adipose tissue remodeling has been assessed and to potential differences in obese rodent models vs. humans. As noted, the adipose tissue expansion involves adipocyte hyperplasia and hypertrophy, followed by subsequent inflammatory consequences that arise from excess adipose tissue. It could be thus hypothesized that, in the early phase of obesity, ASCs, and hyperplastic adipocytes secrete more SFRP5, but less WNT5A, and that a high ratio of SFRP5/WNT5A fosters the adipose tissue expansion. As adipocytes reach their maximal storage capacity, cell death occurs leading to activation of inflammation and fibrosis [102]. In this late phase, hypertrophic adipocytes and macrophages may secrete more WNT5A, but less SFRP5, leading to activation of Wnt signaling, which in turn increases inflammation and impairs insulin signaling bringing about IR. These changes in the WNT5A–SFRP5 axis could potentially mark the limited expansion of SAT, thus promoting the development of unhealthy obesity with increased fat accumulation in the visceral depot (Fig. 1).

Bone morphogenetic proteins (BMPs) signaling

Wnt signaling is necessary but not sufficient to induce commitment of ASCs, since additional signals need to be turned on or repressed to start adipocyte differentiation. The bone morphogenetic proteins (BMPs) have been shown to play an important role for the induction of both white and brown adipogenesis. The BMPs belong to the transforming growth factor β (TGFβ) superfamily, a group of homologous signaling proteins that play different and important roles in embryogenesis, organogenesis, cell proliferation, and lineage-specific differentiation of MSCs [103, 104]. There are at least 14 types of BMPs in humans and rodents, and each of them exerts distinct but overlapping biological functions [105]. Among BMPs, BMP2 and BMP4 have been reported to play a role in the induction of white adipogenesis [106–108], while BMP7 appears to be a regulator of brown adipogenesis [109].

Several studies have demonstrated that BMP2 is involved in promoting the commitment of MSCs in the white adipogenic lineage. The role of BMP2 in promoting adipogenic differentiation has been initially proven using in vitro cell systems such as murine 3T3-L1 preadipocytes [106], murine C3H10T1/2 cells [110], and human mesenchymal cells [111, 112]. Later, Jin et al. demonstrated that BMP2 can induce adipogenesis in vivo, since ablation of *Schnurri2*, a BMP2-activated gene that induces the expression of PPARG2 in cooperation with SMAD1/4, reduced WAT in mice; moreover, in vitro adipogenic differentiation of *Shnurri2*$^{-/-}$ mouse embryonic fibroblasts was prevented [113].

More recently, by analyzing 547 paired SAT and VAT samples of subjects with varying levels of BMI, Guiu-Jurado and colleagues showed that adipose tissue *BMP2* mRNA levels were higher in VAT compared to SAT and related to obesity. In addition, in subjects with morbid obesity, *BMP2* mRNA levels were found to be higher in visceral SVF as compared to subcutaneous stromal cells. These findings suggest that, with positive energy balance, *BMP2* expression may contribute to partition excess circulating triacylglycerol into visceral fat depots and thus predispose to development of metabolically unhealthy obesity [114] (Fig. 1; Table 1). Following binding to its receptor, BMP4 activates the downstream transcription factor SMAD4 and induces terminal differentiation of preadipocytes by stimulating transcription of PPARG. Specifically, BMP4 induces the dissociation of an intracellular complex consisting of the PPARG transcriptional activator zinc finger protein-423 (ZNF423) and the mesenchymal cell canonical WISP2, thereby allowing nuclear entry of ZNF423, PPARG induction, and consequent commitment of precursor cells into the adipocyte lineage [115]. Thus, BMP4 signaling and its cross-talk with canonical WNT/WISP2 are an essential component of the induction of adipogenesis and could be considered as a potential mechanism involved in pathological expansion of WAT.

