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Review

Developmental Origin of Fat: Tracking Obesity to Its Source

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The development of obesity not only depends on the balance between food intake and caloric utilization but also on the balance between white adipose tissue, which is the primary site of energy storage, and brown adipose tissue, which is specialized for energy expenditure. In addition, some sites of white fat storage in the body are more closely linked than others to the metabolic complications of obesity, such as diabetes. In this Review, we consider how the developmental origins of fat contribute to its physiological, cellular, and molecular heterogeneity and explore how these factors may play a role in the growing epidemic of obesity.

We are in the midst of a worldwide epidemic of obesity—a major factor in the development of common medical conditions such as type 2 diabetes, dyslipidemias, fatty liver, cardiovascular disease, Alzheimer's disease, gallstones, and even some cancers. These conditions occur in large part as a result of insulin resistance induced by obesity and the fact that adipose tissue not only serves to store energy but is also the body's largest endocrine organ, secreting hormones, cytokines, and proteins that affect the function of cells and tissues throughout the body.

What Causes Obesity?

Obesity develops when energy intake exceeds energy expenditure. Although the number of fat cells can increase throughout life (Prins and O'Rahilly, 1997), individuals with adult-onset obesity in general exhibit increased adipocyte size, whereas individuals with early-onset obesity have both adipocyte hypertrophy and hyperplasia (Hirsch and Batchelor, 1976). Fat distribution also plays an important role in metabolic risk. Increased intra-abdominal/visceral fat (central or apple-shaped obesity) promotes a high risk of metabolic disease, whereas increased subcutaneous fat in the thighs and hips (peripheral or pear-shaped obesity) exerts little or no risk (Kissebah and Krakower, 1994) (Figure 1, top). Both fat mass and distribution can be measured by magnetic resonance imaging (MRI) and Dual-Energy X-Ray Absorptiometry (DEXA), but for most purposes, simpler surrogate measurements such as body mass index (BMI = weight in kg/height in meters^2) and waist-hip ratio (WHR) or waist circumference are used.

The past two decades have shed considerable light on the role of factors controlling food intake and energy expenditure in body weight regulation (Flier, 2004) and on the transcriptional control and cell biology underlying conversion of preadipocytes to adipocytes (Farmer, 2006; Rosen and MacDougald, 2006). Surprisingly little is known, however, about the developmental origins of adipose tissue; the control of brown versus white preadipocyte commitment; the control of the relative amounts and functional heterogeneity among white fat cells in different depots; and the exact pathways and intermediates between the embryonic stem cell and the mature fat cell. It is these processes that are the primary focus of this Review.

Adipocytes and the Adipose Organ

Virtually all animal species, from *C. elegans* to *Homo sapiens*, have found a way to store excess energy in the form of fat for future needs. *C. elegans* store fat in intestinal epithelium (McKay et al., 2003) and sharks store fat in the liver (Van Vleet et al., 1984)—both tissues of endodermal origin. But in most species, fat storage occurs in a mesodermal tissue, namely white adipose tissue (WAT). WAT location varies between species. For invertebrates, amphibians, and many reptiles, the largest fat stores are intra-abdominal; in seals and whales, most fat is subcutaneous, whereas mammals and birds have both intra-abdominal and subcutaneous WAT. This does not simply reflect an evolutionary adaptation for thermal insulation, as similar fat distribution is observed in arctic and tropical mammals of similar body mass (Pond, 1992). In humans, WAT is dispersed throughout the body with major intra-abdominal depots around the omentum, intestines, and perirenal areas, as well as in subcutaneous depots in the buttocks, thighs, and abdomen (Figure 2, bottom). In addition, WAT can be found in many other areas, including in the retro-orbital space, on the face and extremities, and within the bone marrow. Some adipose tissue is responsive to sex hormones, such as adipose tissue in the breasts and thighs, whereas other depots, such

