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Utility of transplantation in studying adipocyte biogenesis and function

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Abstract

Adipose tissue plays important roles in the regulation of energy homeostasis and metabolism. Two features distinguish adipose tissue from other organs - the ability to greatly expand its mass, via increases in cell size and/or number, and the wide anatomical distribution. While adipose tissue function is greatly affected by adipocyte size and anatomic location, regulations of adipocyte size, number, and body fat distribution are poorly understood. Transplantation of either mature adipose tissue or adipocyte progenitor cells has been used in studying adipocyte function and biogenesis. In this review, we will attempt to summarize methodological considerations for transplantation, including selections of donor material, transplantation site and the length of transplantation study, as well as effects of these factors and vascularization and innervation on the function of transplants. Specific studies are also reviewed to illustrate the utility of adipose tissue transplants in studying adipose tissue function and biogenesis. The focus is on studies in three areas: 1) use of transplants in demonstrating adipose tissue function, such as effects of adipose tissue transplants on metabolism and energy homeostasis of the recipient animals and depot-specific differences in adipose tissue function; 2) use of transplantation to dissect direct or cell-autonomous from indirect or non cell-autonomous effects of leptin signaling and sex on adipocyte size; 3) use of transplantation in the identification of adipocyte progenitor cells and lineage analysis. Finally, future applications of transplantation in studying depot-specific adipocyte biogenesis, and genetic and hormonal effects of sex and age on adipocyte biogenesis and function are discussed.

Keywords

Transplantation; body fat distribution; preadipocyte; gender; leptin; cell-autonomous function

Introduction

Adipose tissue transplantation is widely used for soft tissue reconstructive surgeries in part because adipose tissue transplants re-establish readily at new locations. In the late 70's and early 80's of the last century, Ashwell and her colleagues used this unique feature of adipose

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tissue to investigate the respective roles of “nature vs. nurture” (cell autonomous v. physiological environment) in determining adipocyte size in genetically or pharmacologically-induced obese mice. In a series of adipose tissue transplantation experiments between inbred mouse strains they demonstrated a strong effect of physiological environment (Ashwell and Meade, 1981; Ashwell et al., 1977; Ashwell et al., 1986). Around the same time, Green and Kehinde used transplantation in immuno-deficient nude mice to test the ability of adipocyte progenitor cells to differentiate into mature adipocytes *in vivo* (Green and Kehinde, 1979). Growing interest in the role of adipose tissue as an endocrine organ and the contribution of anatomic location of fat stores to medical morbidities has renewed interest in the use of transplantation as an experimental technique. The goal of this review is to introduce transplantation as a uniquely useful approach to study adipose tissue function and biogenesis *in vivo*. The review covers some major methodological considerations for transplantation experiments, and summarizes the results of the transplantation studies that are relevant to the regulation of metabolism and energy homeostasis by adipose tissues.

Methodology

Factors that may affect the function of adipose tissue transplants include the type of donor tissues (or cells), site and length of transplantation, and vascularization and innervation of transplants. Depending on the particular study design, some of these factors may have more impact than others on the function of transplants. In the following section, methodological aspects of various transplantation studies in the literature are reviewed to provide an overview of adipose tissue transplantation technique.

1. Donor materials

Adipose tissue fragments have been widely used as donor materials in transplantation studies. Adipose tissue fragments ranging from 100-150 mg per piece have yielded functional grafts (Ashwell and Meade, 1981; Gavrilova et al., 2000; Lacy and Bartness, 2005; Rooks et al., 2004). Purified adipocyte progenitor cells and total stromal vascular cells (SVCs) isolated from adipose tissue are also able to develop into mature and functional adipose tissue when transplanted to nude or isogenic mice (Guo et al., 2009; Rodeheffer et al., 2008; Tang et al., 2008; Van and Roncari, 1982). Additionally, lipid-depleted, cultured adipocytes or immortalized preadipocytes (3T3-F442A) can also develop into mature adipose tissue in nude and isogenic recipient mice (Green and Kehinde, 1979; Mandrup et al., 1997; Tavassoli, 1982; Yagi et al., 2004). Immortalized multipotent mesenchymal stem cells (C3H10T1/2) are able to differentiate to white or brown adipocytes *in vivo* when the cells are pre-treated with bone morphogenetic protein 4 (BMP4) or BMP7 prior to transplantation (Tang et al., 2004; Tseng et al., 2008). The main advantage of using adipose tissue fragments as donor material is that adipose tissue is readily available without additional isolation and/or culturing, and that the transplants are functional, to certain degrees, within days or weeks after transplantation (Colombo et al., 2002; Gavrilova et al., 2000; Klebanov et al., 2005; Rodeheffer et al., 2008). Thus, adipose tissue fragments have been preferred donor materials for studying specific functions associated with mature adipose tissue (Colombo et al., 2002; Gavrilova et al., 2000; Klebanov et al., 2005; Lacy and Bartness, 2004, 2005; Rooks et al., 2004). However, genetic manipulation in mature adipose tissue is not feasible, and adipose tissue transplants are in general not suitable for studying adipocyte biogenesis. In contrast, transplantation of adipocyte progenitor cells, which can be genetically or biochemically manipulated *in vitro* prior to transplantation, is well suited for studying adipocyte biogenesis as well as function although it may take weeks to months for transplanted progenitor cells to develop into mature adipose tissue (Guo et al., 2009; Mandrup et al., 1997; Rodeheffer et al., 2008; Tang et al., 2004; Tang et al., 2008; Tseng et al., 2008).

