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Commentary

New approaches targeting brown adipose tissue transplantation as a therapy in obesity

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Abbreviations

AT-MSCs, adipose tissue-derived MSCs; BAT, brown adipose tissue; BH4, tetrahydrobiopterin; BMP, bone morphogenetic protein; DHEA, dehydroepiandrosterone; eWAT, epididymal white adipose tissue; FAs, fatty acids; G3PHD, glycerol 3-phosphate dehydrogenase; HFD, high-fat diet; IL-6, interleukin-6; iPSCs, induced pluripotent stem cells; LPL, lipoprotein lipase; Myf5+, myogenic factor 5-positive; NOD, non-obese diabetic; scWAT, subcutaneous white adipose tissue; TNFα, tumor necrosis factor α; UCP1, uncoupling protein 1; WAT, white adipose tissue

ABSTRACT

Brown adipose tissue (BAT) is raising high expectations as a potential target in the fight against metabolic disorders such as obesity and type 2 diabetes. BAT utilizes fuels such as fatty acids to maintain body temperature by uncoupling mitochondrial electron transport to produce heat instead of ATP. This process is called thermogenesis. BAT was considered to be exclusive to rodents and human neonates. However, in the last decade several studies have demonstrated that BAT is not only present but also active in adult humans and that its activity is reduced in several pathological conditions, such as aging, obesity, and diabetes. Thus, tremendous efforts are being made by the scientific community to enhance either BAT mass or activity. Several activators of thermogenesis have been described, such as natriuretic peptides, bone morphogenic proteins, or fibroblast growth factor 21. Furthermore, recent studies have tested a therapeutic approach to directly increase BAT mass by the implantation of either adipocytes or fat tissue. This approach might have an important future in regenerative medicine and in the fight against metabolic disorders. Here, we review the emerging field of BAT transplantation including the various sources of mesenchymal stem cell isolation in rodents and humans and the described metabolic outcomes of adipocyte cell transplantation and BAT transplantation in obesity.

Keywords

Brown adipose tissue, transplantation, obesity

Contents

1. Introduction

Current society embraces high calorie intake and a sedentary lifestyle, which is triggering an alarming increase in obesity and associated metabolic diseases, such as type 2 diabetes, insulin resistance, Alzheimer's, cardiovascular diseases, and some forms of cancer. Despite the efforts made at various levels (government, education and research) to promote healthy policies, the rise in obesity is far from being stopped or decreased.

In this milieu, new, successful, long-term efficient therapies need to be developed urgently. In the last decade, an attractive target has emerged in the field. This is the last tissue to be discovered in humans: brown adipose tissue (BAT). BAT utilizes fuel such as fatty acids (FAs) to control body temperature by thermogenesis. Thus, BAT dissipates energy to produce heat. Initially described as exclusive to rodents and human neonates, BAT is now commonly accepted to be present and active in adult humans [1–8]. Interestingly, women have both higher BAT mass and activity [3]. Importantly, BAT activity decreases with aging, obesity, and type 2 diabetes [3]. It has been reported that in these conditions BAT shows signs of hypertrophy and a blunted response to adrenergic stimulus [6,9]. The decrease in cold-activated BAT activity in the elderly may be associated with accumulation of adiposity with age [10]. Recently, it has been also described that BAT from obese and hyperglycemic mice shows higher levels of oxidative damage and inflammation (macrophages and T cells infiltration) [11]. Thus, tremendous efforts are being made by the research community to enhance BAT thermogenesis and fight against obesity and its associated metabolic diseases.

2. Classification and distribution of brown adipose tissue

2.1. Types of adipose cells and function: white, brown, beige and pink

In addition to being the largest energy reservoir in the body, adipose tissue is a complex endocrine organ that plays important roles in controlling food intake, endocrine and reproductive

functions, inflammation and immunity. It also regulates whole-body energy homeostasis with a relevant role in glucose and lipid metabolism [12].

Adipose tissue is mostly composed of adipocytes. However, other cell types can be found grouped in the stromal vascular fraction (SVF). They include preadipocytes, fibroblasts, mesenchymal stem cells (MSCs), endothelial cells, and immune cells such as T cells, B cells, macrophages, and dendritic cells [13]. Differentiated adipocytes are mostly composed of lipid droplets, since their main function is the storage of energy in the form of triglycerides. There are several adipose cell types that differ in their morphology, gene expression and metabolic functions. Despite some controversy, they are commonly named white, brown, beige and pink adipocytes.

Morphologically, white adipocytes present a large unilocular lipid droplet that takes up most of the intracellular space, displacing the rest of the organelles to the periphery. These adipocytes are found in white adipose tissue (WAT), which is the main lipid storage organ, providing energy during postprandial fasting and thus regulating energy homeostasis. WAT is also an endocrine organ secreting hormones such as leptin, adiponectin, and resistin, and cytokines such as tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) [14]. It is characterized by some genetic markers such as lipoprotein lipase (LPL) or glycerol 3-phosphate dehydrogenase (G3PHD) [15], surface transporter ASC-1 [16], and the specific transcription factor Tcf21 [17].

In contrast to white adipocytes, brown adipocytes present small multilocular lipid droplets and are composed of many mitochondria that give the BAT its characteristic color. In response to cold exposure, brown adipocytes maintain body temperature by dissipating energy in the form of heat in a process known as non-shivering thermogenesis. This process is carried out by uncoupling protein 1 (UCP1) in response to sympathetic nervous system adrenergic stimulation. Thus, UCP1 generates heat by uncoupling electron transport from ATP production. UCP1 as well as the transcription factor Zic 1 are considered specific BAT markers [17,18].