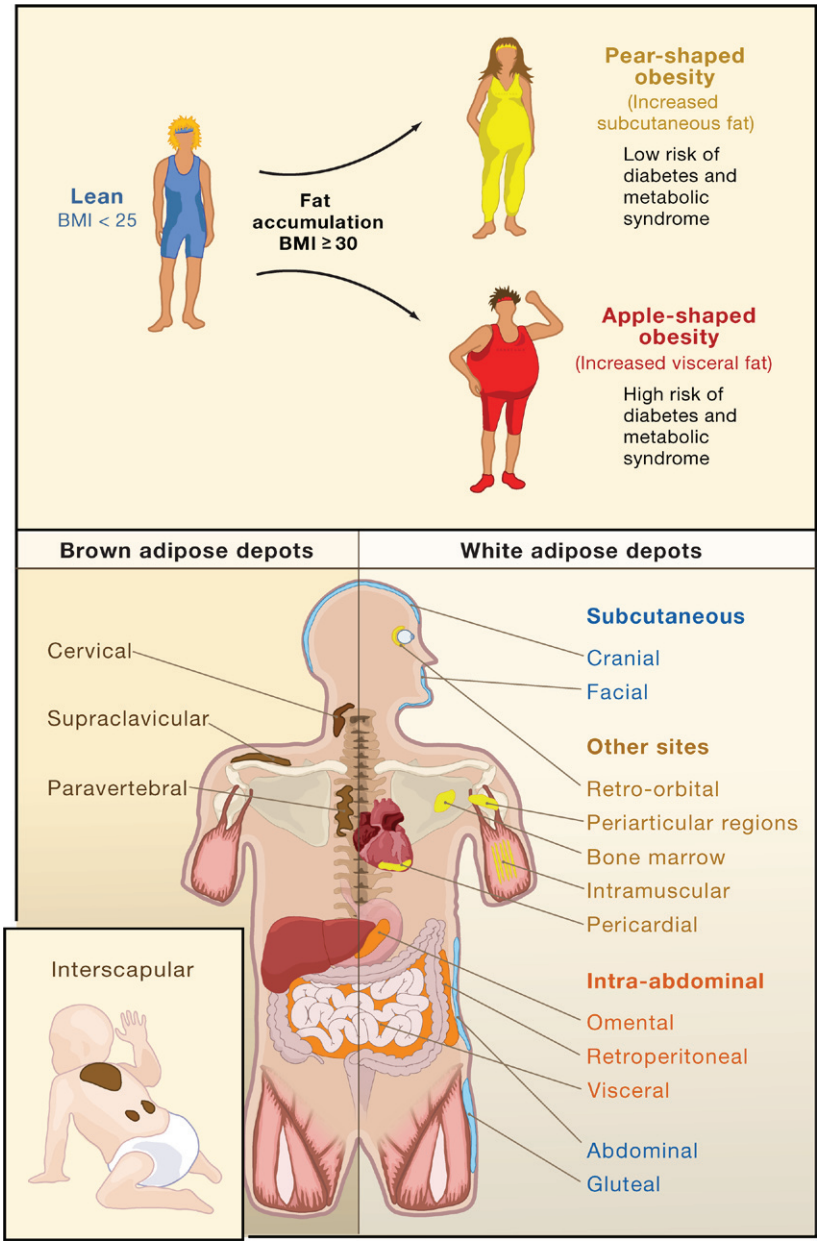


Figure 1. Fat Distribution Influences Risks Associated with Obesity in Humans

(Top) Obesity is the consequence of an excess in fat accumulation and is defined by a body mass index of ≥ 30. Fat distribution can be estimated by measurements of the ratio of waist to the hip circumference (WHR). Obese individuals with low WHR, (subcutaneous or pear-shaped obesity) are at low risk for metabolic complications of obesity, whereas individuals with a high WHR (visceral or apple-shaped obesity) are at high risk for these complications (Kissebah and Krakower, 1994). (Bottom) In humans, depots of white adipose tissue are found in areas all over the body, with subcutaneous and intra-abdominal depots representing the main compartments for fat storage. Brown adipose tissue is abundant at birth and still present in adulthood but to a lesser extent.

as fat on the neck and upper back, are more responsive to glucocorticoids—forming a so-called "buffalo-hump" in humans with an excess of glucocorticoids, such as in Cushing's disease. Interestingly, the acquired form of lipodystrophy associated with treatment for HIV produces a similar kind of buffalo-hump (Miller et al., 1998). Fat distribution, even in thin individuals with steady body weight and stable BMI, changes with age, decreasing in retro-orbital fat and subcutaneous fat and increasing in intra-abdominal fat.

Genetics play an important role in both obesity and distribution of WAT. Twin and population studies have revealed that both BMI and WHR are heritable traits, with genetics accounting for 30%–70% of the variability (Nelson et al., 2000). For example, such genetic control of body fat distribution is evident in Hottentot/Khoisan women, who have marked accumulation of fat in the buttocks (steatopygia). Striking differences in WAT distribution can also be observed in individuals with heritable forms of partial lipodystrophy (Agarwal and Garg, 2006) (see below).

In mammals the adipose organ is composed not only of white adipocytes, which are the primary site of triglyceride/energy storage, but also brown adipocytes, which are important in both basal and inducible energy expenditure in the form of thermogenesis (Figure 2). This occurs through expression of uncoupling protein-1 (UCP-1), a 32 kDa protein found in the inner mitochondrial membrane, that allows dissipation of the proton electrochemical gradient generated by respiration in the form of heat (Cannon and Nedergaard, 2004). In rodents, brown adipose tissue (BAT) is most abundant in the neonatal period and is most concentrated in the interscapular region. Brown adipocytes can also be found in other areas, including typical WAT depots, following cold exposure (Cinti, 2005). Although some "warm-bodied" fishes (such as swordfish) maintain brain temperatures higher than the environment due to the presence of brown fat-like "heater cells" around the brain, BAT itself develops relatively late in the course of evolution in parallel with the development of homeothermy and the capacity for nonshivering thermoregulation. Boney fish, such as carp (*Cyprinus carpio*), express UCP-1 in the liver, and in

Species	 *Caenorhabditis elegans*	 *Drosophila melanogaster*	 *Carcharodon carcharias*	 *Cyprinus carpio*	 *Xenopus laevis*	 *Gallus gallus domesticus*	 *Mus musculus*	 *Homo sapiens*
Fat storage	Stored in intestinal cells	Stored in the "fat body"	Stored in liver	Stored in WAT	Intra-abdominal WAT (no subcutaneous WAT)	Subcutaneous and internal WAT	Subcutaneous and internal WAT	Subcutaneous and internal WAT
Leptin	No	No	No	Yes	Yes	Yes	Yes	Yes
BAT	No	No	No	No	No	No	Present throughout life	Present at birth; reduced in adults
UCP	UCP-like protein (ucp-4)	No	?	UCP-1 in liver	UCP-4 in oocytes	Avian UCP in muscle	UCP-1 in BAT	UCP-1 in BAT
Thermo-regulation	**Ectotherm**	**Ectotherm**	**Ectotherm**	**Ectotherm**	**Ectotherm**	**Endotherm** Shivering and nonshivering thermogenesis	**Endotherm** Shivering and nonshivering thermogenesis	**Endotherm** Shivering and nonshivering thermogenesis

Figure 2. Evolution of Adipose Tissue Depots

Adipose tissue distribution and fat storage have changed dramatically with the process of evolution. In the worm *C. elegans* fat is stored in the intestine, whereas *Drosophila* have a defined fat body. Sharks are devoid of adipose tissue and use their liver to store fat. Intra-abdominal white adipose tissue (WAT) becomes apparent, coinciding with the presence of leptin in boney fishes, such carp (*Cyprinus carpio*), amphibians and fast-moving reptiles. Differentiation of subcutaneous and internal WAT occurs in higher species, such as birds and mammals, exemplified here by the chicken (*Gallus gallus domesticus*), mouse (*Mus musculus*), and human (*Homo sapiens)*. Thermoregulation also appears in the course of evolution but independently of the appearance of brown adipose tissue (BAT). Estimated to have emerged 150 millions years ago, BAT is only present in higher mammals, although UCP-1 expression appears independently of BAT in boney fish. Endotherm refers to an animal that produces its own heat from within versus ectotherm, an animal that does not. These terms are interchangeable with homeotherm, i.e., an animal that can maintain a specific body temperature or is warm blooded versus poikilotherm, i.e., an animal that has a body temperature that varies with the ambient temperature or is cold blooded.

contrast to mammals, UCP-1 expression is diminished in response to cold exposure (Jastroch et al., 2005). So far no tissue resembling BAT has been reported in amphibians or reptiles, although expression of UCP-4 has been found in frog oocytes and in head muscle and the pharynx of *C. elegans*, where it negatively regulates ATP production (Iser et al., 2005). Interestingly, birds are homeotherms, but devoid of BAT, and depend on UCP expression in muscle for nonshivering thermoregulation (Mozo et al., 2005).

In human fetuses and newborns, BAT is found in axillary, cervical, perirenal, and periadrenal regions (Cannon and Nedergaard, 2004) but decreases shortly after birth and has traditionally been considered insignificant in adults, except perhaps in patients with pheochromocytoma, where adrenergic activity is extremely high (English et al., 1973), or in outdoor workers in northern climes subject to prolonged cold exposure (Huttunen et al., 1981). However, recent morphological and scanning studies have shown that brown fat in humans may not be as rare as once believed. Indeed, [^{18}F]-2-fluoro-D-2-deoxy-D-glucose (FDG) positron emission tomography (PET) can detect areas of metabolically active brown fat in the cervical, supraclavicular, axillary, and paravertebral regions of normal individuals (Nedergaard et al., 2007) (Figure 1, bottom). UCP-1 mRNA can be detected in human WAT and is further induced by the antidiabetic drugs thiazolidinediones (Digby et al., 1998), suggesting some admixture of BAT in WAT depots.