On the other hand, human studies showed that alterations in the BMP4 pathway may have negative effects in subcutaneous adipogenesis, with inability to recruit and differentiate new adipocytes, thus promoting hypertrophic obesity and dysfunctional adipose tissue with subsequent risk of developing metabolic diseases [115–117]. Indeed, inappropriate expansion of SAT may result from preadipocyte resistance to BMP4 as a consequence of increased secretion of Gremlin1 (GREM1) [116] (Fig. 1), a potent extracellular and intracellular inhibitor of BMP4 [118, 119], also involved in fibrosis and arthritis development [120]. While cellular BMP4 transcript and protein levels were reported to be increased in hypertrophic obesity, the BMP4-induced recruitment and differentiation of new adipose cells may be antagonized by GREM1, which is up-regulated in this condition [116] (Table 1). In a secretome analysis of several human adipokines from SAT, comparing lean and obese subjects, *WISP2* turned out as a gene that is up-regulated

in obesity [121] (Table 1). Indeed, WISP2 is a recently described adipokine that is highly expressed in early fat precursor cells, as well as in human abdominal SAT of subjects with hypertrophic obesity, visceral fat accumulation, and IR [115]. Moreover, the WISP2 protein appears to be involved in the cross-talk between Wnt and BMP4 signaling pathways, since WISP2 prevented adipogenic commitment and PPARG-dependent differentiation by retaining ZNF423 in the cytosol, a key transcriptional activator of PPARG, and directly inhibiting PPARG activation [115].

Taken together, these findings support the role of impaired BMP2 and BMP4 signaling in hypertrophic obesity, allowing the adipose precursor cells to remain in an uncommitted state and fostering fat storage outside SAT.

Sirtuins

The family of enzymes known as Sir2-related proteins or sirtuins are highly conserved NAD-dependent deacetylases and/or ADP ribosyl transferases that target histones, transcription factors, and co-regulators, to adapt gene expression and metabolic activity in response to changes in cellular energy state [122]. There are seven mammalian sirtuins, SIRT1-7, which share a conserved central catalytic domain capable of binding NAD$^+$, but have different N- and C- terminal and display distinct subcellular localization suggesting different biological functions [123]. SIRT1, SIRT6, and SIRT7 are localized in the nucleus. SIRT2 is the only mammalian sirtuin localized mainly in the cytoplasm, and consistent with its role in cell cycle regulation, shuttles to the nucleus during the G2/M transition [124]. SIRT3, SIRT4, and SIRT5 are localized in mitochondria and regulate the activity of metabolic enzymes involved in oxidative damage [125].

The sirtuin family is known to play a key role in the maintenance of glucose and lipid homeostasis, the control of insulin secretion and sensitivity, the promotion of fat mobilization, the regulation of oxidative stress and inflammation, and the modulation of circadian clock in metabolic tissues [126]. Specifically, several studies have demonstrated a key role for SIRT1 and SIRT2 as cellular energy sensors and mediators of the beneficial effects of calorie restriction [127]. SIRT1 and SIRT2 are expressed in WAT and modulate adipogenesis by affecting the transcriptional activity of PPARG, the master regulator of this process [122]. In rodent preadipocytes, *Sirt1* or *Sirt2* overexpression limits adipocyte differentiation by inhibiting the nuclear receptor PPARG and genes mediating fat storage [128] and by increasing the ability of FOXO1 to repress PPARG transcriptional activity [124]. Consistent with the role of SIRT1 as a negative modulator of adipogenesis and lipogenesis in 3T3-L1 cells [128], mice moderately overexpressing *Sirt1* were leaner than controls and more metabolically active, and displayed