The amount of donor tissues to be used may vary depending on the functional parameters that one is interested in. About one gram of normal adipose tissue, an amount similar to that found in normal lean mice, is sufficient to fully reverse the diabetic phenotype of A-Zip lipodystrophic mice, but has only modest effects on plasma FFA and TG levels after three months (Gavrilova et al., 2000). Similarly, the amounts of adipose tissue transplant required to normalize insulin and glucose metabolism are lower than those required to prevent obesity in *Lep^{ob/ob}* mice (Klebanov et al., 2005). On the other hand, transplantation of adipose tissue in the amount of 10% of total body fat is sufficient to cause a significant reduction in the endogenous adipose tissue mass in recipient mice (Rooks et al., 2004).

Depot origin, sex, and age affect adipocyte tissue function (Anderson et al., 2001; Bjorntorp, 1974; Craig et al., 1987; Engfeldt and Arner, 1988; Jamdar and Osborne, 1981; Kirkland et al., 1990; Lemonnier, 1972; Li et al., 1997; Lonnqvist et al., 1990; Pedersen et al., 1994; Porter et al., 2004; Shi et al., 2007; Sjostrom et al., 1972). Adipose tissue transplants retain some of the specific features of donor tissues or cells, such as effects on glucose and insulin metabolism, handling fatty acids, adipokine secretion, susceptibility to atherosclerosis, and capacities for replication and differentiation (Caserta et al., 2001; Ohman et al., 2008; Perrini et al., 2008; Tchkonja et al., 2004; Tran et al., 2008). In fact, the retention of donor-specific features by the transplants is particularly useful for studying depot-related or sex-related differences in adipose tissue function. Thus, it is important that these factors are taken into consideration in selecting donor material for transplantation.

2. Transplantation sites

Isogenic animals and immuno-deficient nude mice are often used as recipients for adipose transplants (Ashwell and Meade, 1981; Gavrilova et al., 2000; Green and Kehinde, 1979; Guo et al., 2009; Mandrup et al., 1997; Rooks et al., 2004). Adipose tissue transplants can survive and function at various anatomic locations, including underneath the kidney capsule (Ashwell and Meade, 1981; Ashwell et al., 1977) and various subcutaneous sites (Gavrilova et al., 2000; Guo et al., 2009; Lacy and Bartness, 2004; Mandrup et al., 1997; Rooks et al., 2004). The intraperitoneal cavity, epididymal fat pads, and superficial intramuscular sites can also support adipose transplants (Konrad et al., 2007; Tran et al., 2008; Van and Roncari, 1982). Transplants can be easily tracked at locations, such as the kidney capsule and sternal subcutaneous site, where there is no endogenous adipose tissue (Ashwell and Meade, 1981; Ashwell et al., 1977; Guo et al., 2009; Mandrup et al., 1997). Molecular markers, such as GFP and lacZ, make it possible to identify small grafts (10-50 mg) derived from transplanted progenitor cells that are embedded in the endogenous adipose tissue (Guo et al., 2009; Rodeheffer et al., 2008; Tang et al., 2008). Transplantation site may affect the function and morphology of the transplants (Lacy and Bartness, 2005; Meade and Ashwell, 1979; Tran et al., 2008). Subcutaneous adipose tissue grafts in the visceral cavity are more effective than those at a subcutaneous site in reducing blood glucose and improving insulin sensitivity of the recipient mice (Tran et al., 2008). In Siberian hamsters, lipectomy induces compensatory increase in the mass of the nonexcised fat tissue, and transplantation of epididymal fat subcutaneously exaggerates such compensatory growth. Interestingly, the effect of epididymal fat transplants on the growth of nonexcised fat tissue is observed only when the grafts are in direct contact with the endogenous subcutaneous adipose tissues (Lacy and Bartness, 2005). Such transplantation site-related differences may be due to differences in the vascularization and innervation of the transplants and/or interactions between the transplants and the surrounding tissues of the recipients.

3. Length of transplantation study

Adipose tissue transplants survive for long time *in vivo*. The length of transplantation studies ranges from a few weeks to a year depending on the function of transplants to be tested.

Transplantation of normal adipose tissue fully corrects the abnormality of glucose and insulin homeostasis in A-Zip lipodystrophic mice in ~3-4 weeks (Colombo et al., 2002; Gavrilova et al., 2000). Adipocyte differentiation of transplanted adipocyte progenitor cells, Lin(-):CD29(+):CD34(+):Sca-1(+):CD24(+), and effects of the ensuing transplants on blood glucose level in A-Zip lipodystrophic mice can be detected as early as 2 weeks after transplantation, and increase through the entire 12-week study period (Rodeheffer et al., 2008). In a cross-transplantation study, adipocyte size of obese grafts from obese mice or hamsters is assimilated to that of typical lean adipose tissue in one month when transplanted in lean recipients, and vice versa (Ashwell et al., 1977; Meade et al., 1979). Similarly, assimilation in adipocyte size between epididymal fat grafts and the endogenous subcutaneous fat peaks at two weeks after transplantation, but significant decreases in fat mass and adipocyte size of the endogenous epididymal and retroperitoneal fat pads, in response to the addition of epididymal fat grafts, are detected only after five weeks (Rooks et al., 2004). In humans, the adaptation of triglyceride fatty acid composition in gluteal fat transplants to that of the endogenous facial fat is complete by the 4th month and maintained up to 12 months after transplantation (Sadick and Hudgins, 2001). We have recently shown that transplanted adipocyte progenitor cells are fully differentiated and reach the size of the endogenous adipocytes after three months of transplantation. These adipocytes can survive for at least 6 months in nude mice without significant morphological changes after 3 months (Guo et al., 2009). Similarly, mature adipose tissue grafts can survive and function for up to 10 months in mice once established (Klebanov et al., 2005). However, adipose tissues derived from transplanted immortalized 3T3-F442A cells, while morphologically similar to the native adipose tissues of nude mice 8 weeks after transplantation, tended to be resorbed after three months (Mandrup et al., 1997). The cause for the quick resorption of 3T3-F442A-derived adipose tissue *in vivo* is unclear. It is possible that 3T3-F442A adipocytes are more susceptible to apoptosis than primary adipocytes, or lack of proliferating progenitor cells may prevent the continuous renewal of the tissue.

