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Transplantation of Adipose Tissue and Adipose-Derived Stem Cells as a Tool to Study Metabolic Physiology and for Treatment of Disease

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Abstract

Humans and other mammals have three main fat depots - visceral white fat, subcutaneous white fat, and brown fat - each possessing unique cell-autonomous properties. In contrast to visceral fat which can induce detrimental metabolic effects, subcutaneous white fat and brown fat have potential beneficial metabolic effects, including improved glucose homeostasis and increased energy consumption, which might be transferred by transplantation of these fat tissues. In addition, fat contains adipose-derived stem cells that have been shown to have multilineage properties which may be of value in repair or replacement of various cell lineages. Thus, transplantation of fat is now being explored as a possible tool to capture the beneficial metabolic effects of subcutaneous white fat, brown fat, and adipose-derived stem cells. Currently, fat transplantation has been explored primarily as a tool to study physiology, with the only application to humans being reconstructive surgery. Ultimately, the application of fat transplantation for treatment of obesity and metabolic disorders will reside in the level of safety, reliability, and efficacy when compared to other treatments.

The adipose organ is the largest organ in the body. Even lean adult men and women have at least 7 to 10 pounds of fat, and in very obese individuals, fat can represent 100 pounds or more of body weight. The adipose organ is complex, with multiple depots of white fat involved in energy storage, hormone (adipokine) production and local tissue architecture, as well as small depots of brown fat involved in burning energy to create heat (nonshivering thermogenesis).

While excessive accumulation of white fat in obese individuals creates insulin resistance and risk of many metabolic disorders, the realization that white fat may produce beneficial adipokines and that brown fat may have beneficial effects on metabolism has raised the possibility that transplantation of adipose tissue can play an important role in understanding its physiological roles and may even have therapeutic benefits. Adipose tissue has also proved to be a major source of adult-derived multipotent stem cells. This review will summarize our current knowledge about the biology of these fat depots and how transplantation of adipose tissue or adipose-derived stem cells may provide new insights into the physiological roles of adipose tissue and the beneficial effects in disease management.

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Properties of various fat depots

Visceral and subcutaneous white fat depots

White adipose tissue is distributed throughout the body, with the two major depots being subcutaneous and intraabdominal or visceral white fat. These two major fat depots in the body have differential metabolic effects. Epidemiological studies have found that increased visceral fat, i.e. central obesity, as measured by large waist circumference or high waist-hip ratio, is associated with adverse health risks such as insulin resistance, type 2 diabetes, dyslipidemia, hypertension, atherosclerosis, hepatic steatosis, cholesterol gallstones, and overall mortality [1–7] (Fig. 1). Consistent with this notion that visceral fat produces adverse metabolic effects, omentectomy, i.e., removal of visceral fat, results in decreased insulin and glucose levels in humans [8], as well as decreased serum cholesterol and triglyceride levels, improved hepatic and peripheral insulin sensitivity, and increased life span in animal models [9–12]. By contrast, peripheral obesity, i.e. increased subcutaneous fat mainly in the gluteofemoral region, appears to be associated with improved insulin sensitivity and a lower risk of developing type 2 diabetes [13,14] (Fig. 1). Indeed, individuals with combined peripheral and central obesity have lower levels of plasma glucose, insulin, and triglycerides, increased glucose uptake into tissues, and lower aortic atherosclerosis scores than individuals with pure visceral obesity [15,16]. Not surprisingly, therefore, removal of subcutaneous fat by liposuction without changes in lifestyle factors, does not result in improvement in any aspect of the metabolic syndrome [17,18], and may even lead to increased intraabdominal fat accumulation (R. Eckel, personal communication).

The mechanisms responsible for the protective effects of subcutaneous fat and detrimental effects of visceral fat have been ascribed to differential levels of adipokines; differential expression of developmental, metabolic signaling molecules, and microRNAs (miRNAs); and differences in degree of inflammation, and response to insulin-sensitizing compounds. For example, the adipokine adiponectin, and especially the high molecular weight form of adiponectin, has insulin-sensitizing [19,20], anti-atherosclerotic [21], and anti-inflammatory properties, and is secreted more abundantly from subcutaneous fat than visceral fat depots [22–24]. Indeed, when obese ob/ob mice are engineered to overexpress adiponectin in adipose tissue, there is improved insulin sensitivity, increased lipid clearance, improved diacylglycerol levels, reduce hepatic steatosis, and improved function of β-cells despite a massive further increase in subcutaneous fat [25]. By contrast, resistin and retinol binding protein (RBP) 4 are adipokines involved with insulin resistance and type 2 diabetes and are more abundantly secreted from visceral than subcutaneous fat [26–29].

Recent studies suggest that the properties of adipocytes in different fat depots may represent an intrinsic heterogeneity of adipocytes, and that these properties and the distribution of fat in different depots might be regulated by fundamental developmental genes [30]. For example, T-box 15, a mesodermal developmental gene, is more highly expressed in visceral than subcutaneous adipocytes of lean individuals and less expressed in visceral fat of obese individuals, whereas the glycoinositol phosphate-linked membrane protein, glypican 4, shows the opposite pattern and these patterns are also observed in preadipocytes from the same area [30] (Fig. 1). In addition, adipose function and distribution may be affected by

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molecules involved with signal transduction. For example, the neurotrophic tyrosine kinase receptor type 2 (NTRK2), is more highly expressed in subcutaneous fat than visceral fat [31], and mutations of NTRK2 have been found in severely obese children [32]. MicroRNAs (miRNAs), i.e. small non-coding RNAs that can regulate biological processes, have also been shown to have a fat depot-specific expression. miRNA-92, miRNA-95, miRNA-181a, and miRNA-311 are expressed in human subcutaneous fat and are all significantly negatively correlated with adipocyte volume, whereas miR-145 is highly expressed in omental fat in subjects with type 2 diabetes [33] (Fig. 1). Adipose tissue is a major site of inflammation. The visceral fat depot has higher levels of macrophages, T cells, and natural killer cells [34], and releases more inflammatory cytokines, such as monocyte chemotactic protein-1 (MCP1) [35], plasminogen activator inhibitor-1 (PAI-1) [36], interleukin (IL)-6 [37], IL-8 [38], and IL-10 [39], than does subcutaneous fat depot. Thus, increased inflammation produced by excess visceral fat depot increases risk of obesity-related diseases and mortality.

Finally, subcutaneous and visceral fat depots have intrinsically different responsiveness to drugs, such as the insulin sensitizing thiazolidinediones (TZDs). TZDs bind to peroxisome proliferator-activated receptor (PPAR) γ, a nuclear receptor involved in adipocyte differentiation. Subcutaneous fat has higher basal levels of PPARγ 1 and 2, and are more responsive to TZDs than visceral fat [40]. Thus, TZD treatment results in increased subcutaneous fat [41–43], which is associated with increased insulin sensitivity. Treatment with TZDs also increases adiponectin content and secretion from subcutaneous fat, but not from visceral fat of humans [44,45]. Taken together, these data suggest that the beneficial metabolic properties of subcutaneous fat are due to intrinsic differences in adipokine secretion, developmental programming, and responsiveness to insulin-sensitizing compounds.

Subcutaneous and visceral fat have cell-autonomous properties due to inherently different progenitor cells in their fat depots. This was demonstrated by the depot-specific rates of replication, apoptosis, lipid accumulation, and gene expression profiles that persisted for 40 population doublings in preadipocyte strains derived from single subcutaneous, mesenteric, and omental human preadipocytes with stably expressed telomerase [46,47]. These cell-autonomous properties could account for the differential metabolic properties between subcutaneous and visceral fat. One potential approach to promote these beneficial metabolic effects of subcutaneous fat is by increasing subcutaneous fat mass by transplantation.

Brown fat depot

In addition to white fat, mammals have brown fat. This fat differs from white fat by its high levels of mitochondria, multilocular, rather than unilocular, lipid droplets, high degree of vascularization, sympathetic innervation, and most importantly, expression of uncoupling protein (UCP) 1. UCP1 creates a leaky proton channel in the mitochondria that uncouples oxidative phosphorylation which results in inefficient storage of energy as ATP and increased release of heat as part of the process of nonshivering thermogenesis. Thus, the primary metabolic function of brown fat is to increase energy expenditure and heat (Fig. 1).

Brown fat is localized to the interscapular and paraspinal areas in rodents and newborn humans. In adult humans, UCP1-positive brown fat could be identified at autopsy, but this

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brown fat was thought to be non-functional [48–50]. However, recent studies using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) and computer tomography (CT) have revealed significant activity in the brown fat located in the neck, supraclavicular, mediastinal, paraspinal and suprarenal area of adults [51–56]. In individuals studied under ambient conditions, active brown fat, i.e. adipose tissue with high uptake of ^{18}F-FDG in PET/CT scans, is found in 3 % of men and 7% of women [53]. In individuals subjected to two-hour cold exposure, the prevalence of detectable active brown fat in lean individuals increases up to 96% [51,55,56].