lower serum levels of cholesterol, pro-inflammatory adipokines, insulin, and fasting glucose [129]. In addition, *Sirt1* adipocyte-specific knockout mice exposed to a prolonged high-fat diet developed an augmentation of epididymal fat due both to lipogenesis and adipogenesis, which was not seen in SAT [130]. Also in multiple human studies, an association between decreased SIRT1 [131, 132] and SIRT2 [133] expression and obesity and/or T2D has been demonstrated. In a recent study, we have extended these findings, showing that SIRT1 and SIRT2 expression was specifically down-regulated in the VAT in humans and inversely correlated with BMI and waist circumference [23]. The observed obesity-related reduction of SIRT1/2 in VAT was found to be associated with reduced mRNA and protein levels of SIRT1 and SIRT2 also in the VAT ASCs, and these cells displayed increased adipogenic potential with augmented rates of triglyceride accumulation, number of lipid droplets, and capacity to generate new adipocytes in vitro [23]. In addition, stably forced expression of SIRT1 or SIRT2 in ASCs isolated from VAT of obese individuals limited their adipogenic potential, and was also associated with reduced expression of early and late transcriptional factors or markers of adipogenic differentiation (i.e., *CEBPA*, *PPARG*, *SLC2A4*, *ADIPOQ*, *FASN*, and *SREBF1C*), highlighting a causal role of the reduction of SIRT1 and SIRT2 levels in the observed obese adipocyte phenotype. By contrast, in SAT, SIRT1 and SIRT2 mRNA levels were not correlated with either BMI or waist circumference, and the resident ASCs did not show any changes in expression levels of SIRT1 and SIRT2 or lipogenesis/adipogenesis rates in obesity [23]. Furthermore, knockdown of *SIRT1* or *SIRT2* protein levels in ASCs from VAT of lean subjects fostered adipocytes differentiation [23]. These findings indicate that reduced SIRT1 and SIRT2 expression occurs in a depot-specific and obesity-related manner and is tightly linked to increased adipogenic differentiation of VAT ASCs [23] (Table 1).

As previously reported, Wnt/β-catenin signaling is an important regulator of adipocyte differentiation [79], and it is down-regulated by the family of SFRPs through the formation of inhibitory complexes [134, 135]. Interestingly, in obese individuals, *SFRP4* expression was found to be significantly increased in VAT but not in SAT, and this positively correlated with BMI and IR [23, 94]. More recently, *Sfrp4* knockdown in preadipocytes isolated from epididymal adipose tissue of C57BL/6 J mice was shown to reduce lipid accumulation and adipocyte differentiation in association with diminished mRNA levels of adipogenic markers, including *Pparg* and *Slc2a4* [23, 136]. Since SIRT1 deacetylates SFRPs and represses their expression, thus activating Wnt signaling and suppressing adipogenesis [137], it can be postulated that reduced levels of SIRT1 in VAT ASCs from obese subjects may increase SFRP4 levels that foster VAT expansion by early induction of adipogenic

transcription factors. All these findings suggest that SIRT1/2 and SFRP4 may be identified as a key mechanism that promotes the development of VAT through adipogenesis of resident ASCs.

Other sirtuins may be also involved in the adipose tissue metabolism and turnover. Among these, SIRT6 is induced by caloric restriction and has been recently implicated in the regulation of aging, genome stability, glucose homeostasis, and lipid metabolism [138]. Consistent with these results, *SIRT1*, *SIRT3*, and especially *SIRT6* mRNA levels were upregulated in SAT from severely obese patients undergoing bariatric surgery after 6 months of extensive weight loss [139] (Table 1). Although these studies suggest that SIRT6 could play a role in the adipose tissue, the exact function and mechanisms remain unclear. More recently, Kuang et al. have shown that knockout of *Sirt6* in mouse embryonic fibroblasts increased phosphorylation and acetylation of FoxO1, which compromises the transcriptional activity of adipose triglyceride lipase (ATGL), a key lipolytic enzyme [140]. Moreover, loss of ATGL reduced lipolysis in adipocyte and was associated with increased adipose tissue mass [141, 142], whereas its overexpression promoted opposite findings [143]. Thus, reduced *Sirt6* expression in adipose tissue may impair ATGL function by regulating FoxO1 activity, fostering the adipose tissue expansion and obesity by lipid accumulation rather than adipocyte conversion [140] (Table 1). Finally, a cross-talk between expression and activity of SIRT1 and SIRT2, and between SIRT1 and SIRT6 may exist [23, 140]. Indeed, a computational analysis performed by Ingenuity Pathway Analysis has observed that SIRT1 seems to interact directly with SIRT2 and SIRT6 [144]; moreover, this interaction was confirmed in vitro where the overexpression of SIRT1 or activation of SIRT1 by resveratrol enhanced the expression of SIRT6 in murine adipocytes [140]. These findings indicate in SIRT1 and other sirtuins attractive therapeutic targets for treating obesity and obesity-related cardiometabolic diseases.