4. Vascularization and innervation of transplants

Adipose tissue is highly vascularized and innervated, qualities which are critically important to many of its functions. Adipose tissue grafts or adipose tissues derived from transplanted progenitor cells are extensively vascularized (Gavrilova et al., 2000; Guo et al., 2009; Rodeheffer et al., 2008). It appears that transplantation-stimulated angiogenesis is a function of mature adipocytes. Neovascularization and endothelial cell chemotaxis on the chicken chorioallantoic membrane are stimulated by the conditioned media of 3T3 adipocytes, but not preadipocytes (Castellot et al., 1982). Transplantation of preadipocytes in a mouse dorsal skin-fold chamber strongly induces angiogenesis, which is accompanied by the differentiation of preadipocytes. In this case, angiogenesis and adipocyte differentiation are interdependent; inhibition of one process hinders the other (Fukumura et al., 2003). Interestingly, when GFP-transgenic adipocyte progenitor cells are transplanted, the mature adipocytes of the transplants express GFP as expected, but the vasculature of the transplants are GFP-negative, suggesting that cells of transplant's vasculature system are derived from the recipient's (Rodeheffer et al., 2008). It would be interesting to determine if the stromal vascular cells of adipose tissue also contain progenitor cells for the vasculature system by examining GFP expression in the vasculature of transplants derived from unpurified stromal vascular cells of adipose tissues of GFP-transgenic mice. Catecholaminergic innervation was observed in both lean and obese brown adipose tissue transplants underneath the kidney capsule of recipient mice after three months (Ashwell et al., 1986). When undifferentiated (white fat-like) hamster BAT was transplanted into the anterior eye chamber of adult hamsters, the transplants were revascularized and reinnervated by the vessels and the nerves of the host iris after 20 days. Even though the nerve fiber density in the grafts was low, the establishment of specific feature of BAT, such as multilocular fat deposits and abundant, well-developed mitochondria, was synchronous with the establishment of the sympathetic innervation, not with the

revascularization process (Nechad and Olson, 1983). Innervation of transplants may also affect adipocyte and preadipocyte proliferation (Cousin et al., 1993; Foster and Bartness, 2006). However, quantitative and functional assessment of innervation in adipose tissue grafts is lacking in most of transplantation studies, which may affect the interpretation of results.

It should be cautioned though that, although adipocyte size of transplants appears to be conform to that of the endogenous adipose tissue of recipient mice fairly quickly (Ashwell et al., 1977; Guo et al., 2009; Meade et al., 1979; Rooks et al., 2004), other functions of transplants, such as the capacity to handle fatty acid flux or producing leptin (Gavrilova et al., 2000; Klebanov et al., 2005), may not be fully restored due to incomplete innervation and/or vascularization of transplants. Additionally, lack of adequate vascularization of transplants may account for the initial decreases in the volume of adipose tissue transplants (Yamaguchi et al., 2005), and may contribute to necrosis and decreased survival rates of transplants, which normally range from 50%-90% (Ashwell et al., 1977; Gavrilova et al., 2000; Guo et al., 2009; Rodeheffer et al., 2008). Morphological and functional analyses, including determination of adipocyte size, gene expression, and/or the degree of innervation and vascularization, should be performed to ensure that the viability and integrity of transplants is considered in interpreting the results. (Ashwell et al., 1977; Ashwell et al., 1986; Gavrilova et al., 2000; Guo et al., 2009; Nechad and Olson, 1983).

A schematic illustration of the transplantation technique for studying adipocyte function and biogenesis is shown in Figure 1.

Utility of transplantation in studying adipocyte function and biogenesis

Transplantation has been used to address various questions concerning the regulation of adipocyte function and biogenesis. Most of transplantation studies address questions falling into one of the following three areas: demonstrating adipocyte function -metabolic effects on the recipient mice, distinguishing cell-autonomous from non cell-autonomous function of adipose tissue, and lineage analysis of adipocyte biogenesis. The following section will review and discuss some of the relevant studies in these areas to illustrate the utility of transplantation technique in studying adipocytes.