Although it is unclear to what extent BAT might play a role in energy balance in adult humans, Rothwell and Stock (1983) have estimated that as little as 50 g of BAT could account for 20% of daily energy expenditure, if maximally stimulated. Furthermore, up to 24% of the increase in metabolism in lean men produced by ephedrine has been attributed to BAT (Astrup et al., 1985). It has been suggested that the age-related decline in thermogenesis and regulatory energy expenditure in humans and rodents is associated with a reduction in the amount of functional BAT. In rodents, targeted ablation of BAT results in diet-induced obesity, diabetes, and hyperlipidemia (Lowell et al., 1993). UCP-1-deficient mice also exhibit increased susceptibility to age- and diet-related obesity (Kontani et al., 2005). Recently, depots of UCP-1-positive brown adipocytes have been identified, interspersed between skeletal muscle bundles in the legs of an obesity-resistant strain of mice, suggesting that "ectopic brown adipocytes" may play an important role in the regulation of whole-body energy homeostasis (Almind et al., 2007). The potential of inducing even small amounts of brown fat in adult humans could provide a new approach to the treatment and/or prevention of obesity and its metabolic complications.

The Origin of Adipose Tissue

Lineage Determination

Adipose tissue, like muscle and bone, is generally regarded as having a mesodermal origin. However, this may be an oversimplification because precise lineage

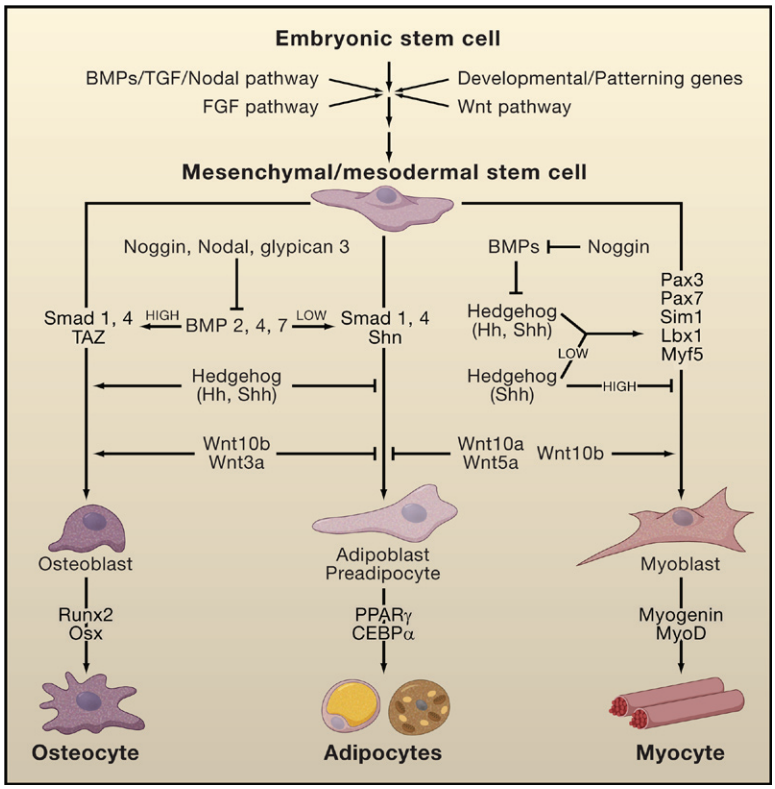


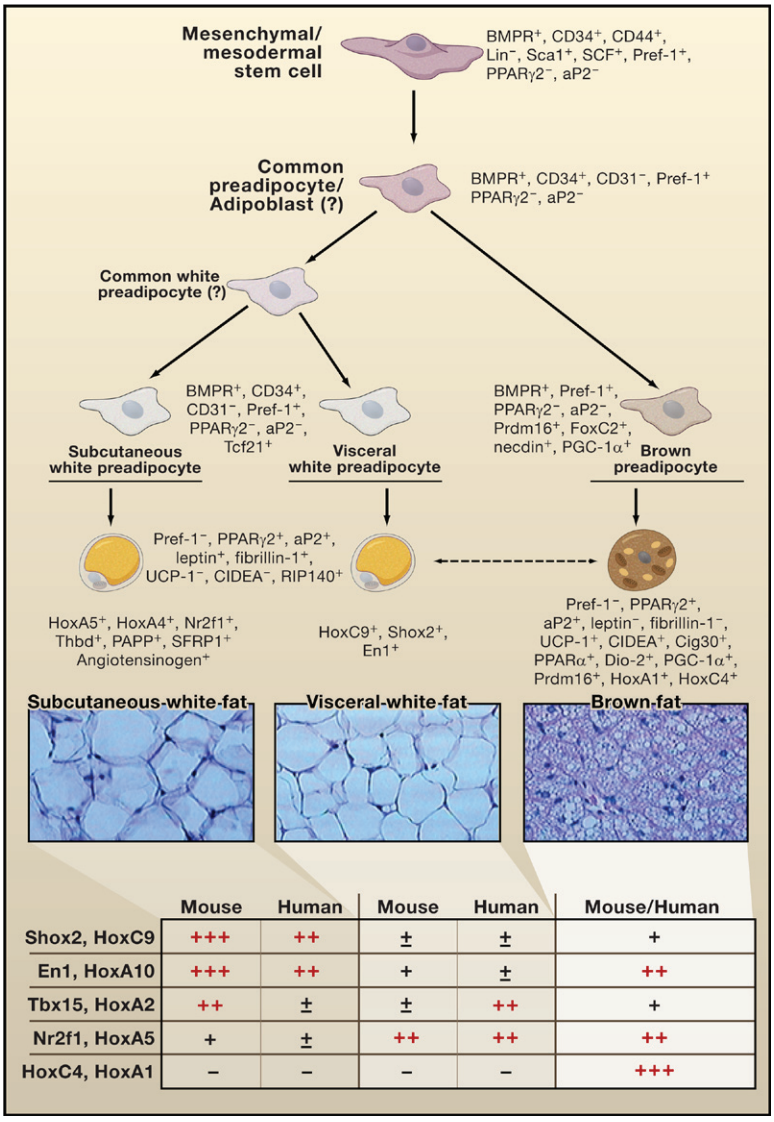
Figure 3. Development of Mesenchymal/Mesodermal Derivatives