Insulin signaling

Insulin exerts a key role in regulating the adipose tissue development and function through activation of its tyrosine kinase receptor (INSR), which consists of two isoforms: INSR-B, the long isoform that is thought to be prevalent in post-mitotic and differentiated cells and is largely responsible for the metabolic action of insulin (i.e., glucose uptake, triglyceride accumulation) via AKT-dependent mechanisms; and INSR-A, predominantly expressed in undifferentiated cells and contributing to prenatal development and tissue growth, as well as in the biology of several human cancers [22]. Insulin promotes anabolic responses in the adipose tissue by stimulating glucose and free fatty acid uptake, inhibiting lipolysis, and stimulating de novo fatty acid synthesis.

In addition, insulin regulates adipose tissue growth and differentiation by enhancing the gene expression of various fat-specific transcription factors, including SREBF1C and PPARG [22]. However, several studies have shown that adipose tissue insulin sensitivity and responsiveness differ in relation to its anatomical site and different grading of BMI [24, 26, 36, 145, 146]. Indeed, insulin signaling is more rapidly and prominently activated in VAT than SAT with greater and earlier activation levels of the INSR, AKT, glycogen synthase kinase-3 (GSK3), and extracellular signal-regulated kinases ($ERK_{1/2}$), as shown by our group [145]. In a later study, we clarified that depot-related differences in insulin signaling were due to innate characteristics of adipose cells rather than to extrinsic factors, such as tissue microenvironment, local circulation, local innervation and/or heterogeneity in cellularity. After insulin stimulation, adipocytes differentiated from visceral stromal cells showed earlier and more transient kinetics of activation of multiple signaling intermediates, including the INSR, insulin receptor substrate (IRS) proteins, AKT and $ERK_{1/2}$, as well as significantly greater glucose transport rates than adipocytes derived from subcutaneous stromal precursors [36]. However, to date, the impact of these depot-specific differences in insulin signaling pathway in the regulation of adipogenesis and adipose tissue expansion in obesity is not fully clear.

Insulin is an important regulator of adipocyte differentiation and function: even though it takes part only in the late phase of differentiation, it is essential to achieve a completely functional adipocyte phenotype in both fat depots [24]. In human ASCs, the presence of insulin did not modify the expression levels of adipogenic markers during both the early and intermediate phases of adipogenesis, while it was found to be essential to achieve a completely functional adipocyte phenotype in the late phase of differentiation [22, 24]. The insulin signaling is modulated by the Wnt signaling pathway. The cross-talk between insulin and the Wnt signaling pathway occurs at multiple levels in murine preadipocytes, including the Wnt co-receptor LRP5 (low-density lipoprotein receptor-related proteins 5) [147] and WISP1 (Wnt1-inducible signaling pathway protein-1) [148] proteins. WISP1 is an adipokine released by mature human adipocytes, which may play a role in glucose homeostasis, as well as in the pathophysiology of obesity and T2D [148, 149]. Several results have demonstrated that *WISP1* mRNA levels in the adipose tissue are positively associated with fasting insulin levels and macrophage infiltration in the adipose tissue, and are associated negatively with insulin sensitivity measured by a hyperinsulinemic–euglycemic clamp [150–152]. WISP1 is more expressed in VAT in both mice and humans [148, 152] in contrast to WISP2, which is preferentially expressed in SAT [115] (Table 1). Moreover, anti-apoptotic, anti-autophagy, and proliferative effects of WISP1 are mediated trough the PI3K/AKT pathway [153],

indicating that WISP1 may be regulated by insulin. Indeed, increased insulin-stimulated gene expression of *WISP1* was abolished when human adipocytes were pretreated with PI3K inhibitors, indicating that chronic exposure to insulin increases *WISP1* expression [148]. In addition, in human ASCs, WISP1 mRNA and protein levels increased during adipocyte differentiation [148]. Thus, it is possible that high circulating insulin levels under conditions of IR, as in morbid obesity, may foster WISP expression and thus further promote VAT expansion.