1. Use of transplantation in demonstrating adipocyte function

Adipose tissue is the main energy storage site in mammals. The ability to store excess energy and to release the stored energy when it is needed is a function of adipose tissue critical in maintaining energy homeostasis. Adipose tissue also produces and secretes leptin, adiponectin, resistin, and other adipokines, which are essential in regulating energy balance and glucose and lipid metabolism (Berg et al., 2001; Leibel, 2002; Stepan et al., 2001; Weisberg et al., 2003; Zhang et al., 1994). The production and release of adipokines and metabolites by adipose tissue are affected by the metabolic status of the tissue, and are believed to be important mechanisms by which adipose tissue communicates with other tissues and organs (Considine et al., 1996; Rosenbaum et al., 1997; Zhang et al., 2002). Transplantation is a powerful approach to demonstrate adipose tissue function or functional differences as shown by several recent studies.

Effects of adipose tissue transplants on energy homeostasis and metabolism of recipients—Impaired adipose tissue function appears to be at the center of metabolic abnormalities associated lipodystrophy and obesity. Like obesity, lipodystrophy in both humans and rodents is associated with hyperphagia, hepatic steatosis, and insulin resistance, which eventually lead to diabetes (Joffe et al., 2001; Reitman and Gavrilova, 2000; Shimomura et al., 1998). Transplantation of the wild-type fat to A-ZIP mice, which have a severe form of lipodystrophic diabetes (Reitman and Gavrilova, 2000), decreases plasma glucose and insulin

levels, and fully corrects the diabetic phenotype in A-ZIP mice after 4 weeks. Other aspects of the A-ZIP phenotype, including hyperphagia, hepatic steatosis, and somatomegaly, are either partially or completely reversed (Gavrilova et al., 2000). The benefits of adipose tissue transplantation in A-ZIP mice are dose-dependent and the full correction of the diabetic phenotype requires the full amount of adipose tissue found in a normal lean mouse (Gavrilova et al., 2000), suggesting that the function of adipose transplants are responsible for the phenotypic correction of A-Zip mice. It was further demonstrated that the secretion of leptin by the transplants is critically important for the correction of the metabolic abnormalities in A-Zip mice. While leptin administration alone is not sufficient to fully correct the diabetic phenotype of A-Zip mice, adipose tissue transplants from leptin-deficient mice (*Lep^{ob/ob}*) failed to correct the diabetic phenotype of A-ZIP mice (Colombo et al., 2002). Transplantation of the wild type adipose tissue is also an effective long-term treatment for leptin deficiency in *Lep^{ob/ob}* mice. Again, the effects of transplants are dose-dependent; transplantation of epididymal fat tissues from 4-8 normal mice to young *Lep^{ob/ob}* mice prevents weight gain, and normalizes insulin sensitivity, and other metabolic abnormalities for up to 10 months (Klebanov et al., 2005). It is not only the mutant mice, such as leptin-deficient and lipodystrophic mice, whose metabolism is affected by adipose tissue transplants; normal mice are also sensitive to additional adipose tissue transplants. Transplantation of epididymal adipose tissue to a subcutaneous site, even as little as 10% of the total fat mass of the recipients, causes a significant reduction in tissue mass and adipocyte size of the epididymal and retroperitoneal fat pads of the recipient C57BL/6J mice. Thus, the recipient mice appear to be able to sense the additional fat mass of the transplants and respond to the change by reducing the endogenous fat tissue mass (Rooks et al., 2004). However, it is important to note that not all rodent species respond to fat transplantation with reduction in the mass of the endogenous fat pads. Transplantation of epididymal fat subcutaneously stimulates the growth of endogenous inguinal and retroperitoneal fat pads in Siberian hamsters (Lacy and Bartness, 2005). Hamsters are hibernating animals and may have the ability to adjust body fat content in a manner that is not available to non-hibernating species such as rats and humans. The mechanism by which fat mass of the transplants is sensed in either mice or hamsters is unclear, but may involve both circulating and/or neural factors. The fact that it takes five weeks for the compensatory reduction in the endogenous fat tissue mass to be detectable suggest that function of transplants may be very limited due to the lack of adequate vascularization and innervation in the initial period following transplantation.

Depot-related differences in adipose tissue function—Abdominal obesity is an independent risk factor of metabolic syndrome and is associated with significantly higher risks for diabetes and cardiovascular diseases, compared to lower body obesity (Bergman et al., 2007; Klein et al., 2007). A possible mechanism for the association of abdominal obesity with metabolic abnormalities is that anatomically distinct adipose tissues are functionally different (Bowers et al., 2004; Engfeldt and Arner, 1988; Eriksson et al., 2000; Montague et al., 1998; Pedersen et al., 1994; Perez-Perez et al., 2009; Perrini et al., 2008; Sztalryd and Kraemer, 1994; Van Harmelen et al., 1997; Wajchenberg et al., 2002). Recently, Tran et al investigated effects of wild type visceral (epididymal) and subcutaneous adipose tissue transplants on the metabolism of recipient C57BL/6J mice. In mice receiving ~1 gram of subcutaneous adipose tissue slices into the peritoneal cavity, weight gain was ~40% less compared to sham-operated mice. Mice with subcutaneous adipose tissue transplants also had significantly reduced plasma glucose and insulin levels, and increased systemic and hepatic insulin sensitivity. In contrast, visceral adipose tissue transplants had no significant effects on body weight or plasma glucose or insulin levels in the recipient mice (Tran et al., 2008). Similar metabolic benefits of subcutaneous adipose tissue were also observed by Hocking et al in a separate study (Hocking et al., 2008). These results demonstrated the physiological relevance of the functional differences between subcutaneous and visceral adipose tissues in mice. However, the