Beige or brite (brown-in-white) adipocytes have intermediate characteristics between white and brown adipocytes in terms of morphology (number of lipid droplets and mitochondria) and UCP1 expression [19]. Beige adipocytes arise from WAT in response to cold temperature or β-adrenergic exposure, through a reversible process called browning or beiging. This process implies increased thermogenesis within the WAT, *i.e.* augmented UCP1 expression and activity in the WAT depots. At least two mechanisms have been hypothesized to occur during the browning process: 1) the transdifferentiation or conversion of white into beige adipocytes, and 2) *de novo* brown adipogenesis. The transdifferentiation has been described in several studies [20–23]. Supporting the second hypothesis, Lee *et al.* have reported a β-adrenergic-induced proliferation and further differentiation of precursors present in WAT [24]. Although further research is needed in this area, most likely the two processes might take place at the same time and to different degrees according to the fat depot or the stimuli received. Besides natural inducers of browning such as cold or exercise, a large number of browning agents have been described [6]. In addition to intermediate expression of UCP1, beige adipocytes express the surface markers PAT2 and P2RX5 [16,25] and the specific markers Cd137, Epsti1, Tbx1, and transmembrane protein 26 (Tmem26) [17,18,26].

Ultimately, a last adipocyte cell type has been described to arise from white adipocyte depots during the gestation and lactation period: pink adipocytes. Their main function is to secrete milk and to store large amounts of lipids in the mammary gland during this period. Differentiation to pink adipocytes is reversed once lactation ends, together with the involution of the mammary glands [27,28]. Pink adipocytes are characterized by the expression of leptin and S-100b [29].

2.2. Distribution in rodents and humans

Adipose tissue is widely distributed in mammals around the entire body. There are two types of adipose tissues that differ based on their location. The most abundant is the WAT, which is

commonly classified into two types: visceral WAT that is located in the abdomen surrounding all organs or viscera, and subcutaneous WAT (scWAT) that is found under the skin [30]. BAT was thought to be exclusive to rodents and human neonates, but in the last decade it was found to be active in adult humans as well [1–5,7,8]. In adult humans, classical BAT is located in the supraclavicular region, but other locations are also found around the neck, axillary, paravertebral and perirenal areas (Figure 1) [8,31].

To obtain a better approximation of the locations of the various adipose tissues, non-invasive techniques are used to determine which of the tissues are thermogenically active. The most widely used substrate is a glucose analog called 2-deoxy-2-[^{18}F]fluorodeoxyglucose that can be detected by positron emission tomography/computed tomography (PET/CT) [32–35]. In a recent study, images obtained by [^{18}F]fluorodeoxyglucose-PET/CT as well as [$^{123/125}$I]-beta-methyl-p-iodophenyl-pentadecanoic-SPECT/CT were compared to identify tissue with high glucose or FA uptake, respectively [36]. By using both methods with and without thermogenesis induction by cold stimulation or a β-adrenergic agonist, thermogenically active areas were detected in mice and humans in all adipose tissues, except epididymal white adipose tissue (eWAT). These results are consistent with the typical morphology observed by histological examination and with the expression of specific genes measured in each of the identified tissues. Ultimately, the authors conclude that in mice the supraclavicular, anterior cervical, axillary, infrascapular, supraspinal and ventral spinal adipose tissues correspond to classical BAT. The eWAT would correspond to classical WAT, and finally the anterior subcutaneous and suprascapular adipose tissues would be encompassed within WAT with a capacity to generate beige adipocytes after exposure to cold or β-adrenergic induction. Finally, a recent, elegant study proposes a non-invasive method to measure *in vivo* local BAT metabolism [37]. Reber and colleagues use an optoacoustic imaging method to measure hemoglobin gradients in mouse and human BAT. The measurements of hemoglobin were correlated to local tissue oxygen consumption. In addition, indirect calorimetry

measured during BAT metabolic activation was performed at the same time as the optoacoustic measurements of hemoglobin. The results were consistent with those obtained by PET and could discern between tissues from healthy and diabetic subjects [37].

2.3. Origin and development of brown adipocytes

Adipose cells have a mesodermal origin and derive from MSCs (Figure 2) [38,39]. MSCs are adherent cells that express various surface markers such as CD29, CD44, CD73, CD90, CD105, CD166, and Stro-1 [40]. Furthermore, MSCs are multipotent cells, *i.e.* in addition to their capacity to generate mature adipocytes, they can differentiate into other cell types such as chondrocytes, osteoblasts, myoblast cells, or connective tissue [39,41].

Brown adipocytes and myoblasts arise from myogenic cell lineage progenitors that express the myogenic transcription factor Myf5+. A recent study demonstrates that the transcription factors MyoD and Myf5 are key players in the repression or activation of the transcription factor PRDM16, determining the differentiation of the brown adipose lineage [42]. In contrast to brown adipocytes, white adipocytes arise from Myf5- precursors. In addition, the expression of transcription factor Tcf21 in these preadipocytes promotes differentiation into the white lineage, inhibiting the myogenic lineage [43]**.** The origin of beige adipocytes remains unclear. Despite some controversy, it is believed that they are derived from the same precursor as white adipocytes, from Myf5- progenitors, or that they are transdifferentiated from pre-existing white adipocytes. However, some studies suggest that both beige and white adipocytes may be derived from positive and negative Myf5 progenitors, and claim that some white adipocytes can be differentiated into beige after stimulation, while classic white adipocytes cannot [44,45]**.**

Bone morphogenic proteins (BMPs) are a family of proteins thought to be related to the development of adipocyte types. Several studies show that the expression of BMP7 induces an increase in the thermogenic protein UCP1, resulting in a brown or beige lineage [46,47]. In

addition to being committed to the white lineage, BMP4 is related to the beige lineage, since its overexpression increases UCP1 levels. These results are more notorious in MSCs derived from mouse than from humans [47].

Another important family of transcription factors involved in the maturation and activity of the three types of adipocytes are the Peroxisome Proliferator-Activated Receptors (PPARs). Importantly, the nuclear receptor PPARγ is essential for adipogenesis [39,43,44] and it is implicated in β-adrenergic-induced BAT thermogenesis [48]. The proliferator-activated receptor c coactivator 1 α (PGC1α) is another important regulator of BAT metabolism that is highly activated after cold exposure, increasing the activity of some transcription factors including PPARs [49]. Moreover, PGC1α promotes mitochondrial biogenesis and respiration, fatty acid oxidation and UCP1 activity in BAT [49,50].