The glucose transporter type 4 (GLUT4) is a key effector of insulin action. GLUT4 protein levels were found to be reduced in adipose cells of both insulin-resistant obese and diabetic subjects [154] (Table 1). The limited insulin responsiveness of the adipose tissue in these conditions appears to lead to an increase of adipocyte size in the presence of higher serum insulin concentrations, thus promoting the development of adipose tissue expansion [155]. Indeed, several studies reported a correlation between adipocyte size and IR thus confirming the "lipid spillover" hypothesis, since hypertrophic adipocytes, especially in SAT, are no longer able to store further lipids, causing the overflow of fatty acids into ectopic sites, resulting in IR [28, 156, 157]. Thus, impaired insulin signaling in the adipose tissue may further favor the development of hypertrophic SAT, expansion of VAT, and adverse metabolic consequences including T2D and CVD.

Family with sequence similarity 13 member A (FAM13A)

Overall adiposity and body fat distribution are both heritable traits and well-established predictors of adverse cardiometabolic and respiratory outcomes, including IR and T2D [158]. While IR is often a consequence of excess adipose tissue, some forms of IR develop without overweight/obesity or are associated with regional lack of fat. Lipodystrophy is a condition characterized by varying degrees of adipose tissue deficiency due to impaired capacity of peripheral fat to expand under a positive energy balance, leading to lipid accumulation at ectopic sites (e.g., skeletal muscle, liver), with severe dyslipidemia and increased risk of hypertension and T2D [159]. This indicates a clear link between limited adipose tissue expandability and adverse cardiometabolic outcomes, underlining the notion that expandable and metabolically flexible adipose tissue is essential for health.

Recently, using a genome-wide association study (GWAS) of large cohorts, several groups independently identified a cluster of common risk variants that are associated with impaired adipose tissue expandability, unfavorable body fat distribution and severe IR [160–162]. A number of intronic SNPs associated with unfavorable body fat distribution are located in or near the family with sequence

similarity 13 member A (*Fam13a*) gene [162, 163]. One of these common non-coding variants of *Fam13a* (rs3822072) identified with GWAS has been associated with higher fasting insulin and lower HDL-cholesterol levels [160, 164]. A second common non-coding *Fam13a* variant rs9991328 (in high linkage disequilibrium with rs3822072) is highly associated with waist-to-hip ratio (WHR) adjusted for BMI [161]. Several studies have indicated that the adipose tissue is the primary site where *Fam13a* plays a key role in the pathological linkage between morbid obesity and adverse metabolic outcomes [165–169]. *Fam13a* modulates adipocytes insulin signaling, showing a negative correlation with diet-induced obesity in mice [165] (Table 1). In a recent study, Lin X et al. provided in vivo and in vitro evidence supporting a key role of *Fam13a* in regulating glucose and lipid metabolism, pinpointing rs2276936 as the possible functional variant regulating hepatic FAM13A expression and the association with metabolic traits such as lower body fat, increased insulin sensitivity, and higher HDL-cholesterol [166]. Interestingly, the metabolic traits associated with rs2276936 were recapitulated in *Fam13a* knockout mice, showing less body weight gain following high-fat diet, increased lean mass, reduced fat mass, lower hepatic lipid accumulation, and improved insulin sensitivity, possibly by increased AMPK activity [166]. Moreover, siRNA-mediated knockdown of FAM13A in human mesenchymal stem cells resulted in increased expression of PPARG, CEBPA and SLC2A4, fostering adipocyte differentiation [167], while FAM13A overexpression caused apoptosis of preadipocytes and largely blocked adipogenesis induced by a standard hormone cocktail [168]. Fathzade et al. have recently reported that some GWAS SNPs in the *Fam13a* locus, such as rs3822072 and rs9991328, were associated with IR traits (e.g., elevated fasting insulin levels, increased WHR, and body fat mass) and with FAM13A expression in SAT, but not in VAT. In men with metabolic syndrome and IR traits, FAM13A expression levels in SAT adjusted for BMI were positively correlated with WHR and fasting insulin, and inversely correlated with fat mass [169] (Table 1). These findings suggest that some *Fam13a* variants may predispose individuals to a normal body weight but with a metabolically unhealthy phenotype, while decreased *FAM13A* expression in SAT is associated with favorable adipose tissue development and function. Compared to wild-type mice, despite increased body weight during high-fat diet, male *Fam13a* knockout mice showed a reduced VAT/SAT ratio, indicating a potential role of *Fam13a* perturbations in driving a shift of fat deposition away from visceral depots [169] (Table 1). The healthy obese phenotype exhibited by *Fam13a* knockout mice was due to increased ability of SAT to generate new adipocytes de novo, functionally active in depositing glucose and responding to insulin, which may help to meet the excess lipid storage needs. Indeed, FAM13A knockdown in both