Lean individuals have been shown to have more easily detected and more active brown fat than overweight or obese individuals [51]. Indeed, activity of brown fat is inversely related to percent total body fat [51,56] and BMI [51,53,56] in most, but not all, studies [57]. Lean individuals also have higher skin temperature than overweight and obese individuals [51]. Obese individuals with active brown fat tend to have improved glucose tolerance suggesting a beneficial effect of active brown fat [58]. Furthermore, increased glucose uptake in brown fat is inversely correlated with fasting glucose [53,59–61]. Lower insulin levels are also weakly, but significantly, associated with activity of brown fat in a group of lean subjects [56]. Overall, these data suggest that upregulation of brown fat activity may contribute to a lean and metabolically healthy phenotype in humans. These findings also suggest that transplantation or stimulation of brown fat may be a therapeutic approach to increasing energy expenditure, lowering white fat mass and improving metabolism.

Transplantation of adipose tissue

Fat transplantation has been the subject of experimentation for over 100 years. The goal of fat transplantation has evolved substantially over the years from improving appearance in reconstructive surgery to learning about the biology of fat to potentially inducing beneficial metabolic effects and treating certain diseases.

Reconstructive surgery

As early as 1893, Neuber reported transplantation of fat from the arm to fill depressions in the face due to tuberculosis [62]. The main technical problem of using fat for reconstructive surgery has been maintenance of graft volume and viability while minimizing inflammatory response [63]. Fat is currently used in reconstructive and other surgery in a variety of structural ways. Unfortunately, there has been no characterization of these individuals, so the metabolic effects of this transplantation are unknown.

Understanding the biology of fat

Experimental fat transplantation in rodents has involved both subcutaneous and visceral white fat, and brown fat (Fig. 2A). The studies have examined the survival, vascularization and innervation of the fat grafts, the metabolic effects of fat transplantation, and the effects of fat grafts on the endogenous fat growth.

Transplantation of white fat pads to study the development of fat in rodents (summarized in Table 1)—Some of the earliest studies were by Hausberger who transplanted “immature” perigonadal fat cells that were devoid of lipid and indistinguishable

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from fibroblasts from five-day old rats into either a subcutaneous or visceral site of recipient rats [64,65] . He demonstrated that these immature fat cells could become whole fat pads that shrank or grew upon starvation or overfeeding respectively, whereas transplanted connective tissue cells from the fascia did not develop into fat pads. Iyama and colleagues showed that fat cells transplanted subcutaneously differentiated more when in proximity to capillaries [66], indicating that vascularization of fat grafts is essential for the growth of fat pads (Table 1). When fat is transplanted between lean and obese mice, the fat cells in the grafts grow or shrink to become similar to those of the host in size and fatty acid composition, indicating that the host environment is more important in determining some aspects of fat cell fate than the initial properties of the transplanted cell [67–69] (Table 1). However, the fat grafts used in these studies were generally small (5–10 mg), and the effects on whole-body metabolism were not examined.

Other studies of whole-fat transplantation examined the regulation of total fat mass (Table 1). Transplantation of perigonadal fat to a subcutaneous site in the recipient mouse initially resulted in a decrease in total fat mass at two weeks, but this difference was no longer significant at five weeks as the fat grew [70]. Generally, it was perceived that removal and/or transplantation of subcutaneous fat to subcutaneous sites induced less growth of total fat mass than did removal and/or transplantation of visceral fat [71,72] (Table 1).

Transplantation of preadipocyte cell lines and stromovascular fraction of fat (summarized in Table 2)—Models of adipocyte development *in vivo* have been made by using preadipocyte cell lines, such as the 3T3-F422A [73–79] (Table 2, part I), and stromovascular fraction (SVF) of fat from rodents [80–86] (Table 2, part II). The SVF, obtained by collagenase digestion of fat pads and centrifugation to remove the mature adipocytes, is a mixture of cells including preadipocytes, fibroblasts, vascular cells and blood cells. The cultured cell studies have revealed that, although these are good models of differentiation *in vitro*, fat pad development *in vivo* after transplantation requires cell lines with very high potential for proliferation and differentiation (e.g. 3T3-F442A are better than 3T3-L1, 3T3-C2 and Ob17 lines [73–76]). Transplantation of SVF of flank, epididymal, omental, and perirenal fat formed fat pads in rodents [80–84], whereas marrow-derived fibroblasts [80] and skin fibroblasts [81] did not form fat pads. In addition, confluent preadipocytes [74], dedifferentiated primary mature adipocytes [85], and SVF [82] engraft better than fully differentiated cells [74] or mature adipocytes [86]. Successful transplantation requires a high degree of vascularization as shown by fat grafts near large blood vessels as well as the absence of fat grafts when intraperitoneal injections of anti-vascular endothelial growth factor (VEGF) antibody were administered [74,76,81]. When preadipocyte cell lines are seeded into a matrix such as the matrigel [77], polyglycolic acid (PGA) scaffold [78], or collagen [81], they have slower rates of maturation, but increased content of DNA and triglycerides, high vascularization, and less necrosis than cells without scaffolds. In some of these experiments, tracking the development of the transplanted preadipocytes has been facilitated by stable transfection with β-galactosidase transgene [74], incubation with the dye PKH26 [82], or expression of green fluorescent protein (GFP) [76]. In an interesting experiment investigating the role of transplantation site on fat cell function and metabolism, Shibasaki, M. *et al*. demonstrated that implantation of 3T3-L1 preadipocyte cells into a subcutaneous site in

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mice improved metabolism as indicated by decreased glucose and insulin levels during a glucose tolerance test, whereas implantation of 3T3-L1 cells into a visceral mesenteric site worsened metabolism as shown by increases in serum insulin, triglycerides, and tumor necrosis factor (TNF)α [79].

Transplantation of brown adipocytes in rodents (Summarized in Table 3)— Successful transplantation of brown fat has been achieved in rodents using small pieces of brown fat tissue [87], whereas small grafts (1–3 mg) [88], isolated preadipocytes or mature brown adipocytes [87] undergo necrosis when transplanted. The development of immature brown fat transplanted into the eye of adult hamsters has been characterized and revealed initial vascularization and proliferation of unilocular brown fat graft, followed by innervation at day 10, and subsequent proliferation of brown adipocyte precursors near capillaries, increased mitochondrial ultrastructure, and development of multilocular lipid droplets [89]. When intraocular transplantation was performed after sympathetic denervation, differentiation of brown adipocytes still occurred, but was slower and less robust [89]. Brown fat has also been transplanted under the kidney capsule [90], but no innervation was observed in this location even after two weeks.

The morphological characteristics of brown fat are different between lean and obese mice. In lean mice, brown adipocytes are small, have multilocular lipid droplets, dense mitochondrial structure, and innervation goes to both the adipocyte and nearby capillaries [91]. In contrast, brown adipocytes of obese ob/ob mice are larger, have unilocular lipid droplets, sparse mitochondria, and innervation goes to the capillaries but much less to the brown adipocytes themselves. Transplantation of brown fat between obese and lean mice showed that morphological transformation of brown fat to that of lean mice could be induced with extreme cold exposure (4°C for five weeks) but not with normal or warm temperature exposure (23°C or 33°C) [91]. Moreover, after the long exposure to 4°C, the brown fat graft still maintained the morphology similar to that of lean mice after being returned to 23°C for another three weeks. Transplantation of brown fat between obese or lean mice also indicated that the host environment of the fat graft, rather than the donor of the fat, determined the fatty acid composition of the graft [92].

Beneficial metabolic effects of transplantation of fat

Transplantation of white fat

Synthesis of fat (summarized in Table 4, part I): Lipodystrophies are genetic or acquired syndromes caused in part by the inability to form lipid droplets in adipocytes. At a clinical level, they are characterized by a significant loss of body fat (either complete or partial), insulin resistance, dyslipidemia, hepatic steatosis, hypertension and/or diabetes [93]. The A-ZIP/F mouse, which carries a dominant negative transcription factor that inhibits adipose differentiation, has virtually no fat and a phenotype resembling that of humans with severe lipodystrophy. Transplantation of perigonadal or subcutaneous fat from a normal mouse to the subcutaneous region of the lipodystrophic mouse greatly improves its metabolism with decreased food intake, reduced glucose and insulin levels, and decreased hepatic steatosis, as well as increased insulin sensitivity and glucose uptake into muscle [94,95] (Table 4, part I). Transplantation of fat also results in improved histology of β-cells and increased insulin

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immunostaining. When fat grafts obtained from leptin-deficient ob/ob mice were transplanted into the lipodystrophic A-ZIP/F mice, no reversal of metabolic abnormalities was observed [96]. Thus, the mechanism by which the transplantation of fat improved metabolism required leptin secretion by the adipocytes and could be reproduced by leptin administration [96]. Subsequently, it has been shown that administration of leptin to humans with lipoatrophic diabetes can also dramatically reverse insulin resistance, hepatic steatosis, and serum triglyceride levels [97]. This treatment, however, is not without side effects, since leptin administration also stimulates the immune system [98,99]. Clearly, if transplantation of adipose tissue could be performed successfully in lipodystrophic patients, then daily leptin injections would no longer be needed.