Mesenchymal stem cells (MSCs) develop from the mesoderm and then commit into different lineages influenced by a number of factors. BMPs through their intracellular mediators (Smad proteins) can trigger MSCs to enter the osteogenic and/or adipogenic lineage, while preventing commitment into the myogenic lineage. Intracellular proteins, such as TAZ and Schnurri-2 (Shn), modify the action of BMP in the determination of the osteogenic and adipogenic lineages. In addition, the action of BMPs can be modulated by Noggin, Nodal, and glypican 3. Wnt and Hedgehog proteins are important for MSC commitment in myogenic and osteogenic lineages and prevent commitment in the adipogenic lineage. Once committed, MSC give rise to undifferentiated precursors (osteoblast, adipoblast/preadipocyte, and myoblast), which upon the expression of key transcription factors enter a differentiation program to acquire their specific functions.

tracing studies have not been performed. The formation of the mesoderm begins with the migration of a layer of cells between the primitive endoderm and ectoderm. This layer spreads along the anteroposterior and dorsoventral axes of the developing embryo giving rise to the axial, intermediate, lateral plate, and paraxial mesoderm. The paraxial mesoderm, after its segmentation into somites, gives rise to the axial skeleton and muscles of the trunk. The lateral plate mesoderm generates the skeleton and muscles of the limbs. Interestingly, the bones and muscles of the skull and face appear to be of ectodermal origin, specifically the neural crest (Bronner-Fraser, 1994). Recently, neural crest stem cells have been reported to be able to differentiate into adipocytes in culture (Billon et al., 2007). Each of these regions are presumed to give rise to local adipose tissue. Such a developmental origin is consistent with the occurrence of various forms of partial lipodystrophy and with the differential expression of genes involved in development and patterning between different fat depots. Brown adipose tissue also appears to be of mesodermal origin. Interestingly, when mesoderm from a 9-day-old rat embryo is engrafted below the kidney capsule of an adult rat, it develops only into BAT (Loncar, 1992). Using genetic fate mapping under control of the homeobox transcription factor Engrailed 1 (En1), Atit et al. (2006) have shown that interscapular brown fat is derived from the paraxial mesoderm.

Mesenchymal stem cells (MSCs) were initially identified in postnatal human bone marrow and have been used to model differentiating mesoderm. MSCs are capable of differentiating into adipocytes, osteoblasts, chondrocytes, myoblasts, and connective tissue. Although the exact number of intermediate stages between a mesodermal/mesenchymal stem cell and a mature adipocyte is uncertain, it is believed that the MSC gives rise to a common early precursor (adipoblast), which in turn develops into committed white and brown preadipocytes that under appropriate stimulatory conditions differentiate into mature adipocytes of different types (Figures 3 and 4). However, as none of these precursor cells possesses any unique morphological characteristics or gene expression markers, it is not clear if separate adipoblasts and/or preadipocytes for brown and white fat exist or if there are different white preadipocytes for different white adipose depots. Nor is it known what factors control progress of the early MSC down these differentiation pathways. In contrast to studies of other well-defined cell lineages, use of monoclonal antibodies and cell sorting in tracing adipocyte development have been severely limited by both the nature of the cells and reagent availability.

The process of terminal adipocyte differentiation during which preadipocytes mature into adipocytes has been extensively studied in mouse 3T3-L1 and 3T3-F442A cell lines and immortalized brown preadipocyte cell lines (Rosen and Spiegelman, 2000). The transition from preadipocyte to adipocyte involves four stages: growth arrest, clonal expansion, early differentiation, and terminal differentiation. These stages are orchestrated by a transcriptional cascade involving the nuclear receptor PPARγ and members of the C/EBPs family (Farmer, 2006). PPARγ plays an important role in adipogenesis and has been shown to be necessary and sufficient for adipocyte differentiation (Rosen and MacDougald, 2006). PPARγ also appears to be required for



	Mouse	Human	Mouse	Human	Mouse/Human
Shox2, HoxC9	+++	++	±	±	+
En1, HoxA10	+++	++	+	±	++
Tbx15, HoxA2	++	±	±	++	+
Nr2f1, HoxA5	+	±	++	++	++
HoxC4, HoxA1	–	–	–	–	+++

Figure 4. Molecular Signature of Adipocyte Lineages

Adipocytes arise from mesenchymal/mesodermal stem cells by a sequential pathway of differentiation. When triggered by appropriate developmental cues, MSCs become committed to the adipocyte lineage. Although some stages are still not clearly defined, this differentiation pathway presumably involves a common preadipocyte or adipoblast, which has the capacity to differentiate into white or brown preadipocytes. The proteins and genes that represent potential molecular markers in cells within the adipocyte lineage are marked with a "+" or "–" sign indicating the relative levels of expression of these markers. The table summarizes the relative expression levels of various developmental and patterning genes in different fat depots in both humans and mice. The data have been compiled from Vohl et al. (2004), Cantile et al. (2003), Gesta et al. (2006), and Tchkonia et al. (2007), as well as our unpublished data. The relative levels of expression have been graded from absent (–) to high (+++).

maintenance of the terminal differentiated state of adipocytes, and expression of a dominant-negative PPARγ in differentiated 3T3-L1 cells induces dedifferentiation with loss of lipid accumulation and decreased expression of adipocytes markers. Likewise, inducible knockout of PPARγ in mature adipocytes in vivo leads to death of both brown and white adipocytes. However, mice with adipocyte-specific knockout of PPARγ generated using an aP2 promoter-Cre still have some white fat development. Two isoforms of PPARγ (PPARγ1 and PPARγ2) are generated by alternative splicing and promoter usage of the *Pparg* gene. Although both are expressed in adipocytes, PPARγ2 has been regarded as a specific marker of fat. Mice with germline knockout of PPARγ2, however, still have some WAT, suggesting that PPARγ1 can compensate for loss of PPARγ2. PPARγ2 null mice are also insulin resistant, suggesting a role for this transcription factor in maintaining insulin sensitivity independent of its effects on adipogenesis (Rosen and MacDougald, 2006).

The C/EBP family consists of five different members, C/EBPα, C/EBPβ, C/EBPδ, C/EBPγ, and CHOP. Sequential expression of these factors is observed during adipocyte differentiation, in which the early expression of C/EBPβ and C/EBPδ promotes expression of C/EBPα and PPARγ (Farmer, 2006). C/EBPβ-deficient mice exhibit reduced adipose mass, whereas mouse embryonic fibroblasts (MEFs) lacking C/EBPβ are still capable of differentiating into adipocytes in vitro, albeit with reduced efficiency. Indeed, some adipose tissue can be found in mice deficient of both C/EBPβ and C/EBPδ.