mechanistic link between abdominal obesity and metabolic disorders remains unclear. Another interesting point of the study is that the addition of adipose tissue transplants improves, rather than impairs, glucose and insulin metabolism in the recipient mice as originally expected (Tran et al., 2008), suggesting that dysfunctional adipose tissue, rather than the excess adipose tissue mass *per se*, may be the culprit for metabolic disorders associated with obesity. Visceral or subcutaneous adipose tissues also confers differential susceptibilities to atherosclerosis induced by inflammation in atherosclerosis-prone apolipoprotein E-deficient ApoE(−/−) mice. While both types of adipose tissue grafts are associated with similarly increased macrophage infiltration and systemic inflammation, ApoE(−/−) mice grafted with visceral adipose tissue developed more atherosclerosis than sham-operated animals, but subcutaneous fat transplants had no effect on atherosclerosis (Ohman et al., 2008). Furthermore, pioglitazone treatment, which decreased inflammation of visceral fat grafts, reduced atherosclerosis associated with visceral fat grafts, but had no protective effect on atherosclerosis in the absence of visceral fat grafts (Ohman et al., 2008). Thus, it appears that, compared to visceral (epididymal) fat, mouse subcutaneous adipose tissue is metabolically beneficial or less detrimental. However, one should be cautious in translating the mouse results to the physiology of abdominal obesity in humans. It is noted that the “mouse visceral fat” used in these studies, i.e., epididymal fat, is distinct from human visceral fat, namely omental and mesenteric fat, in anatomic location, circulatory connection, and likely many other important aspects of adipose tissue biology (see (Harris and Leibel, 2008) for review). Thus, studying the respective human subcutaneous and visceral fat transplants in nude mice may be proven more pertinent to human physiology. Additionally, in Siberian hamsters, transplantation of epididymal adipose tissue, but not inguinal subcutaneous adipose tissue, enhances lipectomy-induced growth of the non-excised epididymal and retroperitoneal adipose tissues (Lacy and Bartness, 2005), again suggesting that epididymal and inguinal subcutaneous adipose tissues are functionally different. However, functional differences between anatomically distinct adipose tissues are relative. In A-Zip lipodystrophic mice, transplantation of epididymal or inguinal fat from the wild type mice was equally effective in reversing the diabetic phenotype (Gavrilova et al., 2000).

2. Use of transplantation in dissecting nature vs. nurture in influencing adipocyte function

Factors such as anatomic location and sex affect adipocyte functions. However, whether there are intrinsic differences in adipocyte function in different fat depots and between sexes that are genetically or epigenetically programmed remains unclear. Adipocyte function is also regulated by numerous hormones, including insulin, growth hormone, catecholamine, leptin, cytokines, gonadal steroids, and corticosteroids. The mechanisms of action for many hormones in influencing adipocyte function are not fully understood. Uses of transplantation in studying effects of direct leptin signaling and sex on adipocyte size are two interesting examples.

Lack of cell autonomous effects of leptin signaling on adipocyte function in vivo

—Leptin has profound effects on adipose tissue metabolism. While the central nervous system (CNS) is believed to be the primary site of action (Ahima et al., 1996; Campfield et al., 1995; Cohen et al., 2001; de Luca et al., 2005; Schwartz et al., 2000), the physiological role of leptin signaling in adipocytes is not clear. Leptin induces lipolysis and fatty acid oxidation in adipose tissue explants or cultured adipocytes *in vitro* (Fruhbeck et al., 1997; Minokoshi et al., 2002; Siegrist-Kaiser et al., 1997; Wang et al., 1999; Zhou et al., 1997). However, results of genetic knockout and complementation studies are conflicting with regard to the importance of adipocyte leptin signaling in regulating adipocyte metabolism and energy metabolism (Cohen et al., 2001; de Luca et al., 2005; Guo et al., 2007; Huan et al., 2003). In order to address this question using a different approach, Guo et al transplanted adipose tissue SVCs from GFP-transgenic wild type and *Lepr^{db/db}* mice pair-wise to the contralateral inguinal subcutaneous sites of nude mice, where they developed into mature adipocytes spontaneously. After three months, the GFP-positive mature adipocytes derived from the transplanted wild type or

Lepr^{db/db} SVCs were morphologically indistinguishable from each other or from the endogenous adipocytes. The average adipocyte size of the wild type and *Lepr^{db/db}* transplants were not significantly different from each other and were highly correlated with endogenous adipocyte size, suggesting that leptin signaling in adipocytes, specifically those mediated by the b isoform, does not affect adipocyte morphology or size independent of the systemic energy balance of the recipient mice (Guo et al., 2009). Similarly, Meade et al transplanted mature adipose tissues from the wild type or *Lepr^{db/db}* mice underneath the kidney capsule of *Lepr^{db/db}* and wild type recipient mice, and observed that adipocytes in the transplants had size and other phenotypes similar to those of endogenous adipocytes of the recipients within a month, regardless the origin of the donor (Meade et al., 1979). Although the expression of beta3-adrenergic receptor in adipocytes is markedly decreased in *Lep^{ob/ob}* or *Lepr^{db/db}* mice when compared to the wild type mice (Collins et al., 1994), adipocyte beta3-adrenergic receptor levels in the wild type and *Lepr^{db/db}* transplants are not significantly different from each other or from the endogenous adipose tissue of the recipient mice (Guo et al., 2009). These results, together with the results from leptin administration and denervation studies and knockout mouse models (Guo et al., 2007; Scarpace and Matheny, 1998), suggest that obesity *per se*, rather than the lack of leptin signal, is responsible for the decreased adipocyte beta3-adrenergic receptor expression in *Lep^{ob/ob}* or *Lepr^{db/db}* mice. Overall, the results of the transplantation study suggest that the effects of direct leptin signaling on adipocyte metabolism, while discernable in *in vitro* studies, are insignificant relative to the effects exerted by central leptin action and other regulators under *in vivo* physiological conditions.