human and mice SAT preadipocytes resulted in upregulation of adipogenesis markers (e.g., CEBPA and PPARG) and increased generation of new adipocytes [169]. The precise molecular mechanism linking FAM13A to adipogenesis is unknown. However, FAM13A has previously been reported to activate the Wnt pathway, since, in human lung cancer, FAM13A knockdown significantly reduced the Wnt signaling activity [170]. Altogether, these results suggest that FAM13A disruption may promote adipocyte differentiation by counteracting the anti-adipogenic effects of Wnt signaling.

microRNAs

microRNAs (miRNAs) are a family of small non-coding RNAs' molecules containing approximately 19–22 nucleotides that regulate gene expression at the post-transcriptional level by preventing translation of their target mRNAs or accelerating their decay via the RNA-induced silencing complex [171]. Several studies provided evidence that miRNAs exist in different tissues and modulate a series of important processes, including early development, cell proliferation, differentiation, and apoptosis [172–174]. miRNAs also play a role in the adipose tissue development and its pathological expansion, by stimulating or inhibiting adipocyte differentiation through fine-tuning of diverse signaling molecules and pathways [175–181]. The key role of miRNAs as stimulators or inhibitors of murine and/or human adipogenesis has been reviewed in detail elsewhere [182–186]. However, some miRNAs exhibit a SAT- or VAT-specific expression pattern during the adipose tissue development and in human obesity. Indeed, a large number of miRNAs are expressed in human WAT, but only a few of them show different adipose tissue levels in obese compared to lean subjects [183]. In addition, a gene expression analysis of 155 miRNAs in human paired omental and subcutaneous adipose tissue from overweight or obese subjects detected 106 miRNAs in both fat depots, of which only 16 were differentially expressed in a fat depot-specific pattern, showing higher expression in VAT than SAT [187] (Table 1). Among these miRNAs, miR-378 was shown to be involved in brown adipogenesis, leading to expansion of brown adipose tissue (BAT), but not WAT, as well as to resistance to both genetic and high-fat diet-induced obesity [188]. miR-378 is generated from a precursor RNA and is located in the first intron of the PPARGC1B gene, with a positive regulation in adipogenesis [189, 190]. PPARG agonists, such as pioglitazone, increase the expression of miR-378 in human SVF cells [191]. Notably, miR-378 promoted adipogenesis of subcutaneous but not of visceral SVF cells, underlying a fat depot-specific regulatory role [191] (Table 1). Altogether, these findings suggest that increased miR-378 expression may be associated with an appropriate expansion of the available SAT