3. Importance of brown adipose tissue in metabolism

The main function of BAT is to control thermogenesis by uncoupling oxidative phosphorylation from ATP production, which generates heat. This fundamental process for survival in mammals is closely related to several metabolic pathways such as lipolysis, lipogenesis, mitochondrial respiration, or FA uptake, transport, storage, and oxidation [6]. Thermogenesis in BAT is under the tight control of the central nervous system [51]. Cold stimulates the sympathetic nervous system and the release of norepinephrine, which ultimately enhances thermogenesis via the activation of UCP1. Since BAT requires plentiful FAs, which are its primary substrate, lipolysis is activated during BAT thermogenesis [9,52,53]. The resulting FAs could be transported into the mitochondria for fuel oxidation, be involved in the regulation of UCP1, or directly activate UCP1 [52]. Interestingly, recent studies have proven that heat can be produced in BAT without intracellular lipolysis [54–56]. FAs could be obtained from 1) endogenous BAT lipolysis, 2) WAT lipolysis, 3) circulating lipoproteins (intestinal chylomicrons or liver-derived VLDL), or

4) *de novo* FA synthesis. Although the classical model states that UCP1 is activated by FAs released upon lipid droplet breakdown in the BAT, Schreiber *et al.* and Shin *et al.* recently studied mice models with BAT-specific elimination of norepinephrine-induced lipolysis and showed that the mice are not cold sensitive when food is present. Thus, these studies propose a new model in which WAT lipolysis (and not BAT lipolysis) might be essential for cold-induced thermogenesis.

The pathophysiology of obesity-induced metabolic disorders has been attributed to several mechanisms such as ectopic fat deposition [57] and WAT dysfunction including increased inflammation [58], endoplasmic reticulum and oxidative stress [59,60], hypoxia [61], mitochondrial dysfunction [62], fibrosis and impaired adipocyte expansion, and angiogenesis [63–66]. While the above mechanisms have been described in WAT, some studies have recently reported BAT derangements during obesity progression. High-fat diet (HFD) treatment has been shown to alter the molecular networks in BAT in a time-dependent manner [67] and to increase BAT inflammation, oxidative stress, and mitochondrial respiration in mice [11].

In the last decade, the scientific community has put a spotlight on BAT as a key player in the control of energy metabolism. In fact, BAT is an active endocrine organ [1–8]. High expectations for the treatment of obesity-induced diseases have emerged with the finding that adult humans have active BAT and that its activity is reduced with age, obesity or diabetes [1–5,7,8,68]. This indicates that BAT plays a key role in both cold-induced and diet-induced thermogenesis. Importantly, several studies in experimental animals have shown that BAT can dissipate caloric energy and reduce both obesity and diabetes [1–5,69–72]. In fact, after short-term cold exposure, BAT is the main lipid clearance organ and it has the highest FA oxidation rate [73,74]. Thus, keeping BAT active and continuously combusting excess food energy might be a potential new approach to treat obesity-induced disorders. Titanic efforts are being made by the scientific community to search for new BAT activators. In addition to natural activators of

thermogenesis such as cold or exercise, several factors have been found (extensively reviewed elsewhere [75,76]) to enhance thermogenesis such as FGF21 [77], BMP7 [78], BMP8b [79], natriuretic peptides [80], norepinephrine [81], meteorin-like [82], bile acids [83], or adenosine [84]. Interestingly, a drug that can enhance BAT thermogenesis has been tested in humans for the first time [85]. Cypess *et al.* administered orally the β3-adrenergic receptor agonist mirabegron in healthy young humans. The compound stimulated BAT thermogenesis and increased resting metabolic rate. However, further research is needed to study mirabegron's mechanism of action and potential off-target effects [86].

4. Source of mesenchymal stem cells in rodents and humans

The population of stem cells has always been associated with embryonic stem cells and stem cells isolated from the bone marrow. However, other potential sources of stem cells in human adults have been identified in recent years (Figure 2).

The umbilical cord and peripheral blood are the most studied sources, due to non-invasive, easy isolation methods. Adipose tissue is also considered a good option, as a high number of MSCs is obtained. Apart from these, there are many other tissues from which MSCs can be extracted, such as bone marrow, cartilage, tendon, muscle, or dental pulp, but they require more invasive procedures [87]. Other studies focus on trying to isolate MSCs from bodily fluids such as synovial fluid [88], Wharton's jelly [89] or even menstrual blood [90].

In a recent study, stem cells isolated from umbilical cord blood and WAT obtained from liposuction were compared [91]. Both cell types were differentiated into white and brown adipocytes. The results showed that adipose tissue-derived MSCs (AT-MSCs) had a greater capacity for adipocyte differentiation, and higher UCP1 expression when differentiated into brown adipocytes [91]. The authors concluded that adipose tissue is a more appropriate source of MSCs for the study of obesity and other metabolic dysfunctions.

Cell transdifferentiation studies are also being carried out to obtain stem cells from differentiated cells for future therapeutic uses. In one of these studies related to adipose tissue, the authors transdifferentiated human mature white adipocytes into fibroblast-like cells that maintained stem cell gene signatures [92].

The source and method of obtaining MSCs can be very similar in rodents and humans. However, the time required and the capacity for differentiation to other lineages can vary between species, hindering their translation to future therapeutic applications [93].

5. Effects of thermogenic adipocyte transplantation in obesity

5.1. Beige or brown cell transplantation

The potential efficacy of AT-MSCs to prevent obesity and other associated pathologies has been proven in several studies summarized in Table 1. Silva *et al.* isolated AT-MSCs from human mediastinal brown fat depots and carried out several studies in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice fed a HFD to evaluate their thermogenic potential *in vivo* [94]. While no differences were seen when undifferentiated AT-MSCs were injected subcutaneously compared to saline controls, the authors observed a reduction in body weight and glucose levels in mice that had been transplanted with differentiated AT-MSCs within scaffolds 5 weeks post-transplantation [94]. Similar results were obtained in another study in which human-derived MSCs, MSC-derived brown adipocytes, and MSC lysate were injected intraperitoneally into obese mice for 10 weeks [95]. Only the injection of MSC-derived brown adipocytes reduced body weight and decreased triglyceride and cholesterol levels. However, all treatments improved non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, glucose intolerance, inflammation, and energy homeostasis.