Diacylglycerol acyltransferase 1 (DGAT1) is a key enzyme in the synthesis of triglycerides in mammals. Fat pads from mice lacking DGAT contain small adipocytes. Transplantation of perigonadal fat from mice lacking DGAT into a subcutaneous site of obese ob/ob mice or Agouti yellow mice improved metabolism as demonstrated by decreased body weight, weight of fat pads, triglycerides in muscles, and serum TNFα, as well as increased insulin sensitivity, energy expenditure, and adiponectin mRNA [100]. Fat grafts lacking DGAT also enhanced glucose tolerance in normal wild-type mice and Agouti yellow mice, but not in ob/ob mice, possibly because the degree of obesity in ob/ob mice was too severe.

Leptin-deficient or leptin receptor defective obesity (summarized in Table 4, part II): Several genetic rodent models of obesity have defects in either leptin or the leptin receptor. For example, the obese ob/ob mouse is leptin deficient due to mutations for the leptin gene. Transplantation of perigonadal fat from normal mice into a subcutaneous site of ob/ob mice restored metabolism with normalization of plasma levels of leptin, insulin, glucose, and corticosterone, improved glucose and insulin tolerance tests, and decreased food intake and body weight [101]. In addition, because of the role of leptin in immune function, this restored immune function including decreased amount of apoptosis of immature thymocytes to normal levels, increased thymus and spleen cell number to normal, and normalized IL-6 levels [102].

Obese Zucker fatty rats (fa/fa or ZDF) and obese db/db mice, on the other hand, have genetic mutations in the leptin receptor and have been used in transplantation experiments to help understand the role of leptin receptor in fat metabolism. Thus, when normal wild-type rats were transplanted with perigonadal fat graft from Zucker Diabetic Fatty rats [103], administration of leptin by adenoviral gene transduction did not deplete fat from the fa/fa graft nor activate STAT3 or CREB in the fa/fa graft, and did not increase plasma catecholamines as compared to rats transplanted with normal fat. These results indicate a role of the leptin receptor in fat in the effect of leptin on STAT3 which leads to mitochondrial oxidation of fatty acids in fat, as well as the indirect effect of leptin on hypothalamus to release catecholamines, increase CREB phosphorylation and stimulate mitochondrial oxidation of fatty acids in fat.

As with the lipodystrophic mice, transplantation of normal fat into obese leptin-deficient mice helps normalize energy balance and metabolism by increasing plasma leptin [101,102]. However, the majority of obese people do not lack leptin production [104], but have some

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degree of leptin resistance. Thus, it is not surprising that administration of recombinant leptin into obese subjects for six months in a placebo-controlled trial did not produce dramatic reduction in body weight in most of the subjects [105]. Hence, transplantation of fat for the sole purpose of increasing leptin levels to treat the majority of obese subjects is not a sufficient reason for fat transplantation. However, certain fat depots have other properties which may produce beneficial metabolic effects (see below).

Subcutaneous versus visceral fat transplantation (summarized in Table 4, part III): Subcutaneous and visceral fat are associated with differential metabolic effects and have differential gene expression profiles. However, until recently, fat transplantation had not been used to examine direct effects of cell-autonomous properties of subcutaneous and visceral fat on metabolism. In a somewhat surprising result considering the evidence that visceral fat is associated with insulin resistance, Konrad *et al*. in 2007 showed that epididymal fat transplanted to the visceral cavity improved glucose tolerance and decreased glucose and insulin levels [106]. However, adipocyte size in the graft was significantly smaller than that of endogenous epididymal fat, and small fat cells are associated with increased insulin sensitivity [107]. Thus the visceral fat graft in this model appears to have lost its detrimental cell-autonomous properties by changing its own metabolic balance.

More recently, we have explored this question by creating a four-way study, transplanting visceral fat into both subcutaneous and visceral depots and subcutaneous fat into both subcutaneous and visceral depots. We found that transplantation of about 1 g of subcutaneous flank fat into the visceral cavity of normal C57BL/6 mice resulted in beneficial metabolic effects, including decreased body weight, total fat mass, plasma insulin and glucose levels, as well as improved glucose tolerance, enhanced whole-body insulin sensitivity, and increased insulin action to suppress hepatic glucose production [108] (Fig. 2B). Since these were allografts, the fat graft did not cause inflammation and there was no increase in gene expression of F4/80 macrophage, IL-6, or TNFα in the fat graft (Fig. 2B). Plasma levels and gene expression of adiponectin and leptin in the fat graft were either unchanged or decreased, thus they are not likely to mediate the beneficial effects of subcutaneous fat in this model. Resistin, an adipokine associated with insulin resistance [26], did decrease in expression in the fat graft, however, it is not clear that this explains the protective metabolic effect of the transplant. Transplantation of subcutaneous flank fat to a subcutaneous site in the recipient also significantly decreased body weight, fat mass, and plasma glucose, as well as increased glucose uptake into fat and hepatic insulin sensitivity, but to a lesser extent than transplantation of subcutaneous fat to the visceral cavity. By contrast, transplantation of epididymal fat into the visceral cavity or to a subcutaneous site had no beneficial metabolic effects, indicating that the effects of subcutaneous fat are due to its cell-autonomous properties. These results indicate that there was cross-talk between the subcutaneous fat graft placed in the visceral cavity and the recipient mouse's liver where insulin's suppression of glucose production improved. The mechanism for this cross-talk is not known, but the most likely is that secreted factors from subcutaneous fat, when present in sufficient concentration, act on nearby tissues in the recipient such as the liver. Hocking *et al*. showed that transplantation of subcutaneous fat to the visceral cavity in mice fed a high fat diet did not affect body weight, but also had beneficial metabolic effects such as

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decreased fat mass and improved glucose tolerance [109]. Thus, transplantation of subcutaneous fat induces several beneficial metabolic effects, but whether transplanted subcutaneous fat would have beneficial metabolic effects in humans is not known.

Transplantation of brown adipose tissue or cells engineered to form brown fat in rodents (summarized in Table 5): The notion of transplanting brown fat to increase energy expenditure and improve metabolism is an appealing one. Since endogenous brown adipose tissue is very limited, identification and manipulation of critical regulators of brown fat differentiation have been employed to engineer brown fat that can help to induce beneficial effects.

Bone morphogenetic protein (BMP)-7 is a member of the transforming growth factor-beta (TGF-beta) superfamily. C3H10T1/2 mesenchymal progenitor cells treated with BMP7 and transplanted into nude mice have been shown to undergo brown adipocyte differentiation that led to increased in energy expenditure, mitochondrial biogenesis, and decreased weight gain [110] (Table 5). Likewise, PRDM16 (PR domain containing 16), a zinc finger protein which forms a transcriptional complex with the active form of C/EBP-β (CCAAT/enhancer-binding protein), has been shown to induce brown adipocyte differentiation from primary mouse myoblasts [111] as well as human and mouse skin fibroblasts [112]. The resultant brown fat pad contained UCP1 positive multilocular and unilocular fat cells, had high glucose uptake on PET scan, and increased basal respiration (Table 5). These and other approaches are being explored as potential therapies for obesity treatment or prevention.

Transplantation of adipose-derived stem cells (ASCs): Adipose-derived stem cells (ASCs) are a population of multipotent cells isolated from adipose tissue by adherence to plastic. ASCs have the ability to undergo self-renewal and can differentiate into various cell lineages, including white or brown adipocytes, osteocytes, chondrocytes, myocytes, leukocytes, endothelial cells, neurons, epithelial cells, hepatocytes, and pancreatic cells [113] (Fig. 3). This multilineage capacity of ASCs offers potential to repair, maintain or enhance various tissues[113]. In rodent models, purer populations of preadipocytes can be isolated from ASCs derived from SVF using cell surface markers and flow cytometry, and these have been shown to form fat in mice [114,115]. The population of ASCs can also be expanded *in vitro* with similar degrees of differentiation, angiogenesis and immune response as the well characterized bone marrow stem cells [116–119].