Cell-autonomous determination of sex-related differences in adipocyte function

—Sexual dimorphism in adipocyte size and function are well documented in humans and rodents (Anderson et al., 2001; Lemonnier, 1972; Pedersen et al., 1994). Adipocyte size of perigonadal fat pads is smaller in virgin females than in age-matched males (Bergmann et al., 1995; Johnson and Hirsch, 1972; Kaplan et al., 1976; Sinnott-Smith and Waddington, 1992). During normal growth, increases in the mass of inguinal subcutaneous fat pads are due to increases in both fat cell size and fat cell number in male Wistar rats, but mainly to increases in fat cell number in female rats (Porter et al., 2004). When subjected to mild-to-moderate caloric restriction, female rats defend fat cell number at the expense of fat cell size, whereas the converse is seen for male rats (Porter et al., 2004). These sex-related differences may be due in part to the effects of gonadal steroids on adipocytes and their progenitor cells (Anderson et al., 2001; D'Eon et al., 2005; Heine et al., 2000; Jones et al., 2000; Krakower et al., 1988), but cell-autonomous function of male and female genetic factors may also contribute to sexual dimorphism independent of gonadal steroids. Guo et al observed that SVCs from female C57BL/6 mice gave rise to smaller adipocytes than those from male mice when they were both transplanted to the sternal subcutaneous site of male nude mice (Guo et al., 2009), suggesting that genetic and/or epigenetic programming in adipocyte progenitor cells contributes significantly to the sexual dimorphism in adipocyte size. Sexual dimorphisms in the structural organization and function of the brain have also been observed, and are believed to be due to both cell-autonomous fulfillment of sex-specific genetic programs and epigenetic effects of prenatal and early postnatal hormonal exposure (Becu-Villalobos et al., 1997; Beyer et al., 1992). Intriguingly, the sexual dimorphism in adipocyte size was not observed in the transplants derived from adipose tissue SVCs of *Lepr^{db/db}* mice (Guo et al., 2009). Since leptin signaling is critically important in regulating puberty and fertility (Ahima et al., 1997; Farooqi et al., 1999; Mantzoros et al., 1997; Montague et al., 1997), it is possible that the lack of leptin signaling in *Lepr^{db/db}* mice may affect sexual development and epigenetic modifications in adipocyte progenitor cells prior to transplantation. Alternatively, it is possible that the genetic or epigenetic effects of sex on adipocyte size may require leptin signaling in the progenitor cells and/or mature adipocytes.

3. Use of transplantation in lineage analysis of adipocyte progenitor cells

Adipocyte biogenesis, which occurs throughout life, is important to normal adipose tissue growth and turnover in both lean and obese subjects (Heine et al., 2000; Hirsch, 1971; Hirsch and Han, 1969; Shillabeer and Lau, 1994; Spalding et al., 2008). Impaired adipocyte biogenesis is the underlying cause of metabolic abnormalities in lipodystrophy (Joffe et al., 2001; Reitman and Gavrilova, 2000; Shimomura et al., 1998; Tran et al., 2008), and may also contribute to the impaired adipose tissue function and metabolic abnormalities in the obese state. Indeed, increased ability to expand adipose tissue mass was associated with improved metabolism in adiponectin-transgenic *Lep^{ob/ob}* mice (Kim et al., 2007). Additionally, differential regulation of adipocyte biogenesis may be a major determinant of body fat distribution, depot-related differences in adipocyte function, and insulin-sensitizing effects of thiozolidinediones (Adams et al., 1997; Akazawa et al., 2000; Anderson et al., 2001; Faust et al., 1976, 1977; Hausman et al., 2003; Kirkland et al., 1994; Krakower et al., 1988). However, the nature of adipocyte progenitor cells and regulation of adipocyte biogenesis *in vivo* is still poorly understood. Transplantation has been used in several recent studies to test adipocyte progenitor cells and in lineage analysis of white and brown adipocytes, which will be discussed below.