Functional brown adipocytes have also been generated by cellular reprogramming procedures using induced pluripotent stem cell (iPSC)-derived embryoid bodies (iBAs) transduced with the

PRDM16 gene or from direct conversion of mouse embryonic fibroblasts (dBAs) by the induction of PRDM16, C/EBP-β and L-MYC genes [96]. Importantly, subcutaneous transplantation of brown adipocytes (obtained from iBAs or dBAs) in HFD-treated mice and in KK-Ay diabetic mice was able to reduce diet-induced obesity, dyslipidemia, and insulin resistance [96].

In addition to the implantation of brown adipocytes, an elegant study shows another potential therapy against obesity through the thermogenic induction of human white adipocytes with adrenergic stimuli [97]. Beige adipocytes were derived from human capillary network progenitors and activated *in vitro* with the adenylate cyclase activator forskolin. These cells showed increased UCP1 mRNA expression and enhanced systemic glucose tolerance when implanted with matrigel subcutaneously into glucose-intolerant NOD-scid IL2rg(null) mice, both in normal chow or HFD [97].

5.2. Brown adipose tissue transplantation

In addition to the transplantation of beige or brown adipocytes, several studies have directly implanted BAT in an attempt to combat metabolic disorders (Table 1). To date, most BAT transplants have been performed in mice. Usually, the tissue originates from healthy adult wild-type donor mice and is implanted into a recipient mouse model for different purposes.

One of the first studies targeting diabetes was carried out in a streptozotocin-induced type I diabetes mouse model where BAT was transplanted from healthy mouse embryos subcutaneously [98]. One month after transplantation, mice showed normal blood glucose levels, an increase in adiponectin and leptin levels, and a reduction in adipose tissue weight and inflammation markers. Importantly, diabetic markers such as polyuria, polydipsia and polyphagia were reversed. However, insulin resistance and blood insulin levels were unchanged [98]. A few years later, the same authors obtained similar results in a non-obese diabetic (NOD)

mouse model in which BAT transplantation prevented diabetes [99]. They highlighted insulin-like growth factor-I (IGF-I) as an enhancer of these adipogenic, anti-inflammatory, and anti-diabetic effects [99].

In obesity-related studies, BAT transplantation is commonly carried out to reduce metabolic derangements associated with high calorie intake. Several studies performed BAT transplants in HFD-induced obese mice, in locations such as the visceral cavity, adjacent to endogenous BAT, or even the subcutaneous space, to determine whether this metabolically active organ can reverse obesity-induced metabolic disorders [100–102]. All these studies observed an improvement in glucose levels, an increase in insulin sensitivity, and a decrease in inflammation. In addition, these results were accompanied by an increase in levels of hormones such as adiponectin or norepinephrine, resulting in a higher metabolic rate, oxygen consumption, and energy expenditure [100–102].

As mentioned above, exposure to cold, a β-adrenergic stimulus, or exercise could induce browning in WAT resulting in beige fat. Stanford *et al.* transplanted scWAT from exercise-trained donors into the visceral cavity of HFD-treated sedentary recipients [103]. The authors demonstrated that exercise-induced beige fat transplantation can improve glucose homeostasis and the HFD-induced metabolic phenotype by increasing the oxygen consumption rate and UCP1 mRNA levels of the transplanted scWAT [103].

Other authors have studied the therapeutic potential of BAT in genetically modified animal models. In 2015, BAT from wild-type mice was transplanted subcutaneously in the dorsal region of leptin-deficient obese mice (*ob/ob*) [70]. The results obtained in this model corroborated those found in previous studies [100–102]: decreased weight gain, increased energy expenditure, and improved insulin sensitivity and hepatic steatosis. In addition, the authors reported an increase in adiponectin levels, expression of the β3-adrenergic receptor, and WAT FA oxidation [70]. Another study used Hph-1 mice, a mouse model of tetrahydrobiopterin (BH4) deficiency [104].

BH4 is involved in the control of BAT's regulatory factors such as norepinephrine and nitric oxide. Hph-1 mice are obese, glucose intolerant, insulin resistant, and have impaired BAT function. Implantation of BAT from wild-type donors into the visceral fat region of Hph-1 mice improved BAT function together with systemic metabolic alterations [104]. BAT transplantation has also been used to study a mouse model of Parkinson's disease by using DJ-1 knockout mice [105].

Metabolic activation after BAT transplantation has also been studied in other obesity-associated pathologies, such as cardiovascular disease, polycystic ovary syndrome, and atherosclerosis. Thoonen *et al*. utilized UCP1-deficient mice subjected to catecholamine-induced cardiomyopathy [106]. BAT transplantation from wild-type donors into the visceral cavity of UCP1-deficient mice improved survival and reduced myocardial injury, indicating a systemic cardioprotective role of functional BAT [106]. In a rat model with dehydroepiandrosterone (DHEA)-induced polycystic ovarian syndrome, BAT from wild-type rat donors was implanted in the dorsal region adjacent to endogenous BAT [107]. In addition to reversion of the DHEA-induced polycystic ovary syndrome characteristics, the authors observed metabolic improvements in glucose tolerance and insulin sensitivity [107]. Finally, BAT but not epididymal WAT (eWAT) transplantation reduced ApoE knockout-induced atherosclerosis in mice [108]. The authors concluded that this was mediated by metabolic improvements such as an increase in oxygen consumption, energy expenditure, and FGF-21, norepinephrine, and adiponectin levels; improvement in glucose tolerance; and a reduction in triglyceride levels [108]. Importantly, serum TNFα levels were increased after BAT transplantation and the implanted BAT showed higher levels of several pro- and anti-inflammatory mediators such as TNFα, IL-6, MCP-1, PAI-1, IGF-1, TFG-β, IL-4 and IL-10. This raises the question of whether the immune cells present in the circulation and/or in the implanted BAT would compromise in part the alleviation of the metabolic phenotype in a long-term study.