In contrast, C/EBPα is required for white adipogenesis. C/EBPα-deficient mice that have been rescued from death by re-expression of *C/ebpa* in liver show an absence of subcutaneous, perirenal, and epididymal WAT but near normal mammary WAT, with somewhat hypertrophied BAT (Linhart et al., 2001). Although both C/EBPα and PPARγ control adipocyte differentiation, PPARγ appears to be dominant. Thus, forced expression of PPARγ can rescue the differentiation of MEFs lacking C/EBPα to adipocytes, whereas forced expression C/EBPα does not rescue differentiation in MEFs lacking PPARγ.

What Defines a Preadipocyte and an Adipocyte?

Although some lipid accumulation can occur in many cell types, including macrophages, hepatocytes, and muscle, adipocytes are morphologically different from other cells due to the presence of large lipid droplets surrounded by a specific protein, perilipin (Greenberg et

al., 1991). The droplets are usually unilocular in WAT and multilocular in BAT. Mature adipocytes in WAT and BAT are also marked by the presence of PPARγ2, markers of terminal differentiation (such as Glut4 and fatty-acid synthase), and insulin-regulated glucose uptake and metabolism (Rosen and MacDougald, 2006). In addition, WAT is characterized by the presence of leptin, whereas BAT is distinguished by the existence of UCP-1 (Figure 4). However, white or brown preadipocytes are indistinguishable from any cell type with a fibroblast-like morphology, making them difficult to identify and study.

The only widely accepted marker of preadipocytes is preadipocyte factor 1 (Pref-1; also known as DLK-1 or *Drosophila* Homolog-like 1) (Villena et al., 2002). Pref-1 is expressed at high levels in both white and brown preadipocytes, and expression markedly decreases upon differentiation. Pref-1, however, is not unique to the preadipocyte and is also expressed in placenta, pituitary, adrenal cortex, fetal liver, and pancreatic islet cells.

Pref-1/DLK-1 belongs to the Notch/Delta/Serrate family of proteins that have epidermal growth factor-like repeats. Pref-1 is synthesized as a transmembrane protein and is cleaved to generate a soluble 50 kDa form that acts to inhibit adipocyte differentiation. Mice lacking Pref-1 show accelerated fat deposition, whereas mice overexpressing soluble Pref-1 in adipose tissue exhibit decreased fat mass and adipocyte marker expression. Pref-1/DLK-1 is in an imprinted region of the genome, and in sheep, the genetic disorder termed callipyge, which changes imprinting, leads to decreased fat deposition, hindlimb muscular hypertrophy, and increased feeding efficiency (Charlier et al., 2001).

Other putative preadipocyte markers are the type VI collagen alpha 2 chain (COL6A2) (Ibrahimi et al., 1993) and a secretory protein related to the Wnt antagonist Frzb, namely FRP2/SFRP2 (Hu et al., 1998). The latter is particularly abundant in subcutaneous fat (Gesta et al., 2006). Like Pref-1, both are more highly expressed in undifferentiated preadipocytes and reduced in mature adipocytes. However, neither is adipose tissue specific.

In addition to adipocytes, fat pads contain preadipocytes, vascular cells, nerves, macrophages, and fibroblasts. When adipose tissue is dispersed, these latter cells are collectively referred to as the stromovascular fraction. Using cell-sorting approaches, Sengenes et al. (2005) isolated preadipocytes from human stromovascular fraction. These $CD34^{+}/CD31^{-}$ cells displayed features distinct from the adult mesenchymal and hematopoietic stem cells. This approach, however, is still in its infancy, and, at present, the only definitive criteria that identifies preadipocytes is their subsequent ability of the cells to accumulate lipid and develop a pattern of adipocyte marker gene expression (Figure 4). It is also important to realize that even cell lines considered good models of WAT do not express all WAT markers. For example, 3T3-L1 cells produce little, if any, leptin compared to normal white adipocytes.

Signals Inducing Mesodermal and Adipose Development

Vertebrate embryonic patterning and evolution of mesodermal tissues such as fat are controlled by several conserved developmental signaling systems, including effects of Nodal, wingless (Wnt), bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs). The exact effects of these factors depend on concentration, stage of differentiation, as well as extrinsic factors, such as matrix-cell and cell-cell interactions, the presence of vasculature, and the level and type of innervation (Figure 3).

Bone Morphogenetic Proteins, Nodal, and the SMAD Pathway

BMPs are members of the transforming growth factor-β (TGF-β) superfamily and play a critical role in the commitment of MSCs into the adipocyte lineage. There are 14 members in the BMP family (BMP-2 to BMP-15). *Bmp4* has been shown to stimulate the differentiation of MSC to adipocytes (Tang et al., 2004), and mice with disruption of the *Bmp4* gene die between E6.5 and E9.5 and show little or no mesodermal differentiation or expression of the early mesodermal marker brachyury (T) (Winnier et al., 1995). In addition, several mouse models with null mutations ablating either ligands, receptors, or downstream components of the BMP signaling system showed defects in mesoderm formation, providing indirect evidence for a role for BMPs in the development of adipose tissue (Tseng and He, 2007). *Bmp2* expression during early development appears to be limited to the visceral endoderm and the extra-embyonic mesoderm, and BMP-2 and BMP-7 have been shown to promote osteogenic and inhibit adipogenic differentiation of MSCs, although these effects are variable depending on concentration (Wang et al., 1993; Asahina et al., 1996) (Figure 3). Mice with disruption of the Schnurri-2 gene, a zinc finger-containing protein that works in cooperation with Smad 1 and 4 and C/EBPα in induction of PPARγ expression following BMP-2 stimulation, have reduced white and, to a lesser extent, brown fat mass (Jin et al., 2006).

Fibroblast Growth Factors

The FGF system consists of four receptors and at least 23 growth factors, a number of which have been implicated in development or differentiation of mesodermal tissues. FGFs 1, 10, 16, and 19 have specifically been implicated in adipose development. FGF-10 mRNA is most abundant in WAT, where it is expressed primarily in preadipocytes (Yamasaki et al., 1999). FGF-10 shows significant mitogenic activity for primary preadipocytes but does not affect their differentiation, suggesting that it may act as a growth factor for WAT preadipocytes. Conversely, FGF-16 is expressed predominantly in BAT and might be important for embryonic development of BAT (Konishi et al., 2000). Transgenic mice overexpressing FGF-19 have increased BAT mass and reduced susceptibility to diet-induced obesity (Tomlinson et al., 2002). FGF-1 enhances adipogenesis of human preadipocytes and also supports development of the vascular tissue within the fat pad (Hutley et al., 2004).

Hedgehog Signaling and GATA Transcription Factors

A potential role of Hedgehog signaling in mesoderm progression into adipose tissue was first suggested by developmental studies of *Drosophila*. In *Drosophila*, activation of the *Hedgehog* (*Hh*) pathway blocks formation of the fat body that is normally present during the larval and pupal periods (Suh et al., 2006), whereas the GATA transcription factor *Serpent* (*srp*), downstream of *Hedgehog*, is the earliest marker for fat body development and is essential for its differentiation.