The possibility of isolation of ASCs from aspirates obtained at liposuction in humans provides a minimally invasive procedure with low morbidity, which allows isolation of stem cells in sufficient quantity for autologous transplantation [120]. Transplantation of ASCs obtained from human lipoaspirates have been performed successfully for reconstructive surgery of breast and to close fistulas associated with Crohn's disease [121,122]. Ongoing clinical trials are examining the safety and efficacy of transplanting ASCs to improve metabolism of patients, such as those with lipodystrophies, type 1 diabetes, type 2 diabetes, ischemic myocardium, or myocardial infarction. Patients who have overcome leukemia during childhood are subsequently at increased risk of developing obesity, diabetes, and cardiovascular disease, hence the possible beneficial effects of ASCs transplantation in these subjects are also being examined (Table 6). Furthermore, thousands of non-expanded

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autologous transplantations of ASCs have been performed in horses and dogs to treat osteoarthritis with minimal systemic effects (www.vet-stem.com). The multilineage function of ASCs was demonstrated when ASCs obtained from brown fat of mice were transplanted into infarct border zone of the heart, and were shown to subsequently express markers for smooth muscle cells, endothelial cells and cardiomyocytes and improve ventricular function [123,124]. Furthermore, human ASCs treated with TZD, a PPARγ agonist, *in vitro* developed into brown adipocytes which expressed UCP1 and had increased oxygen consumption and energy expenditure [125].

Limitations and concerns about fat transplantation

Brown fat

As with any procedure, there are potential limitations and concerns about fat transplantation as a clinical procedure. Supraphysiological levels of brown adipocytes might cause detrimental effects. For example, overexpression of UCP1 in mice increased visceral fat and decreased subcutaneous fat, and only increased energy expenditure when mice had reached a certain threshold of body weight [126]. Furthermore, increased activity of brown fat following stimulation by noradrenaline resulted in increased blood flow and body temperature [127]. For brown fat, function also requires adequate innervation to allow full regulation of energy expenditure [92]. Furthermore, one clinical study found no significant correlation between whole body thermogenesis at rest and uptake of glucose into brown fat [51]. Future studies will need to examine the degree by which brown fat uses fatty acid oxidation versus glucose oxidation in humans, since fatty acids may supply up to 90% of the fuel to brown fat [127–129], and to determine whether fatty acid oxidation in brown fat correlates better with thermogenesis than does glucose uptake.

Adipose-derived stem cells (ASCs)

Although ASCs are multipotential, several factors need to be considered as ASCs are engineered to produce beneficial metabolic effects in humans (Fig. 4). First, optimal ASCs should be from young, healthy donors, have normal karyotype and high potential for proliferation and differentiation *in vivo*, whereas ASCs from donors of older age may lose their capacity to differentiate [130–132] and develop more abnormalities resulting in tumorigenesis [133–137] (Fig. 4, step I).

Secondly, to increase efficiency of brown or white adipogenesis, ASCs may be reprogrammed by forced expression of UCP1, PPARγ or, PRDM16; or by treatment with BMP7 or retinoic acid [138] (Fig. 4, step II). In the future, expression of specific miRNAs may also be utilized to promote adipocyte cell lineage, while simultaneously inhibiting unwanted lineages such as osteogenesis [139]. For animal studies, this type of forced gene expression has often utilized adenoviral vectors, however this can stimulate inflammatory responses [140]. For human use, safer, non-viral reprogramming will need to be achieved using other vectors or other delivery methods, such as microbubbles containing plasmid DNA that can be triggered to release their contents into specific tissues by ultrasound. This has been successfully demonstrated for muscle, vessels, and spines of animals [141], as well as delivery of siRNA into mesenchymal stem cells for transplantation [142].

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Third, delivery of ASCs into the recipient may be carried out by transplantation, by subcutaneous injection, by injection into the injured tissue, as well as by intravenous injection in which ASCs home to injured tissue [143,144] (Fig. 4, step III). For experimental studies, monitoring the migration of ASCs can be followed in real-time with bioluminescence microscopy [143] or by using GFP expressing cells [145]. Ongoing clinical trials are injecting ASCs intravenously and examining metabolic effects in patients with diabetes type 1 or 2 (Table 6), but the migration of intravenously injected ASCs in animal models of diabetes are needed to be determined for these methods.

Increased cell survival and lipid content of ASCs differentiated into fat after transplantation have been reported with the use of hydrogels [146], PLGA (poly(lactic-co-glycolic acid)) [147], and collagen scaffolds [148]. Local delivery of factors to enhance angiogenic, antifibrotic, anti-apoptotic and anti-inflammatory properties, such as VEGF [149,150], hepatic growth factor (HGF) [149,151], fibroblast growth factor (FGF) [152], transforming growth factor (TGF) β [149], platelet-derived growth factor (PDGF) [153], IL-8 [154], or matrix metalloproteinase (MMP) 2 [155], have been shown to increase survival of fat grafts. Whether these scaffolds and growth factors can help increase the survival of brown fat transplants derived from ASCs by increasing proliferation, differentiation, vascularization and innervation (Fig. 4, step IV) in order to produce beneficial metabolic effects, such as increased energy expenditure, decreased body weight, and increased insulin sensitivity (Fig. 4, step V) should be investigated over the long-term.

Future perspectives

The goal of fat transplantation has evolved dramatically from the early uses for esthetic and reconstructive surgery to understanding the biology of fat, and now, to being a potential tool to provide beneficial metabolic effects. The potential for transplantation of brown fat has come with a recognition that active brown fat may have beneficial metabolic effects in humans, such as reducing body weight and fat mass, and lowering glucose and insulin levels. However, better metabolic characterization of brown fat in humans in terms of its fat oxidation, potential adipokines, and mechanisms of brown fat activation in response to stimuli such as cold or drugs are needed. In addition, the identification of critical regulators of brown fat cell fate, such as BMP-7 and PRDM16, has raised the possibility that one could induce other progenitor cells to form brown fat and suggests a second strategy to increase brown fat mass. Likewise, subcutaneous white fat may have beneficial metabolic effects, and its cell-autonomous properties are often studied in relation to its well-known protective adipokines such as adiponectin and leptin. However, more studies are needed to discover and characterize its other properties, such as other adipokines, developmental genes, miRNAs, and its increased responses to insulin-sensitizing drugs, all of which raise the notion that transplantation or induction of specific types of white fat may also induce metabolic improvement. Novel uses of growth factors and regulators of differentiation should be explored in order to better purify, modulate, expand and/or maintain for brown fat, subcutaneous white fat and ASCs. Better understanding of the loss of function of brown fat and ASCs with aging as well as *in vitro* passaging and tumorigenesis will provide new targets for reprogramming of cells for transplantation and maintenance.

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Finally, ongoing and future clinical trials are examining the potential of ASCs in diseases such as lipodystrophy, diabetes, and tissue repair for myocardial infarction. There are completed clinical trials in which autologous bone-marrow stem cells were injected intracoronally into patients with acute myocardial infarction. Long-term beneficial effects such as improved left ventricular function and decreased mortality rate after five years were reported in the BALANCE nonrandomized trial [156], however there was no significant improvement in left ventricular function in the randomized-controlled three-year ASTAMI trial [157] and 18-month BOOST trial [158]. All of these trials reported that transplantation of bone-marrow stem cells was safe. Whether transplantation of adipose tissue and its component cells, with or without tissue engineering, may provide treatment for many disorders beyond classic metabolic diseases is not yet known. The overall value of these types of fat transplantation will ultimately be determined by their long-term benefits and safety as compared to present therapies.