6. Discussion

BAT is a metabolically active organ, involved in the maintenance of energy homeostasis. Therefore, it is an attractive target in the fight against obesity and other associated metabolic diseases. The activities of brown adipocytes are reduced in obese and diabetic patients [3], which provides possibilities of targeting functional BAT that might result in therapeutic benefits. Thus, activation of BAT represents a potential opportunity to enhance energy expenditure and weight loss, and to improve lipid and glucose homeostasis.

Many studies have attempted to increase WAT browning and BAT activity through the use of several activators of thermogenesis (extensively reviewed elsewhere [6,75,76]). Here, we reviewed the potential benefits of transplanting functional BAT. We covered the source of adipocytes, the methods used, and the main metabolic outcomes (Figure 3).

Embryonic stem cells and stem cells isolated from bone marrow have been the traditional sources of MSCs. However, other potential stem cell sources in human adults have been described recently, such as the umbilical cord, peripheral blood, adipose tissue, bone marrow, cartilage, tendon, muscle and dental pulp [87]. The last five require more invasive procedures. However, the umbilical cord and peripheral blood are good options in terms of non-invasive, easy isolation methods. Adipose tissue is currently gaining leadership due to the fact that a large number of MSCs can be obtained, and AT-MSCs have a greater capacity for adipocyte differentiation and higher UCP1 expression when differentiated into brown adipocytes than MSCs isolated from umbilical cord blood [91]. This makes AT-MSCs a more appropriate source of MSCs for the study of obesity and other metabolic dysfunctions.

Transplantation studies have used both beige/brown cells or BAT directly with proven efficacy to prevent obesity and associated pathologies (Table 1 and Figure 3). In the first case, AT-MSCs from mice and humans have been followed in cell reprogramming procedures or under

adrenergic stimuli to obtain functional beige or brown adipocytes [94–97]. In the second case, BAT transplantation has been performed in mouse models of diabetes, diet, and genetic models of obesity, and obesity-associated pathologies, such as cardiovascular disease, polycystic ovary syndrome, and atherosclerosis [70,98,107,108,99–106]. In the vast majority of the studies, the metabolic phenotype including glucose tolerance, insulin sensitivity, and weight gain was efficiently improved (Table 1).

BAT thermogenesis is under the tight control of the brain. Thus, several authors have evaluated whether, in addition to the normal revascularization of the transplanted BAT, it was also irrigated by the sympathetic nervous system [96,101,109]. The evaluation of the re-innervation of the implant was performed by the IHC detection of tyrosine hydroxylase, an essential enzyme for the synthesis of catecholamines such as norepinephrine. These studies suggest that there is a partial sympathetic re-innervation of the transplanted BAT but to a lesser extend compared to the endogenous BAT. In addition, recipient mice showed an increase in sympathetic activity and plasma norepinephrine [101,109]. Altogether, this indicates that the implanted BAT receives the brain regulation and contributes to the sympathetic activity of the endogenous BAT.

Transplantation of functional BAT has been performed mainly in mice. Thus, translation into human therapeutic studies must be carried out with caution. Although the MSCs source and isolation method could be similar in rodents and humans, there might be differences in the duration and capacity for differentiation to other lineages and the outcome obtained. In addition, the accessibility of BAT in humans is challenging due to its distribution in the body. The most likely source of AT-MSCs in humans would be WAT obtained from surgical interventions such as liposuction.

Other potential limitations of the transplantation of functional BAT are related to safety. This raises the question of how tightly controlled is BAT thermogenesis. Enhanced BAT thermogenesis might induce necrosis, an unsafety high temperature, increased resting metabolic

rate or tachycardia that might be contraindicated in the elderly or for those with heart disease. While no signs of necrosis have been reported in the transplantation studies performed in mice, increased resting metabolic rate and tachycardia has been described in humans treated with the β3-adrenergic receptor agonist mirabegron [85,86]. This drug is currently approved to treat overactive bladder and was reported to activate human BAT in healthy and young humans [85]. However, the potential adverse effects should be considered in further human applications. In addition, most of the studies in mice have analysed the increased in BAT thermogenesis in a short-term period. Long-term studies would have to carefully analyse whether the increase in energy expenditure by thermogenesis triggers a compensatory mechanism such as an augmented appetite that might overcome its benefit.

Disclosure statement

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Figure legends

Figure 1. Schematic representation of brown adipose tissue (BAT) distribution in rodents and humans. For many years, BAT was thought to be exclusive to rodents and human neonates, but in the last decade it was discovered that adult humans have active BAT and that its distribution is similar to that found in rodents. The classical BAT is located in the suprascapular region, but it is also found around the neck, axillary, paravertebral and perirenal areas.

Figure 2. Sources for mesenchymal stem cell (MSC) isolation and differentiation into white, beige or brown adipocytes. Cells with MSC-like characteristics have been isolated from the placenta, amniotic fluid, and umbilical cord and from several adult tissues, including muscle, dermis, blood, bone marrow, gingival tissue and adipose tissue. The most common phenotypical markers of MSCs include CD29, CD44, CD37, CD90, CD105, CD116 and Stro-1. MSCs have the capability to self-renew and have multilineage potential to differentiate into many mesodermal cell types. These include myoblasts, osteoblasts, and adipocytes. Brown adipocytes and myoblasts arise from myogenic cell lineage progenitors that express myogenic transcription factor Myf5+. In contrast, white adipocytes arise from Myf5- precursors. The origin of beige adipocytes is still under scrutiny. Existing theories indicate that they are either derived from the same precursor as white adipocytes or transdifferentiated from pre-existing white adipocytes.