Mammalian homologs of *Hedgehog*, i.e., *Sonic hedgehog* (*Shh*), *Indian hedgehog* (*Ihh*), and *Desert hedgehog* (*Dhh*), strongly influence the fate of MSCs. During embryogenesis, *Shh* produced by the notochord and neural tube exerts ventralizing influences on somitic cells controlling their fate into osteogenic, chondrogenic, myogenic, and presumably adipogenic lineages. Activation of the *Hh* pathway by *Shh* and *Ihh* can block the progression of mesenchymal C3H10T1/2 cells to the adipogenic lineage, whereas inhibition of the *Hh* pathway with an antagonist of the *Hh* receptor *smoothened* (*smo*) or using a dominant-negative form of the downstream transcription factor *Gli2* stimulates adipogenesis (Suh et al., 2006). This antiadipogenic action of *Hh* appears to be mediated by transcription factors of the GATA family. Constitutive expression of GATA-2 and GATA-3 suppresses differentiation of 3T3-F442A preadipocytes. This occurs through the interaction between the GATAs and C/EBPα or C/EBPβ, resulting in suppression of PPARγ2 promoter activity (Tong et al., 2005). Conversely, embryonic stem cells lacking both copies of the GATA-3 gene demonstrate an enhanced capacity to differentiate into mature adipocytes in vitro.

Wnt Signaling

Wnts are a 19-member family of secreted signaling proteins, which have a major influence on embryonic development and tumorogenesis. Wnt proteins bind to receptors of the frizzled family, which in turn activate β-catenin-dependent and β-catenin-independent actions. Although Wnt signaling is important in mesodermal development, once the MSC is formed Wnt acts to suppress adipogenic differentiation and favors myogenic or osteogenic differentiation. Overexpression of *Wnt3a* in C3H10T1/2 mesenchymal stem cells triggers differentiation of these cells toward osteoblasts and inhibits their ability to become adipocytes (Kennell and MacDougald, 2005), whereas conditional deletion of β-catenin in the developing mouse uterus results in a switch from myogenesis to adipogenesis (Arango et al., 2005). Recently, BMP-2 has been shown to increase the expression of TAZ, a WW domain-containing transcriptional modulator, which acts similarly to β-catenin and modulates MSC fate favoring osteogenic gene transcription, while repressing PPARγ-dependent adipogenic gene transcription (Hong et al., 2005).

Sustained activation of the Wnt signaling pathway by overexpression of *Wnt1*, *Wnt10b*, or a β-catenin mutant with increased stability in mouse fibroblasts prevents adipogenic differentiation by blocking the cascade of gene expression regulated by C/EBPα and PPARγ. Mice overexpressing *Wnt10b* specifically in adipose tissue exhibit decreased total fat mass and increased bone mass (Longo et al., 2004). Interestingly, in these mice, the interscapular BAT has a visual appearance of WAT but expresses neither brown nor white adipocyte markers. Conversely, myoblasts isolated from *Wnt10b*-deficient mice show increased expression of genes involved in lipid storage and increased adipogenic potential (Vertino et al., 2005). Recently, a C256Y mutation in *Wnt10b* has been identified in a human subject with early-onset obesity (Christodoulides et al., 2006). This mutation abrogates *Wnt10b's* ability to activate WNT signaling and block adipogenesis. Expression of *Wnt10a* and *Wnt5a* is also significantly increased in brown preadipocytes that are unable to differentiate due to impaired insulin signaling (Tseng et al., 2004, 2005), suggesting a differential role of members of the Wnt family in the regulation of brown versus white adipogenesis.

Adipocyte Heterogeneity among Different WAT Depots

The distribution of WAT not only varies considerably between species but also between individuals of the same species. In humans, variations of white fat distribution have gained considerable interest due to their association with metabolic disorders (Figure 1). There are two major, and not mutually exclusive, theories about why these different fat distributions are differentially linked to metabolic complications. The first is based on anatomy and the fact that visceral fat drains its products (free fatty acids and various adipokines) into the portal circulation where they can act preferentially on the liver to affect metabolism (Bjorntorp, 1990). The second is rooted in cell biology and is based on the concept that fat cells in different depots have different properties causing them to be linked to a greater or lesser extent to the development of metabolic disorders (Lafontan and Berlan, 2003). Indeed, expression profiling has revealed significant differences in expression of hundreds of genes between different depots of adipose tissue in both rodents (Gesta et al., 2006) and humans (Vidal, 2001; Vohl et al., 2004). These differences may not only help explain the impact of different fat depots on the development of metabolic complications but also suggest possible differences in developmental origin of these fat cells.

Differential origin of adipocytes in different WAT depots is also suggested by striking differences in adipose tissue distribution among normal individuals and especially in individuals with various forms of lipodystrophy (Agarwal and Garg, 2006). For example, in congenital generalized lipodystrophy (Berardinelli-Seip Syndrome),

adipose tissue is almost completely absent from subcutaneous depots, intra-abdominal depots, intrathoracic regions, and bone marrow. However, these individuals still have a relatively normal amount of adipose tissue in the retro-orbital area, buccal region, palms and soles, and other areas. In most families, this syndrome is due to mutations in *seipin*, a gene that encodes a 44 kDa protein of unknown function. By contrast, familial partial lipodystrophy of the Dunnigan type is due to mutations in the Lamin A/C gene and is characterized by a marked loss of subcutaneous adipose tissue in the extremities and trunk but no loss of visceral, neck, or facial adipose tissue. Some lipodystrophies appear to have a segmental or dermatomal distribution.

The notion that various WAT depots may be derived from distinct precursors is supported by a number of lines of evidence. First, different WAT depots have variations in chronology of appearance. In rodents, WAT develops mainly after birth, being present first in the perigonadal and subcutaneous depots, and only later in the omental depot. In humans, WAT development begins early in the second trimester of gestation and by birth is well developed in both the visceral and subcutaneous depots. Second, as noted above, visceral fat and subcutaneous fat exhibit substantial differences in patterns of gene expression. Similarly, variations in gene expression have been observed in preadipocyte fractions from different adipose depots (Gesta et al., 2006). Likewise, administration of monoclonal antibodies raised against adipocyte plasma membranes to chick embryos significantly reduces the weight of abdominal adipose tissue without affecting femoral or pectoral fat depots (Wu et al., 2000), suggesting that these depots express different membrane protein antigens than those of other depots. Third, these depot-specific variations in gene expression and adipose tissue function appear to be intrinsic (Hauner and Entenmann, 1991). Thus, isolated cloned human preadipocytes from subcutaneous adipose tissue exhibit a greater ability to differentiate in culture than those from intra-abdominal adipose. These differences are conserved over multiple cell generations and are associated with different patterns of gene expression, suggesting that these adipose depots could result in part from different precursor cells (Tchkonia et al., 2006).