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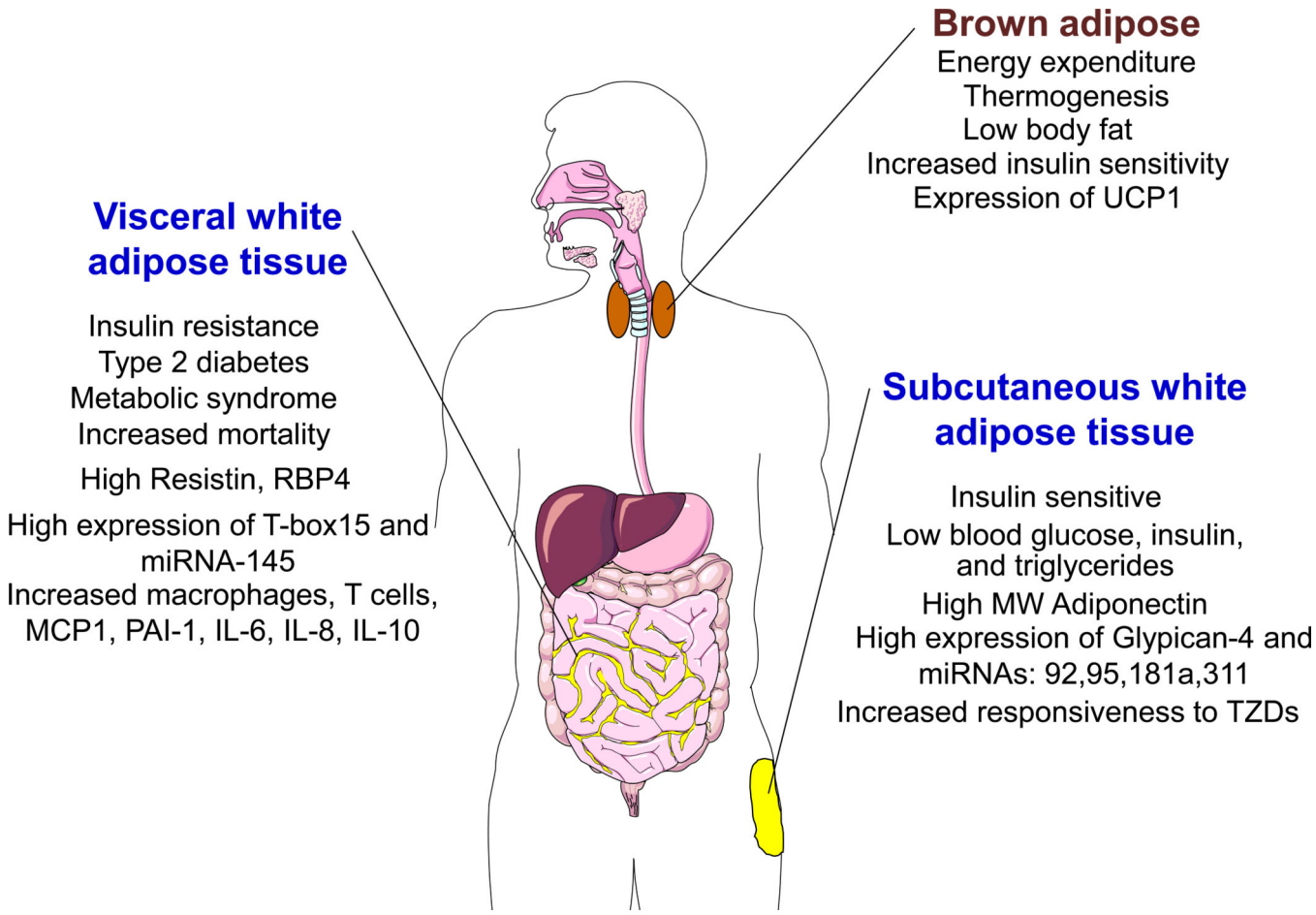

Figure 1. Adipose tissue in human

Visceral white adipose tissue is associated with increased risk of several metabolic conditions, diseases, and mortality, whereas subcutaneous and brown fat is associated with improved metabolism. Visceral fat secretes higher levels of the adipokines, resistin and retinol binding protein (RBP) 4, which are associated with insulin resistance, whereas subcutaneous fat secretes higher levels of high molecular (MW) adiponectin which is associated with improved metabolism. The developmental gene T-box 15 is more highly expressed in visceral fat of lean individuals, whereas glypican-4 is more highly expressed in the subcutaneous fat of lean individuals. Gene expression of the uncoupling protein 1 (UCP1) is specific to brown fat. miRNA-145 is more highly expressed in the visceral fat of individuals with type 2 diabetes, whereas several miRNAs in the subcutaneous fat are associated with smaller adipocyte size. Visceral fat has higher levels of inflammatory cells and cytokines. Subcutaneous fat is more responsive to the insulin-sensitizing drugs, such as thiazolidinediones (TZDs), than visceral fat.

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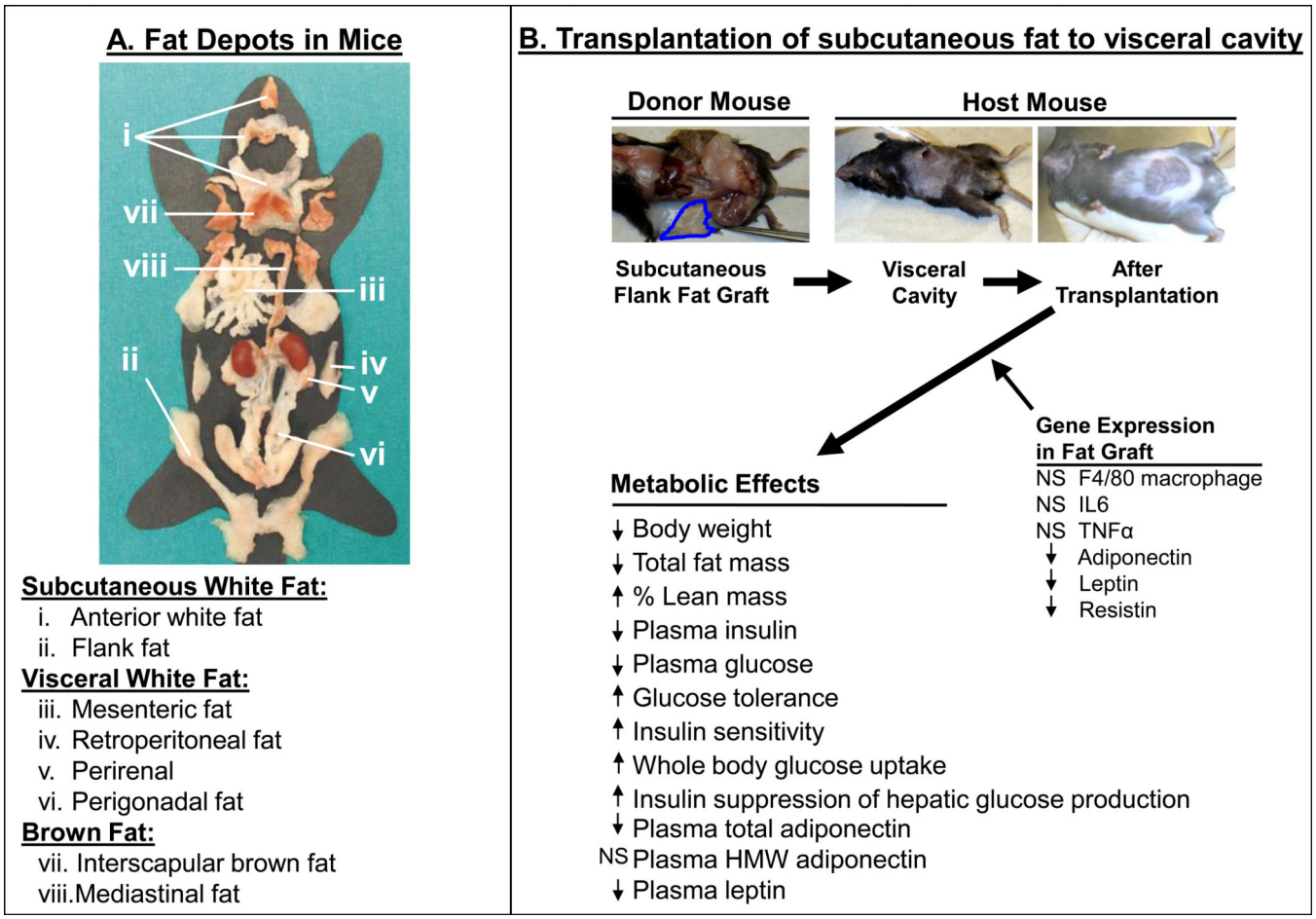

Figure 2. Fat depots and transplantation of subcutaneous fat in mice

A. The subcutaneous fat depots, visceral fat depots, and brown fat depots are shown in a mouse model, as reprinted from Murano *et al*[159] and Cinti S. [160] (used with permission). **B**. Transplantation of subcutaneous flank fat into the visceral cavity of mice induced several beneficial metabolic effects such as decreased body weight, decreased fat mass and improved insulin sensitivity. These beneficial effects were not mediated by inflammation, adiponectin, or leptin, but might be mediated by decreased levels of resistin.