Figure 3. Thermogenic adipocytes transplantation as a therapeutic strategy to fight obesity. Schematic representation of the strategy to transplant thermogenic adipocytes for therapeutic purposes. 1) The first step would be surgical resection of WAT from an obese patient. 2) Next, the tissue would be disaggregated using collagenase to obtain a single cell suspension. 3) Mesenchymal stem cells (MSCs) would be separated from the stromal vascular fraction (SVFs) and cultured and expanded *in vitro*. 4) The next step would be the lineage-

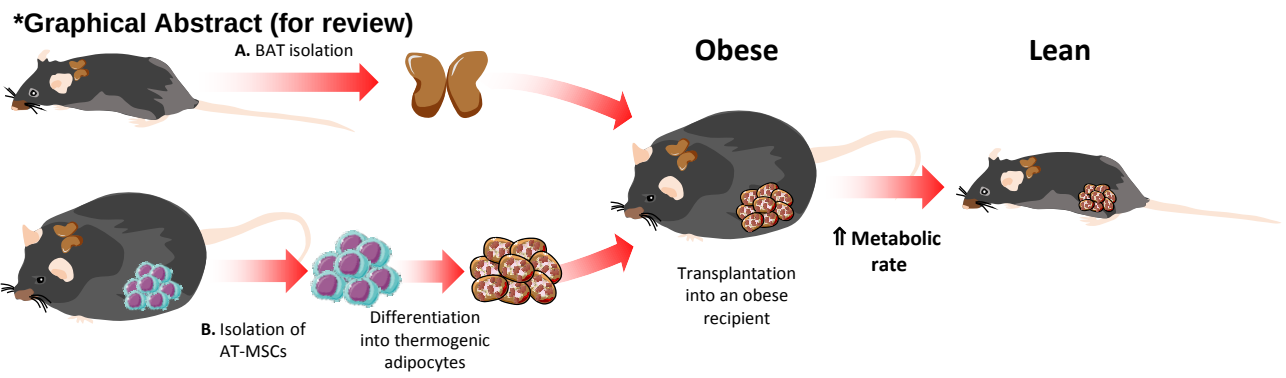
specific differentiation of these MSCs into thermogenic (beige) adipocytes. 5) Finally, the last step would consist in the transplant of differentiated adipocytes into the obese patient. These thermogenic adipocytes would work as metabolically active cells and would increase the metabolic rate of the patient, which in turn could result in weight loss.

Source	Recipient	Transplant localization	Main outcomes	Refs
BEIGE OR BROWN CELL TRANSPLANTATION				
Precursor cells from human mediastinal BAT	Autoimmune-mediated type 1 diabetic mice fed with HFD	Sc injection	Reduced glucose levels and body weight.	[94]
Brown adipocyte-derived stem cells and MSCs lysate	HFD-induced obese mice	Injected intraperitoneally	Reduced body weight. Improved non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, glucose intolerance, inflammation, and energy homeostasis.	[95]
Brown adipocytes from iPSC-derived embryoid bodies (iBAs) and from mouse embryonic fibroblasts (dBAs)	HFD-induced obese mice and in a type 2 diabetes mouse model	Sc	Decreased weight gain. Improved glucose tolerance and insulin sensitivity (dBAs).	[96]
Beige adipocyte-derived progenitors of human capillary networks	Autoimmune-mediated type 1 diabetic mice	Sc	Improved glucose tolerance and decreased hepatic steatosis.	[97]
BAT TRANSPLANTATION				
Mouse embryonic BAT (E16.5–E17.5)	Streptozotocin-induced type 1 diabetes mouse model	Sc region	Normalized glucose tolerance, loss of adipose tissue and reduced inflammation. Increased adiponectin, leptin and IGF-1 levels and decreased glucagon levels.	[98]
12-week old mouse BAT	HFD-induced obese mice	Visceral cavity	Improved glucose tolerance, insulin sensitivity, and lower body weight. Increased norepinephrine, FGF21, and IL-6 levels.	[100]
6-week old mouse BAT	6-week old HFD-induced obese mice	Adjacent to BAT	Reduced weight gain, improved insulin sensitivity and glucose homeostasis. Increased body temperature, energy metabolism, oxygen consumption, and respiratory ratio.	[102]
8-week old mouse BAT	8-week old HFD-induced obese mice	Dorsal sc region	Loss of body weight and fat, increased energy expenditure, and oxygen consumption. Increased sympathetic activity.	[101]
Mouse embryonic BAT (E15–E18)	Autoimmune-mediated type 1 diabetic mice	Sc region	Euglycemia. Increased adiponectin and IGF-1 levels and decreased inflammation and glucagon levels.	[99]
6-week old mouse BAT	*Ob/Ob* mice	Dorsal sc region	Improved insulin resistance and hepatic steatosis. Reduced weight gain, increased oxygen consumption, energy expenditure, FA oxidation in scWAT and eWAT, and increased adiponectin levels and β3-adrenergic receptor expression.	[70]
12-week old mouse BAT	Catecholamine-induced cardiomyopathy in *UCP1*$^{-/-}$ mice	Visceral cavity	Improved glucose homeostasis. Cardioprotection.	[106]
11-week old exercise-trained mouse scWAT	12-week old HFD-induced obese mice	Visceral or sc cavity	Improved glucose homeostasis, increased oxygen consumption rate, and UCP1 levels. Increased glucose uptake in skeletal muscle and BAT.	[103]

3-week old rat BAT	Dehydroepiandrosterone (DHEA)-induced polycystic ovary syndrome rat	Adjacent to BAT	Improved insulin sensitivity and fertility. Activated endogenous BAT and increased adiponectin levels.	[107]
8-week old mouse BAT	Hph-1 mice fed with HFD	Visceral fat regions	Improved BAT function. Decreased adiposity, glucose intolerance, and insulin resistance.	[104]
DJ-1 knockout-induced obese resistant mice BAT	HFD-induced obese mice	Sc region	Ameliorated body mass gain. Reversed hepatic steatosis, induced UCP1 expression in endogenous BAT, and improved glucose tolerance and insulin sensitivity.	[105]
12-week old mouse BAT	12-week old *ApoE*$^{-/-}$ mice	Visceral cavity	Decreased atherosclerotic lesion area and triglyceride levels. Increased oxygen consumption, energy expenditure, and FGF-21, norepinephrine and adiponectin levels. Improved glucose tolerance.	[108]

Table 1. Models for beige or brown cell transplantation and BAT transplantation and their main outcomes in metabolism

BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; Sc, subcutaneous; scWAT, subcutaneous white adipose tissue


*Graphical Abstract (for review)
A. BAT isolation
Obese
Lean
B. Isolation of AT-MSCs
Differentiation into thermogenic adipocytes
Transplantation into an obese recipient
⇑ Metabolic rate