adipose cells and thus with a healthy adipose tissue development (Fig. 1). Among those miRNAs with a depot-specific pattern, miR-181a-5p and miR-23a-3p were also found to be significantly reduced in VAT from obese compared with non-obese controls, while no differences were observed in SAT according to a wide BMI range, from 25.7 to 43.7 kg/m^2 [192] (Table 1). Furthermore, miR-181a-5p and miR-23a-3p expression levels were inversely correlated with adiposity (measured by BMI and waist circumference) in VAT, whereas this correlation was found only for miR-23a-3p in SAT. The same correlation was observed with both miRNAs and HOMA-IR [192]. Previous studies found that overexpression of miR-181a-5p in cultured porcine primary preadipocytes accelerated accumulation of lipid droplets, increased the amount of triglycerides, and fostered adipocyte differentiation, whereas reducing miR-181a-5p levels had opposite effects [193]. On the other hand, Heneghan et al. found that omental and circulating levels of miR-17-5p and miR-132 were significantly decreased in obese individuals compared with non-obese subjects, and that miR-17-5p expression levels were inversely correlated with BMI. Interestingly, the expression of these two miRNAs in VAT and blood from obese subjects correlated significantly with glycosylated hemoglobin, leptin, and fasting blood glucose [194] (Table 1). In vitro data indicate that overexpression of miR-17-5p in 3T3-L1 preadipocytes accelerated their adipogenic differentiation via targeting the Wnt signaling cascade effector Tcf7l2 [195]. However, additional studies will be needed to establish the molecular mechanism through which miR-181a-5p and miR-17-5p modulate the network of adipogenesis transcription factors and their potential role in human obesity and VAT expansion.

Conclusions

Studies of mouse and human adipose tissue provide strong evidence that the inability of SAT to recruit ASCs and promote their adipogenic differentiation in response to the demand for increased energy storage leads to inflammation of the adipose tissue, ectopic fat accumulation with unfavorable body fat distribution, IR, and adverse cardiometabolic and respiratory outcomes. In contrast, the ability of SAT to recruit new adipose cells is protective against cardiometabolic diseases, underlining the notion that an expandable and metabolically flexible SAT is essential for developing healthy obesity (Fig. 1). Adiposity and body fat distribution are both heritable traits, and thus, individuals with a genetic predisposition for unhealthy obesity exhibit their inability to expand SAT, a high VAT/SAT ratio, IR, dyslipidemia, and fatty liver. Multiple signaling pathways and master genes appear to mediate the link between the inability of SAT to expand appropriately and the unfavorable

body fat distribution. The Wnt/BMP pathway is one of such pathways, which allows the ASCs to remain in an uncommitted state promoting fat storage outside SAT. Consistent with this concept, reduced adipose tissue expression of SIRT1 and SIRT2 in human obesity is another key event, which occurs in a depot-specific manner, i.e., exclusively in VAT, and mediates an increased adipogenic potential of visceral ASCs. Hence, the SIRT1/SIRT2 and Wnt/BMP pathways are involved in VAT expandability in humans and could be considered as useful therapeutic targets to counteract unhealthy obesity. On the other hand, understanding how SAT can be turned into a suitable lipid storing is another major challenge that could help to counteract the adverse cardiometabolic consequences of VAT expansion. In this regard, FAM13A and miR-378 appear as newly identified regulators of fat distribution and metabolic traits through their ability to increase the recruitment and differentiation of subcutaneous ASCs, fostering SAT development; this could be particularly important when there is a demand for increased energy storage (Fig. 1). Further understanding of the mechanisms regulating in vivo recruitment and adipocyte differentiation of ASCs in distinct adipose tissue depot and in healthy compared to unhealthy obesity could potentially lead to identify novel therapeutic targets to favorably manage the excess lipid storage needs, and thus uncouple fat accumulation from the adverse cardiometabolic outcomes of obesity.

Compliance with ethical standards

Conflict of interest The authors have no conflicts to disclose in relation to the content of this manuscript.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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