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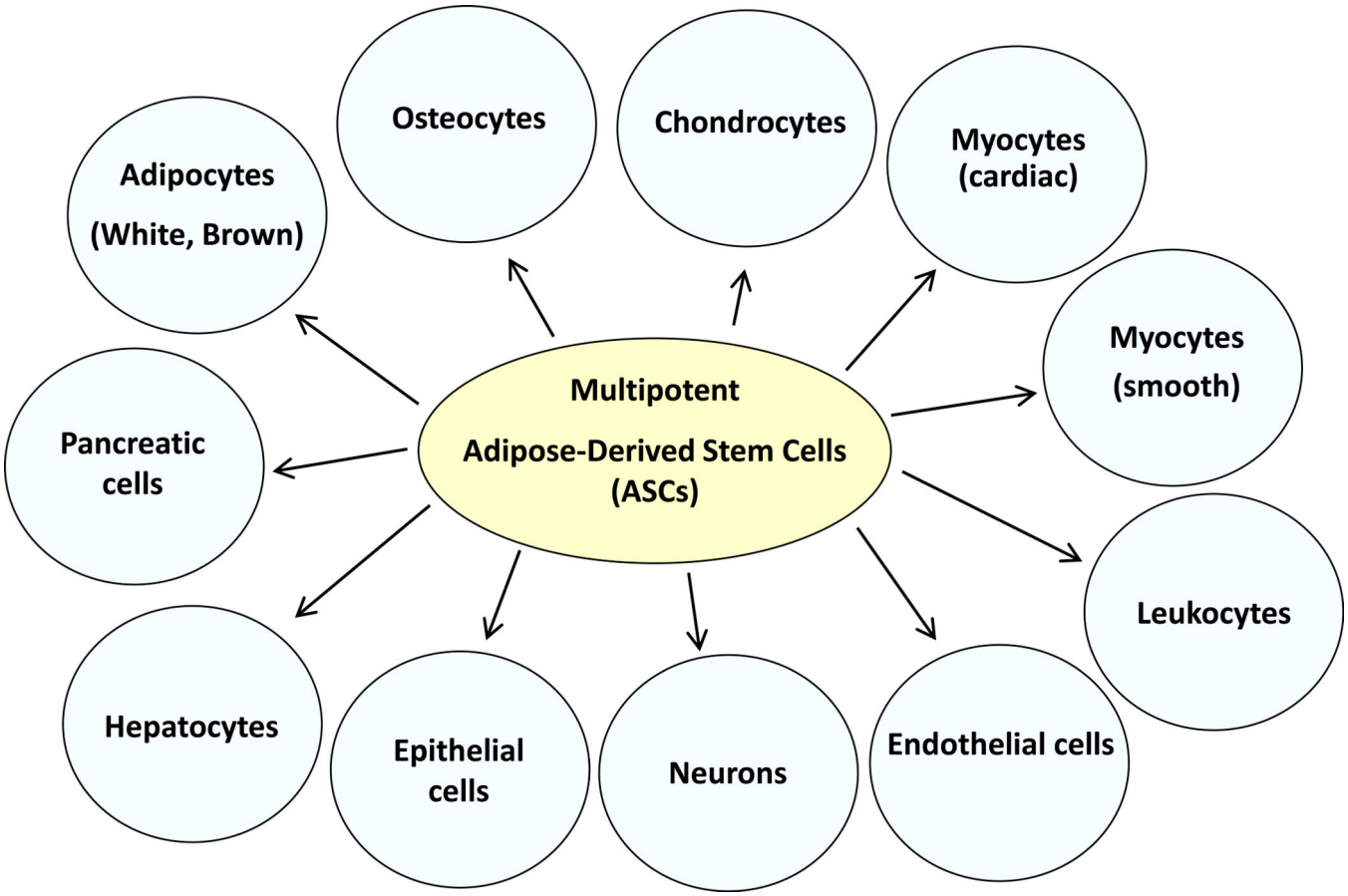

Figure 3. Differentiation of human adipose-derived stem cells (ASCs) into various phenotypes for clinical applications

The multipotent ASCs have high self-renewal capacity and can differentiate into several cell lineages, such as white and brown adipocytes, osteocytes, chondrocytes, myocytes, leukocytes and endothelial cells from the mesoderm layer; neurons and epithelial cells from the ectoderm layer; as well as hepatocytes, pancreatic cells and epithelial cells from the endoderm layer. This multipotent potential of ASCs may contribute to tissue repair, maintenance, and/or enhancement of various tissues.

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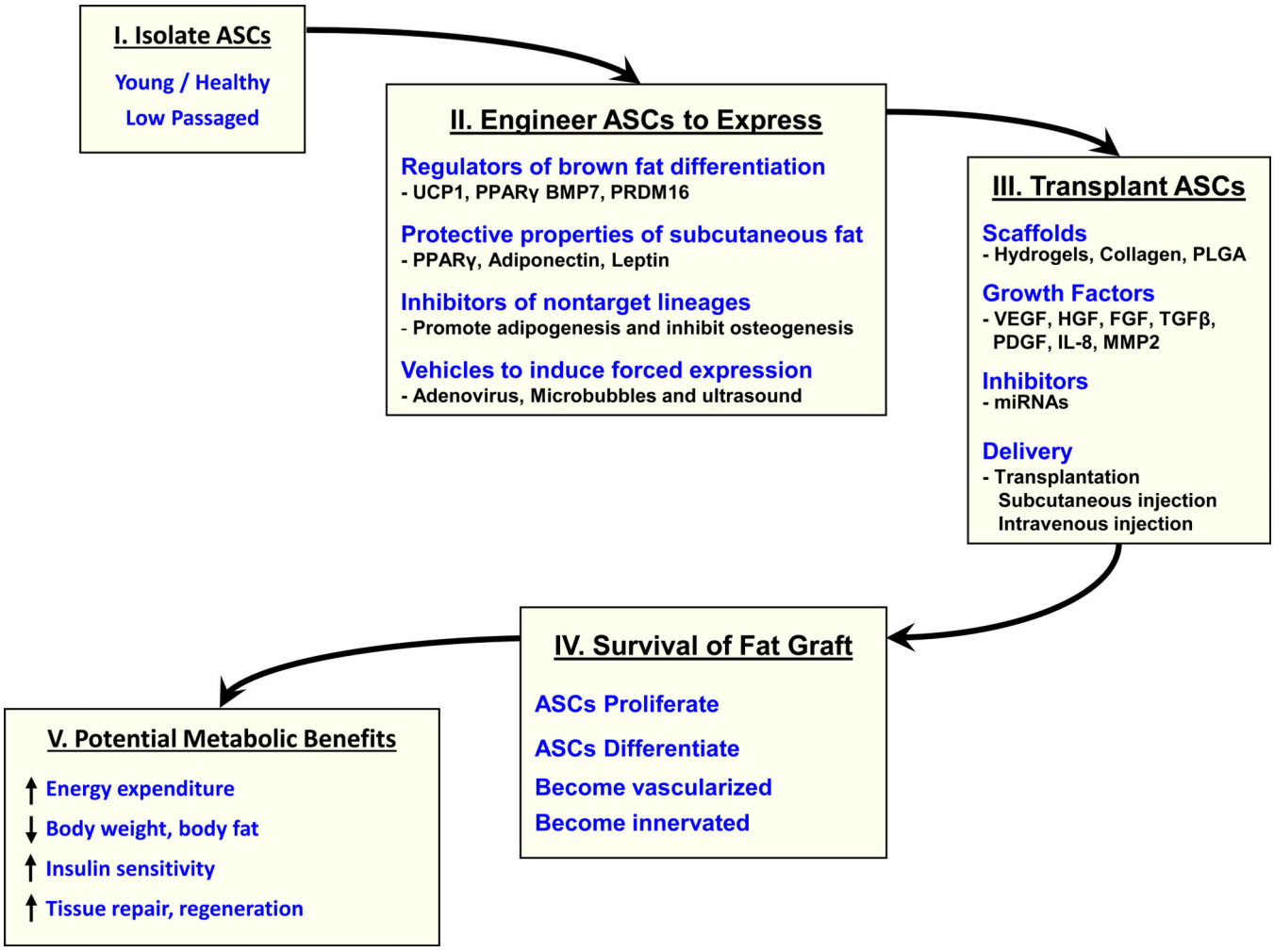

Figure 4. Potential Effects of Transplantation of Adipose-Derived Cells Expressing Properties of Subcutaneous White Adipocytes and Brown Adipocytes

Several steps are to be considered as ASCs are engineered to induce beneficial metabolic effects *in vivo* **I**. Optimal sources of isolated ASCs are young, healthy, low passaged cells with high potential for proliferation and differentiation without tumorigenesis. **II**. ASCs are engineered to express regulators of brown fat differentiation or beneficial properties of subcutaneous fat, with the help of inhibitors of nontarget lineages, by various methods such as those involving adenoviral vectors in animals or microbubbles containing plasmid DNA that are triggered to release into specific tissues by ultrasound in humans. **III**. Scaffolds, growth factors, and inhibitors can be used to promote the growth of engineered ASCs that are delivered *in vivo* by transplantation during surgery, subcutaneous injections or intravenous injections. **IV**. The ASCs proliferate, differentiate, and become vascularized and innervated to form functional fat grafts. **V**. The fat grafts derived from engineered ASCs may then induce potential beneficial metabolic benefits. Abbreviations: BMP7: bone morphogenetic protein; FGF: fibroblast growth factor; HGF: hepatic growth factor; IL: interleukin; MMP: matrix metalloproteinase; PDGF: platelet-derived growth factor; PLGA: (poly(lactic-co-glycolic acid)); PPARγ: peroxisome proliferator-activated receptor;

PRDM16: PR domain containing 16; TGF: transforming growth factor; UCP1: uncoupling protein 1;VEGF: vascular endothelial growth factor.