Identification of adipocyte progenitor cells—While multipotent mesenchymal stem cells isolated from bone marrow and adipose tissue can be induced to undergo adipose differentiation *in vitro* with adipogenic stimuli (Conget and Minguell, 1999; Pittenger et al., 1999; Prockop, 1997; Zuk et al., 2001), bone marrow-derived stem cells are probably not the progenitor cells for native adipocytes. Bone marrow stem cells can be induced to migrate to adipose tissue where these cells undergo adipocyte differentiation after thiozolidinedione treatment. However, the bone marrow-derived adipocytes maintained a multilocular morphology through the entire 6-month study period, and are thus distinct from the unilocular native adipocytes (Crossno et al., 2006; Guo et al., 2009). In order to characterize and localize progenitor cells for native adipocytes, Tang et al generated two strains of “PPAR γ -reporter” mouse on the premise that PPAR γ is a key transcription factor that regulates terminal adipocyte differentiation (Tontonoz et al., 1994). In one of the strains, PPAR γ -expressing cells and all descendants can be permanently marked with indelible lacZ expression, which, however, can be repressed by doxycycline treatment; in the other, the proliferation of PPAR γ -expressing cells can be monitored by doxycycline-induced repression of GFP expression (Tang et al., 2008). By following lacZ and GFP expression, they identified a population of proliferating and PPAR γ -expressing cells that exist prenatally. These cells reside in the mural compartment of adipose tissue vasculature, and carry markers of stem cells, such as Sca-1 and CD34, as well as markers of mural cells, such as PDGFR and SAM. Most importantly, when transplanted to nude mice, these cells developed into mature adipocytes with a unilocular morphology (Tang et al., 2008). Taking a different approach, Rodeheffer et al used a fluorescent activated cell sorting method to separate subpopulations of stromal vascular cells of mouse adipose tissue based on their surface markers. A subpopulation of cells, Lin(-):CD29(+):CD34(+):Sca-1(+):CD24(+), which constitute about 0.08% of the stromal vascular fraction, were able to reconstitute a normal white adipose tissue depot and rescue the diabetic phenotype in A-Zip lipodystrophic mice when transplanted (Rodeheffer et al., 2008). In contrast, a closely-related and more abundant subpopulation of cells, which were CD24-, were unable to form adipose tissue *in vivo* when transplanted into A-Zip mice, even though they were able to differentiate into adipocytes *in vitro* (Rodeheffer et al., 2008). Expression of adipocyte-specific genes in the transplanted progenitor cells paralleled improvements of the metabolic phenotype of A-Zip recipient mice (Rodeheffer et al., 2008), suggesting that adipocyte differentiation of the transplanted progenitors is directly responsible for the correction of the metabolic phenotype of A-Zip mice.

These are the first reports on the molecular identity of progenitor cells of white adipocytes. However, the relationship between Lin(-):CD29(+):CD34(+):Sca-1(+):CD24(+) progenitor cells and the proliferating, PPAR γ -expressing cells described by Tang et al is unclear. The two populations of progenitor cells share the same stem cell markers, Sca1- and CD34 (Rodeheffer et al., 2008; Tang et al., 2008). An interesting distinction between the two, however, is that Lin (-):CD29(+):CD34(+):Sca-1(+):CD24(+) cells are not able to develop into mature adipocytes when transplanted into the wild type mice without additional stimulation of high fat feeding, whereas the PPAR γ -expressing cells are able to differentiate to mature adipocytes in nude mice without high fat diet or other stimulation. PPAR- γ is a transcription factor required for terminal adipocyte differentiation and maintenance of many adipocyte-specific properties (Spiegelman, 1997). Thus, it is possible that Lin(-):CD29(+):CD34(+):Sca-1(+):CD24(+) cells may be at an earlier stage of the adipocyte lineage than the PPAR γ -expressing cells. It would be of interest to determine if Lin(-):CD29(+):CD34(+):Sca-1(+):CD24(+) cells express PPAR- γ , and where they are located in adipose tissue.

Our recent study confirms that the stromal-vascular fraction of adipose tissue indeed contains adipocyte progenitor cells. Transplanted stromal-vascular cells isolated from the adipose tissue of GFP transgenic mice developed into mature adipose tissue at the site of transplantation in nude mice. The mature adipocytes derived from transplanted SVCs were morphologically similar to native adipocytes, predominantly unilocular as compared to the multilocular adipocytes originating from bone marrow (Crossno et al., 2006), and could only be distinguished from native adipocytes by their GFP expression. The transplants consisted of uniformly GFP-positive adipocytes, suggesting that there was little recruitment of adipocyte precursor cells from the recipients. These results are consistent with the idea that progenitor cells for native adipocytes are likely produced and/or stored locally within adipose tissue. Thus, differences in the proliferative capacity and/or the pool size of adipocyte progenitor cells and in the production of adipogenic paracrine factors may influence the growth, turnover, and function of adipose tissues in a depot-specific manner (Adams et al., 1997; Akazawa et al., 2000; Anderson et al., 2001; Faust et al., 1977; Hausman et al., 2003; Kirkland et al., 1994; Krakower et al., 1988; Soret et al., 1999). Obesity and aging are associated with decreased proliferation and differentiation of preadipocytes and decreased lipogenic activity of adipocytes (Gregoire et al., 1995; Hotta et al., 1999; Karagiannides et al., 2001; Kirkland and Dobson, 1997; Permana et al., 2004; Van Harmelen et al., 2004), which may contribute to the deterioration of adipose tissue function and metabolic abnormalities in obese and older individuals.

Determination of white and brown adipocyte lineage—Brown adipose tissue (BAT) plays an important role in regulating body temperature and energy expenditure in rodents, and to a lesser degree in humans. Increased BAT function is a potentially important mechanism in increasing energy expenditure and promoting weight loss (Almind et al., 2007; Cypess et al., 2009; Hamann et al., 1996; Marette et al., 1991; Stehling et al., 1997). While white and brown adipocytes clearly share some common adipocyte characteristics, the lineage of brown and white adipocytes appears to be separated before the preadipocyte stage (Hansen et al., 2004; Moulin et al., 2001; Tai et al., 1996; Tseng et al., 2008). Bone morphogenetic proteins play important roles in determining white and brown adipocyte lineage. Pre-treatment of C3H10T1/2 cells - which are immortalized multipotent mesenchymal stem cells- with BMP4 promotes white adipocyte differentiation when the treated cells are transplanted in nude mice (Tang et al., 2004). In contrast, BMP7 stimulates the differentiation of brown adipocyte progenitors but not white adipocyte progenitor cells (Tseng et al., 2008). BMP7-treated C3H10T1/2 cells are committed to brown adipocyte differentiation both *in vitro* and *in vivo*, which is associated with the activation of the program of mitochondrial biogenesis and the expression of uncoupling protein 1 (Tseng et al., 2008). On the other hand, 3T3-F442A cells appear to be committed to white adipocyte lineage. When 3T3-F442A cells were transplanted