Figure

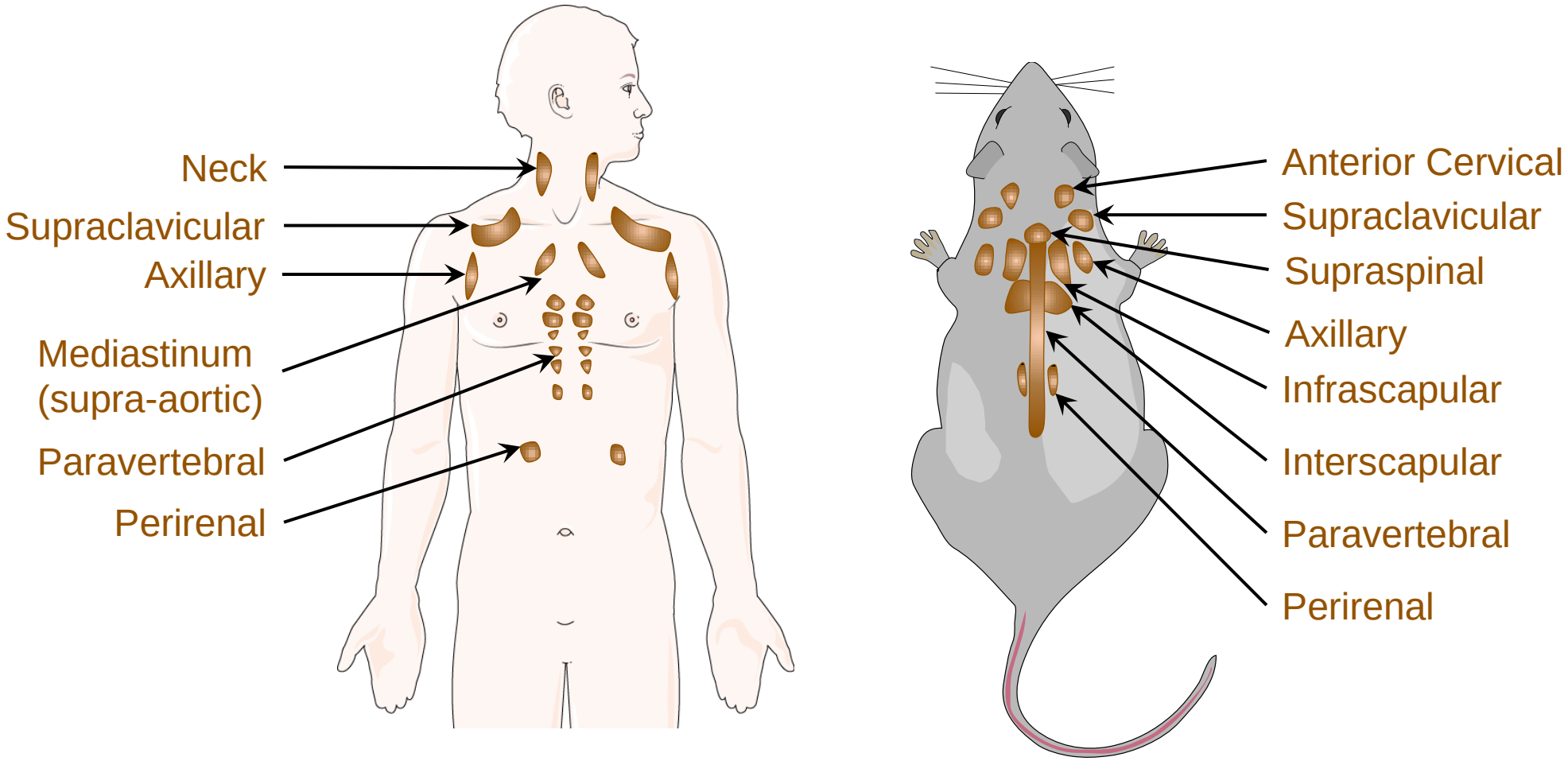


Figure 1

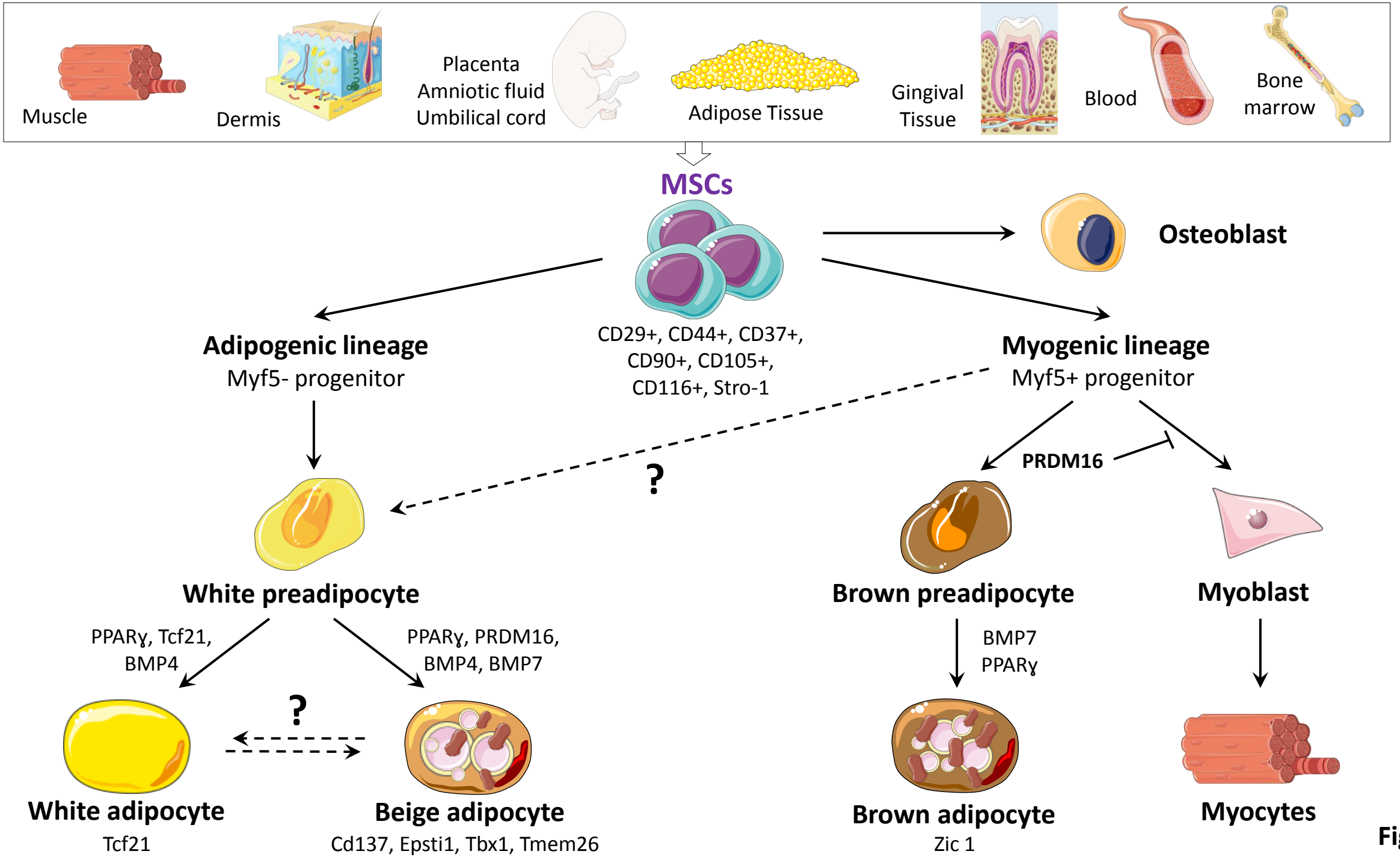
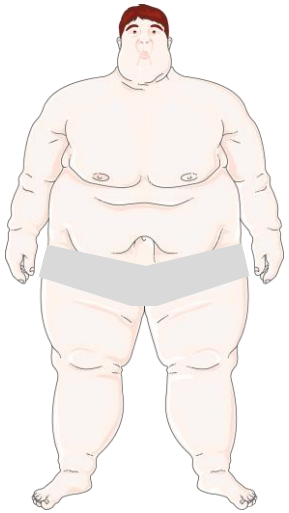
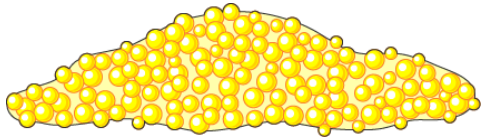

Muscle
Dermis
Placenta
Amniotic fluid
Umbilical cord
Adipose Tissue
Gingival Tissue
Blood
Bone marrow
MSCs
Osteoblast
CD29+, CD44+, CD37+,
CD90+, CD105+,
CD116+, Stro-1
Adipogenic lineage
Myf5- progenitor
Myogenic lineage
Myf5+ progenitor
?
PRDM16
White preadipocyte
Brown preadipocyte
Myoblast
PPARɣ, Tcf21,
BMP4
PPARɣ, PRDM16,
BMP4, BMP7
BMP7
PPARɣ
?
White adipocyte
Tcf21
Beige adipocyte
Cd137, Epsti1, Tbx1, Tmem26
Brown adipocyte
Zic 1
Myocytes