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Table 1

Transplantation of whole white fat pads to study morphological development of fat in rodents

Ref.	Fat Graft	Transplantation Site	Animals (Fat graft → Host)	Observations
64, 65	1) Immature epididymal 2) Connective tissue cells of fascia	Subcutaneous or Visceral	WT → WT rats	1) Formed fat pads that shrank or grew with starvation or overfeeding. 2) Did not develop into adipose tissue.
66	Immature epididymal	Subcutaneous	WT → WT rats	More preadipose cells differentiated near capillaries.
67	1) Epididymal 2) Flank	Under kidney capsule	ob/ob or WT → ob/ob or WT mice	Cell size of fat graft became similar to that of the host.
68	Epididymal	Under kidney capsule	Gold thioglucose obese or WT → obese or WT mice	Following calorie restriction, cell size of fat graft became similar to that of the host.
69	Flank	Under kidney capsule	ob/ob or WT → ob/ob or WT mice	Fatty acid composition of fat graft became similar to that of the host.
70	1) Epididymal 2) Epididymal	Subcutaneous	WT → WT mice	1) NS or decreased endogenous fat mass when repeated twice. 2) NS endogenous fat mass.
71, 72	1) Remove epididymal 2) Remove flank 3) Transplant epididymal 4) Transplant flank 5) Remove + Transplant epididymal 6) Remove + Transplant flank 7) Sham	1, 2, 7) None 3 to 6) Subcutaneous	WT → WT hamsters	1) Increased endogenous fat mass. 2) NS endogenous fat mass. 3) Increased endogenous fat mass. 4) Increased endogenous fat mass, but to less extent than group 3 5) Increased endogenous fat mass. 6) Decreased endogenous fat mass. NS body weight across all groups.

Numbers 1), 2), 3), etc in each row correspond to the same group of animals for each study.

Abbreviations: NS: No statistically significant difference in; WT: wild-type.

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Table 2

Transplantation of preadipocyte cell lines and stromovascular fractions (SVF) of into rodents

Ref.	Fat Graft	Transplantation Site	Recipient Animals	Observations
I. Transplantation of preadipocyte cell lines:				
73	1) 3T3-F442A 2) 3T3-C2	Subcutaneous	Athymic mice	1) Formed fat pads *in vivo*. Similar histology and size of adipocytes as endogenous adipocytes. 2) Did not form fat pad.
74	1) 3T3-F422A labeled with β-galactosidase 2) 3T3-L1	Subcutaneous	Athymic mice	1) Labeling with β-galactosidase proved that 3T3-F422A cell line developed into fat pads *in vivo* Better to implant cells near confluency, whereas fully differentiated cells did not form fat pads. 2) Did not form fat pad.
75	1) Ob17 (from ob/ob mouse) 2) Ob17 –OR11 (mutant clones)	Subcutaneous	Athymic mice	2) Labeling with Ob17-OR11 mutant cell line proved that it developed into fat pads *in vivo* However, fat pads formed in only 2 of 6 mice.
76	1) 3T3-F442A/GFP 2) 3T3-F442A/GFP/PPARγ-DN 3) 3T3-F442A/GFP + anti-VEGF R2 Ab	Subcutaneous	Athymic mice	1) Formed fat pads *in vivo* 2,3) Did not form fat pad. No angiogenesis. PPARγ and VEGF were essential to form fat pads *in vivo* but VEGF was not required for differentiation *in vitro*.
77	1) 3T3-F442A 2) 3T3-F442A + Matrigel	Subcutaneous	Athymic mice	1) Formed fat pads *in vivo* 2) Formed larger fat pads. Slower differentiation, but increased DNA and triglyceride content over time.
78	1) PGA scaffold 2) Undifferentiated 3T3-L1 + scaffold 3) Differentiated 3T3-L1 + scaffold	Subcutaneous	Athymic mice	1, 2) Did not form fat pad. 3) Formed fat pad.
79	1) 3T3-L1 2) 3T3-L1	1) Subcutaneous 2) Visceral	Athymic mice	1) Enhanced glucose tolerance, decreased insulin levels, thus improving metabolism. 2) Increased serum insulin, triglycerides, and TNFα, thus worsening metabolism.
II. Transplantation of SVF or mature adipocytes:				
80	1) SVF from omental and perirenal fat 2) Marrow-derived fibroblast	Spleen	Rats	1) Formed fat pad *in vivo* 2) Formed vascularized fibrotic nodules, but did not accumulate lipid.
81	1) SVF from epididymal fat 2) Skin fibroblasts	Subcutaneous	Rats	1) Formed fat pads *in vivo*. Size of adipocytes were similar to that of host's endogenous fat. Capillaries were near fat cells. Collagenous matrix from culture was needed to form fat pad. 2) Did not form fat pad.
82	SVF from epididymal fat	Visceral	Rats	Labeling with PKH26 proved that SVF can give rise to fat pads *in vivo*. Fat graft had similar cell size as that of endogenous fat, but still had multilocular lipid droplets, indicating incomplete differentiation.
83	1) SVF from flank fat of GFP mice, passaged with induction medium, 2) Same as group 1, but no induction	Subcutaneous	Athymic mice	1) Labeling with GFP proved that SVF gave can give rise to fat pads *in vivo* Cells accumulated lipid droplets, but did not fully differentiate. 2) Did not form fat pad.
84	1) SVF from flank fat of WT 2) SVF from flank of db/db mice	Subcutaneous	Athymic mice	1, 2) Formed fat pads *in vivo* 2) Lack of signaling after leptin receptor did not affect formation of fat pad.
85	1) Dedifferentiated mature adipocytes 2) Sham operation	Subcutaneous	Athymic mice	1) Formed fat pads *in vivo*.

Ref.	Fat Graft	Transplantation Site	Recipient Animals	Observations
86	Mature adipocytes from epididymal fat	Subcutaneous or Visceral	Rats	Cells became fat-depleted for 3 months. Labeling with PKH26 showed that survival rate of cells was 30% and 15% when transplanted to the subcutaneous and visceral sites respectively.

Numbers 1), 2), 3), etc in each row correspond to the same group of animals for each study.

Abbreviations: GFP: green fluorescent protein; anti-VEGFR2 Ab: anti-vascular endothelial growth factor receptor 2 antibody; PPARγ –DN: peroxisome proliferator-activated receptor γ-dominant negative mutant receptor; PGA: polyglycolic acid; WT: wild-type.

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Table 3

Transplantation of brown fat to study development of fat pads in rodents

Ref.	Fat Graft	Transplantation Site	Animals (Fat graft → Host)	Observations
87	1) Clusters of brown fat 2) Isolated brown preadipocytes 3) Isolated brown adipocytes	1) Intramuscular 2,3) Under the kidney	WT→ WT mice	1) Formed fat pad. 2, 3) Did not form fat pad.
88	Brown fat (1–3 mg)	Subcutaneous	WT→ WT mice	Did not form fat pad.
89	1, 2) Immature brown fat	1) Into eye 2) Denervation of iris, then implanted into eye	WT→ WT hamsters	1) Initial vascularization and proliferation of unilocular cells. Innervation started at day 10, and reached 17% of the endogenous levels by 6 weeks. As number of adrenergic fibers increased, differentiation increased. 2) Denervation delayed appearance of normal brown fat to 20 days.
90	1) Brown fat 2) White fat 3) Brown fat exposed to cold 4) Brown fat 5) White fat	1,2) Under the kidney 3–5) none	WT→ WT mice	1) No innervation after 2 weeks. Increased cell size and lipid content. 2) No innervation after 2 weeks. Decreased cell size and lipid content 3) NS cell size. Decreased lipid content. Increased vascularization.
91	1–4) Brown fat	1–4) Under the kidney	1)ob/ob or WT→ob/ob or WT 23μC for 5 wk 2)ob/ob or WT→WT 4μC for 5 wk 3)ob/ob or WT→ob/ob 33μC for 5 wk 4)ob/ob or WT→ob/ob or WT 4μC for 5 wk+23μC for 3 wk	1, 3) Ambient temperature partially transformed lipid droplet size and mitochondrial structure to that of host. Very few innervations to adipocytes. 2) Cold temperature completely transformed size of lipid and mitochondrial structure to that of WT host. Innervation to both blood vessels and adipocytes. 4) Cold then ambient temperature still maintained complete transformation to that of WT host. Innervation to both vessels and adipocytes.
92	Brown fat	Under the kidney	ob/ob or WT→ob/ob or WT	Fatty acid composition of graft changed to that of host.