into interscapular BAT pad of nude mice, they differentiated into adipocytes with unilocular morphology and no detectable expression of UCP-1 amidst of native brown adipocytes of nude mice (Our unpublished observation). Retinoblastoma protein may also play a role in determining the switch between white and brown adipocyte gene expression patterns. Deficiency or down-regulation of retinoblastoma gene expression results in differentiation of mouse embryonic fibroblasts or stem cells into adipocytes with a gene expression pattern and mitochondrial content resembling brown adipose tissue *in vitro* (Hansen et al., 2004). Recent work by Spiegelman and his colleagues suggests that brown adipocytes and skeletal muscle share common progenitors. PRDM16, a transcription factor, plays a critical role in controlling the cell fate switch from myoblastic precursors to brown fat cells by forming a transcriptional complex with the active form of C/EBP-beta (Kajimura et al., 2009; Seale et al., 2008). They have shown that forced expression of PRDM16 and C/EBP-beta is sufficient to induce a fully functional brown fat program in naive fibroblastic cells, including skin fibroblasts from mouse and man. Transplantation of fibroblasts expressing these two transcription factors into mice gives rise to an ectopic fat pad with the morphological and biochemical characteristics of brown fat (Kajimura et al., 2009). It would be of interest to investigate whether altering the expression of PRDM16 or retinoblastoma protein in mesenchymal stem cells, such as C3H10T1/2, or even committed white adipocyte progenitor cells, such as 3T3-F442A, will lead to the formation of brown adipose tissue when the genetically-manipulated cells are transplanted in nude mice. Cross transplantation of stromal vascular fraction of brown and white adipose tissues to white and brown fat pads may also reveal how much the intrinsic nature of the progenitor cells and the specific microenvironment of BAT contribute to the determination of the specific features of mature brown adipose tissue.

Perspectives

Body fat distribution, independent of the overall adiposity, is an important determinant of co-morbidity of obesity; abdominal obesity is associated with increased risk for diabetes and cardiovascular diseases (Bergman et al., 2007; Despres et al., 1990; Rader, 2007; Reisin and Alpert, 2005; Tchernof, 2007). Studies have shown that subcutaneous and intra-abdominal adipose tissues are functionally distinct (Hocking et al., 2008; Ohman et al., 2008; Tran et al., 2008), and some of the depot-related differences in adipocyte function may have been programmed in the respective progenitor cells (Adams et al., 1997; Caserta et al., 2001; Kirkland et al., 1996; Krakower et al., 1988; Pedersen et al., 1996; Sztalryd et al., 1991). Factors that regulate depot-related differences in adipocyte biogenesis and function are still poorly understood. Genetic factors, including sex, clearly play a role in determining body fat distribution (Bouchard, 1997; Fried and Kral, 1987; McTernan et al., 2000; Rosenbaum et al., 2001; Wajchenberg, 2000); stress and age may also impact body fat distribution and adipocyte function in a depot-specific manner via hormonal and/or neuronal mechanisms (Bjorntorp and Rosmond, 2000; Vicennati and Pasquali, 2000). Understanding differential regulation of adipocyte biogenesis and function in anatomically distinct fat depots would be critical to the prevention and treatment of abdominal obesity and its associated metabolic co-morbidities. As the studies in this review have shown, transplantation is a particularly useful experimental paradigm in demonstrating specific function of adipose tissue or in distinguishing effects of cell autonomous-function versus “environmental” factors on adipocyte biogenesis and function, and thus could be used to investigate mechanisms by which anatomic location, sex, and age affect adipose tissue biogenesis and function. With the availability of many genetically modified mouse strains, transplantation of mature adipose tissue or adipocyte progenitor cells from or to genetically modified mice would be a fruitful approach to investigate the role of candidate genes in the differential regulation of adipocyte biogenesis and function. Moreover, as the likelihood that mouse and human differ in important aspects of adipose tissue biology is great, transplantation of human adipose tissue or SVCs to nude mice may have the added advantage in the study of human adipose tissue provenance and development.

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Figure 1.

A schematic illustration of the transplantation technique for studying adipocyte biogenesis and function. Transplantation can be used to assess effects of specific cellular elements of adipose tissue (or intact tissue) on the physiology of recipient mice, such as energy intake and expenditure and disease susceptibility; influences of recipient mice on the transplants; or cell-autonomous functions of transplants. Whole adipose tissue, preadipocytes, or stromal vascular cells of adipose tissue (SVCs) can be used as donor materials. Various anatomic locations in recipient mice - as indicated - can be used as transplantation sites. Factors that influence the function and morphology of transplants and the effects of the transplants on recipient mice include anatomic location of the donor tissue and transplants, and sex, age, and genetic makeup of donor and recipient mice. The degree of vascularization and innervation of the transplants also affect their survival and function.