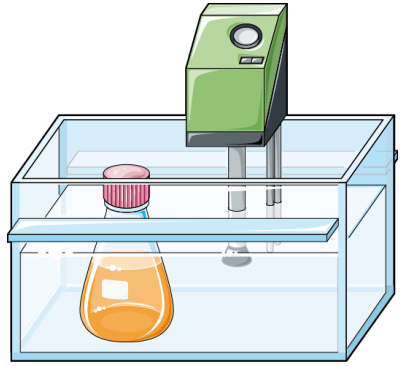
Figure 2





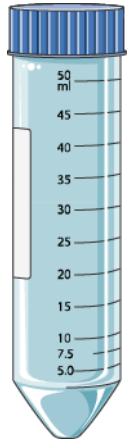
1. Surgical resection

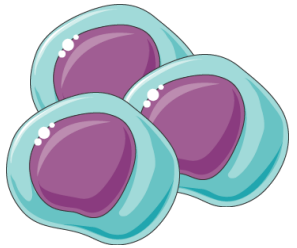
Resection of white adipose tissue from obese patient



2. Tissue digestion

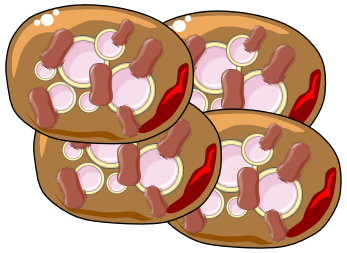
Using collagenase, the tissue is disaggregated to obtain a single cell suspension





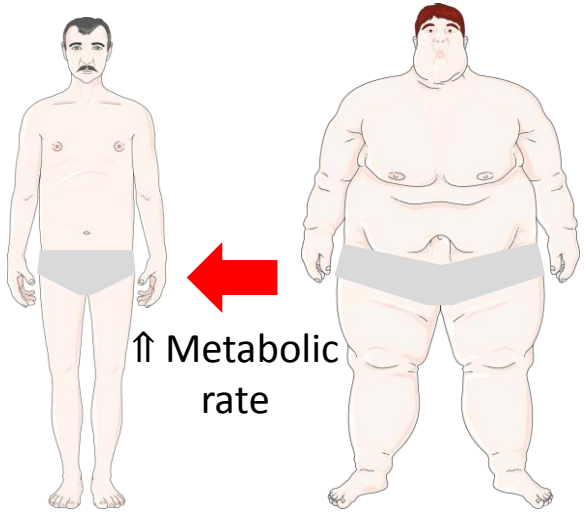
3. Isolation and expansion of MSCs

MSCs are separated from SVFs and cultured to allow proliferation



4. Lineage specific differentiation of MSCs

MSCs are differentiated into thermogenic adipocytes



5. Transplant of thermogenic adipocytes to obese patient

Figure 3