Numbers 1), 2), 3), etc in each row correspond to the same group of animals for each study.

Abbreviations: NS: no statistically significant change in; wk: weeks; WT: wild-type.

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Table 4

Transplantation of white fat to study systemic metabolic effects in rodents

Ref.	Fat Graft	Transplantation Site	Animals (Fat graft → Host)	Observations
I. Synthesis of Fat:				
94,95	1) Ovarian 2) Flank 3) Sham	1,2) Subcutaneous 3) Sham	1,2) WT→A-ZIP/F-1 mice 3) A-ZIP/F-1	1+2) Normalized metabolism in lipodystrophic mice. Decreased food intake and hepatic steatosis. Increased whole-body and hepatic insulin sensitivity. Improved histology of β-cells.
96	1) Ovarian 2) Ovarian 3) Sham	1) Subcutaneous 2) Subcutaneous 3) Sham	1) WT → A-ZIP/F-1 mice 2) ob/ob → A-ZIP/F-1 3) WT or A-ZIP/F-1	1) Normal fat graft normalized metabolism in lipodystrophic mice. 2) NS in metabolic effects. Thus, leptin is mediating metabolism in lipodystrophic mice.
100	1–3)Perigonadal	Subcutaneous	1) DGAT1−/− or WT → WT mice 2) DGAT1−/− or WT → Agouti yellow 3) DGAT1−/− or WT → ob/ob	DGAT1−/− decreased body weight, fat mass, muscle triglycerides, and serum TNFα. DGAT1−/− increased insulin sensitivity, energy expenditure, and adiponectin mRNA. DGAT1−/− increased glucose tolerance in WT and Agouti but not severely obese ob/ob.
II. Leptin-deficient or leptin receptor defective obese mice:				
101	1) Epididymal 2) Sham	1) Subcutaneous 2) Sham	1) WT → ob/ob mice 2) WT or ob/ob	1) Normal fat graft restored metabolism in leptin-deficient obese mice. Decreased body weight, food intake, and serum insulin.
102	1) Epididymal 2) Sham	1) Subcutaneous 2) Sham	1) WT → ob/ob mice 2) WT or ob/ob	1) Normal fat graft restored immune and inflammatory responses.
103	Epididymal	Subcutaneous	WT or ZDF → WT or ZDF rats + Adenoviral delivery of leptin	Hyperleptinemia depleted fat from normal grafts but not from ZDF grafts. Hyperleptinemia activated STAT3 and CREB in normal grafts but not in ZDF grafts.
III. Subcutaneous versus visceral fat depots:				
106	1) Epididymal 2) Sham	1) Visceral cavity 2) Sham	1) WT → WT mice 2) WT	1) Decreased plasma glucose and insulin. Improved glucose tolerance. However, cell size of visceral fat graft was decreased as compared to endogenous fat. Thus, visceral fat graft lost its detrimental properties in this model.
108	1,2) Flank 3,4) Epididymal 5) Sham	1) Visceral cavity 2) Subcutaneous 3) Visceral cavity 4) Subcutaneous 5) Sham	1–5) GFP WT→ WT	1) Decreased body weight and fat. Improved whole-body and hepatic insulin sensitivity. 2) Similar improvements as group 1, but to a lesser extent. 3, 4) NS metabolic effects. Thus, cell-autonomous properties of subcutaneous fat improved metabolism.
109	1,2) Flank 3,4) Epididymal 5) Sham	1) Visceral cavity 2) Subcutaneous 3) Visceral cavity 4) Subcutaneous 5) Sham	1–5) WT → WT mice All fed high fat diet.	1) Decreased fat mass, improved glucose tolerance, but NS body weight. 2, 3, 4) NS metabolic effects. Thus, cell-autonomous properties of subcutaneous fat improved metabolism.

Numbers 1), 2), 3), etc in each row correspond to the same group of animals for each study.

Abbreviations: CREB: cAMP response element binding;DGAT1−/−: diacylglycerol acyltransferase 1–deficient; GFP: green fluorescent protein; NS: not statistically significant difference in; STAT3: signal transducer and activator of transcription;TNFα: tumor necrosis factor α; WT: wild-type; ZDF: Zucker Diabetic Fatty rats

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Table 5

Transplantation of engineered cells to form brown fat in rodents

Ref.	Fat Graft	Transplantation Site	Recipient Animal	Observations
110	C3H10T1/2 cells with or without BMP7 in medium	Subcutaneous	Athymic mice	- Formed brown fat pad with UCP1-positive multilocular and unilocular fat cells. - Energy expenditure increased. Body weight gain decreased.
112	MEFs transduced with retroviral PRDM16 and C/EBP-β or control	Subcutaneous	Athymic mice	- Formed brown fat pad with UCP1-positive multilocular and unilocular fat cells. - Glucose uptake into fat pad. Increased basal respiration.

Abbreviations: BMP7: bone morphogenetic protein 7; C/EBP: CCAAT-enhancer-binding proteins; MEFs: mesenschymal embryonic fibroblasts; PRDM16: PR domain containing 16; UCP1: uncoupling protein 1

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Table 6

Ongoing Human Clinical Trials Assessing Safety and Efficacy of Using Adipose-Derived Stem Cells (ASCs)

Disease/Condition	Delivery of ASCs	Endpoints	Design Follow-Up Time	Patient no.	Site/Company
Reconstructive Surgery:					
Lumpectomy (RESTORE-2)	Transplantation of autologous ASCs to reconstruct breast deformities	Functional and cosmetic results of reconstructive breast surgery	Phase IV 12 months	70	Belgium, Italy, Spain, UK. Cytori Therapeutics Inc.
Renal failure (Vesico-Ureteral Reflux)	Transplantation of autologous adipocytes to treat defective volume	Radiography of urethra and bladder. Presence of kidney or ureter infection	Phase III Non-randomized 10 years	14	Strasbourg, France. University Hospital.
Perianal Fistulas without Crohn's Disease (FATT1)	Fibrin adhesives with or without ASCs during surgery	Closure of fistulas (abnormal connection between structures)	Phase III. Randomized multicenter, single blinded. 26 weeks	207	Spain, Germany, UK. Cellerix Ltd.
Perianal Fistula	Fibrin glue with or without autologous ASCs from lipoaspirates	Closure of fistulas	Phase II Randomized, multicenter. 1 year	50	Spain. Cellerix Ltd.
Diabetic lower extremity & venous stasis wounds	Subcutaneous injection of lipoaspirate into wounds	Wound healing	Phase I/II. Randomized, single blinded. 12 months	250	USA. Washington D.C. Veterans Affairs Medical Center
Metabolic:					
Lipodystrophy (AADSCTPL trial)	Transplantation of autologous lipoaspirate enriched with ASCs	Clinical evaluation of transplanted area. Tissue viability, neovascularization, degree of resorption of fat graft	Phase I 1 year	10	Brazil. Hospital Irmandade Santa Casa de Misericordia de Porto Alegre
Type 1 Diabetes Mellitus	Intravenous autologous ASCs	Dose of insulin-dependent and anti-hyperglycemic medicine, glycosylated hemoglobin (HbA1c), C-peptide	Phase I/II 12 months	30	Philippines, Hong Kong. Adistem Ltd.
Type 2 Diabetes Mellitus	Intravenous autologous ASCs	Lower blood glucose (fasting, random, post-prandial)	Phase I/II 48 weeks	34	Philippines, Adistem Ltd.
Ischemic Myocardium (PRECISE trial)	Injection of autologous ASCs or placebo	Cardiac function, major adverse cardiac and cerebral events	Phase I. Randomized, double blinded, placebo. 36 months	36	Denmark, Netherlands, Spain. Cytori Therapeutics Inc.
Myocardial Infarction (APOLLO-01)	Injection of autologous ASCs or placebo	Cardiac function, major adverse cardiac and cerebral events	Phase I. Randomized. 6 months	48	Netherlands, Spain. Cytori Therapeutics Inc.
Leukemia survivors	Transplantation of ASCs after total body irradiation versus no treatment	Obesity, fat depots, blood pressure, cholesterol, diabetes	Observational prospective. 12 months	60	USA, Canada. Memorial Sloan-Kettering Cancer Center

Source: www.clinicaltrials.gov