Some studies have also suggested that preadipocytes of obese humans replicate faster in culture than those of lean individuals and secrete more basic FGF (Lau et al., 1987); however, this has not been observed in all studies (Permana et al., 2004). All of these studies are complicated by the lack of distinct markers of the preadipocyte population and the difficulty defining precisely where in the commitment and differentiation process a given cell lies. Extrinsic factors may also differentially affect adipocyte development in different depots. For example, transgenic mice overexpressing 11-β hydroxysteroid dehydrogenase in fat have the same level of enzyme in various fat depots but develop only visceral obesity (Masuzaki et al., 2001).

Developmental and Patterning Genes in WAT Depots

The notion that developmental and patterning genes might play a role in the differential development of various adipose tissue depots has been suggested by several recent studies (Vohl et al., 2004; Cantile et al., 2003; Gesta et al., 2006; Tchkonia et al., 2007). Intra-abdominal adipocytes express higher levels of *HoxA5*, *HoxA4*, *HoxC8*, *Glypican 4* (*Gpc4*), and *Nr2f1* (*nuclear receptor subfamily 2 group F member 1* or *Coup-TF1*), whereas subcutaneous fat has higher levels of *HoxA10*, *HoxC9*, *Twist1*, *Tbx15*, *Shox2* (*Short stature homeobox 2*), *En1 (Engrailed 1)*, and *Sfpr2*, and in most cases, these differences are observed in both rodents and humans (Figure 4). Similar differences in development gene expression are observed in preadipocytes isolated from different adipose depots of rodents (Gesta et al., 2006) and humans (Tchkonia et al., 2007), suggesting that different regions of mesoderm might give rise to precursors in different adipose depots. These differences in gene expression are large in magnitude (up to 1000-fold), appear to be intrinsic, and persist during in vitro culture and differentiation, indicating that they are cell autonomous and independent of the tissue microenvironment. In addition, Gesta et al. (2006) have shown that three of these developmental genes (*Tbx15*, *Glyp4*, and *HoxA5*) exhibit changes in expression that closely correlate with the extent of obesity (BMI) and the pattern of fat distribution (WHR).

T-box15 (Tbx15) is a transcription factor from the phylogenetically conserved family of T-box genes. To date, 18 different mammalian T-box genes have been identified, many of which have orthologs in lower organisms. These T-box transcription factors share a characteristic sequence similarity within the DNA-binding domain (T-domain). The first T-box gene characterized was *Brachyury (T)*, a gene known to play a major role in mesoderm development in vertebrates. In rodents, Tbx15 shows a dorsoventral patterning that mirrors the distribution of the agouti protein (Candille et al., 2004), and loss of function of Tbx15 in mice results in abnormal skeletal development. In humans, Tbx15 is higher in visceral than in subcutaneous fat and correlates negatively with BMI and WHR in visceral adipose tissue and positively in subcutaneous adipose tissue (Gesta et al., 2006).

Glypican 4 (Gpc4) is a cell-surface heparan sulfate proteoglycan linked to the plasma membrane by a glycosylphosphatidylinositol linkage. Glypicans can modify the activity of a number of morphogens and growth factors involved in mesodermal development, such as BMPs, Wnts, Hhs, and FGFs. In humans, visceral adipose tissue Gpc4 expression correlates positively with both BMI and WHR, whereas in the subcutaneous adipose tissue, the inverse is true (Gesta et al., 2006). In humans with the Simpson-Golabi-Behmel syndrome, part of the X chromosome carrying glypican 3 and 4 is deleted. These infants display macroglossia, macroso-

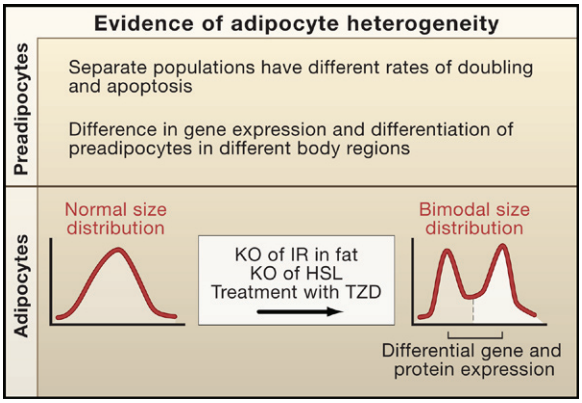


Figure 5. Evidence for Heterogeneity of Preadipocytes and Adipocytes

Evidence for two distinct subtypes of preadipocytes in human subcutaneous fat can be found in differences in replication rates, differentiation capacities, and susceptibilities to TNF-α-induced apoptosis (Tchkonia et al., 2005). Preadipocytes isolated from different regions of the body show differential patterns of gene expression and capability in differentiation (Gesta et al., 2006). The presence of heterogeneity of adipocytes is also suggested by a bimodal size distribution in cells isolated from mice with fat-specific knockout (KO) of the insulin receptor (IR) (Bluher et al., 2002) or hormone-sensitive lipase (HSL) (Fortier et al., 2005), from Zucker rats treated with thiazolidinediones (TZD) (de Souza et al., 2001), and by differences in gene and protein expression between large and small adipocytes.

mia, and renal and skeletal abnormalities. Interestingly, fibroblasts from patients with the Simpson-Golabi-Behmel syndrome show an increased tendency to differentiate into adipocytes in culture (Wabitsch et al., 2001).

Hox genes encode conserved transcription factors expressed along the anteroposterior axis of vertebrates and serve as determinants of embryonic cell fate. These genes share a 180 bp homeobox that encodes a 60 amino acid DNA-binding homeodomain and are organized in 4 clusters (HoxA, HoxB, HoxC, and HoxD). Disruption of HoxA5 gene in mice causes early postnatal lethality due to respiratory tract defects. However, surviving animals present diverse skeletal and gastrointestinal abnormalities (Aubin et al., 2002). HoxA5 expression positively correlates with BMI and WHR in both visceral and subcutaneous human WAT (Gesta et al., 2006). Thus, HoxA5, Gpc4, and Tbx15 expression vary with fat distribution and expression of the latter two is an excellent marker for visceral fat accumulation.

Potential Heterogeneity within a Single Fat depot

In addition to differences between fat cells in adipose tissue depots, recent work has suggested that there may be heterogeneity of adipocytes and preadipocytes within the same depots (Figure 5). Mice with fat-specific knockout of the insulin receptor (FIRKO) or hormone-sensitive lipase (HSL) exhibit a heterogeneous phenotype in adipocytes, with two populations of adipocytes—one with a small diameter (<50 μm) and the other having a large diameter (>150 μm)—rather than a normal distribution of size (Bluher et al., 2002; Fortier et al., 2005). Characterization of the smaller and larger cells from both FIRKO and normal mice (Bluher et al., 2004) and from normal humans (Jernas et al., 2006) reveals differences in expression of several genes and proteins, supporting the hypothesis that a single fat pad may contain at least two populations. Furthermore, in mice, the response of adipocytes in a single depot to a treatment with thiazolidinedione, an antidiabetic drug and ligand of PPARγ, suggests the presence of at least two populations of different diameters (de Souza et al., 2001). More recently, Tchkonia et al. (2005) have produced more direct evidence of developmental heterogeneity by studying preadipocytes cloned from a single human fat depot. They found two distinct types of clones—one exhibiting higher expression of adipogenic transcription factors and better ability to replicate and differentiate but less sensitive to TNF-α induced apoptosis than the other type. The proportion of these preadipocyte subtypes differed between the different depots studied. Thus, analogous to the situation with white blood cells in various organs, different depots of adipose tissue may contain variable ratios of different types of white adipocytes resulting from separate developmental lineages. This could play an important mechanistic role linking different forms of obesity to metabolic diseases and also opens new possibilities for finding therapeutic targets or pathways to treat obesity and obesity-associated complications.

Specifying Brown versus White Adipose Lineages

The developmental patterns of BAT and WAT are also distinct. BAT emerges earlier than WAT during fetal development. It is at its maximal size relative to body weight at birth, when the requirements for nonshivering thermogenesis are needed, then involutes in both humans and rodents with aging (Cannon and Nedergaard, 2004). Development of WAT, on the other hand, begins in midgestation (in humans) or shortly after birth (in rodents) and gradually increases throughout life. Morphologically, BAT can be distinguished from WAT by multilocular lipid inclusions, rich vascularization, and abundant mitochondrial density.

Many genes are differentially expressed in mature brown and white adipocytes (Figure 4). With the exception of UCP-1, which is generally accepted as the defining marker of brown fat, most other differentially expressed genes show only relative differences between the two types of adipose cells. These include the cell death-inducing DFF45-like effector A (Cidea), which is highly expressed in BAT, where it interacts with and suppresses UCP-1 activity; type 2 iodothyronine deiodinase; the transmembrane glycoprotein Cig30; the fatty-acid-activated transcription factor PPARα; the nuclear coactivator PGC-1α; and factors involved in mitochondrial biogenesis and function. All of these genes are preferentially expressed in BAT, whereas leptin, the nuclear corepressor RIP140, and the matrix protein fibrillin-1 are more highly expressed

in WAT than BAT. Among the developmental genes, homeobox genes HoxA1 and HoxC4 are preferentially expressed in human fetal BAT, whereas HoxA4 and HoxC8 are more abundant in human WAT (Figure 4; Table S1).

At the cellular level, both BAT and WAT appear to originate from mesodermal/mesenchymal stem cells (Figure 4). One important and unsolved question in adipocyte biology relates to defining exactly how and when the differentiation of BAT versus WAT is specified. Most data indicate that white and brown preadipocytes are already committed to their unique differentiation fates. Thus, stromovascular fractions containing preadipocytes isolated from BAT differentiate into UCP-1-expressing cells, whereas similar isolates from WAT differentiate into fat without UCP-1 (Kopecky et al., 1990). In addition, lineage tracking studies using the *UCP-1* promoter have demonstrated that white adipocytes are distinct from brown adipocytes during normal development (Moulin et al., 2001). Indeed, brown preadipocytes have been reported to possess a "myogenic" signature, providing additional evidence for distinct origins of BAT and WAT and a potential link to the energy-burning function of BAT (Timmons et al., 2007). However, in rodents chronically exposed to cold, many white fat depots develop large numbers of brown fat cells (Cinti, 2005), and low levels of UCP-1 can be found in SVF isolated from WAT during in vitro differentiation (Klaus et al., 1995). Whether this represents transdifferentiation of WAT adipocytes or preadipocytes to BAT or simply the presence of brown preadipocytes in many WAT depots is unknown, but the stable nature of white and brown preadipocyte cell lines in culture suggests the latter is more likely the case.

Factors Involved in Brown versus White Lineage Determination

Preadipocytes need to be released from suppression to become committed to terminal differentiation. Among the known inhibitors of this early adipogenic event, several have differential effects on brown versus white fat differentiation, including members of the Wnt and retinoblastoma families. These include pRB, p107, and p130, which selectively suppress the differentiation of brown preadipocytes via repression of the transcription factor FOXC2 and coactivator PGC-1α (Table S2). Necdin, a protein functionally resembling pRB as a growth suppressor, is also highly expressed in brown preadipocytes (Boeuf et al., 2001), and suppression of necdin by insulin may be essential for brown preadipocytes to enter an adipogenic program (Tseng et al., 2005). Recently, a zinc finger-containing protein, PRDM16, has been found selectively expressed in brown fat cells. Forced expression of prdm16 in a white preadipocyte cell line or WAT in vivo activates a robust brown fat phenotype (Seale et al., 2007). However, the major regulators determining brown versus white preadipocyte commitment remain to be determined.

Similar to white adipocytes, the differentiation of brown preadipocytes to brown adipocytes is controlled by a transcriptional cascade involving the transcription factors C/EBPs and PPARγ. Interestingly, however, some of these adipogenic transcription factors appear to have differential effects on brown versus white fat differentiation. Thus, mice lacking C/EBPα in all tissues but liver display a complete absence of WAT, whereas interscapular BAT is nearly normal (Linhart et al., 2001). Conversely, mice with adipose-specific knockout of PPARγ display marked reduction in both WAT and BAT (He et al., 2003). Recently, Gray et al. (2006) reported that a dominant-negative PPARγ mutation (P465L) displays altered brown fat morphology with larger lipid droplets, leading to reduced thermogenic capacity, while WAT appears to be normal.

Factors that Control the Thermogenic Program of BAT

BAT is uniquely distinguished from WAT and other tissues by its function in thermogenic energy expenditure. A number of transcription factors and coregulators appear to play particularly important roles in the final stages of differentiation of BAT and modulation of the expression of thermogenic genes, especially UCP-1. Among these, PGC-1α appears to have a central role by enhancing the transcriptional activity of PPARγ and the thyroid hormone receptor (Puigserver et al., 1998). Indeed, ectopic expression of PGC-1α in white preadipocytes can activate expression of UCP-1 and key components of the mitochondrial respiratory chain (Tiraby et al., 2003). Brown preadipocytes lacking PGC-1α show reduced induction of thermogenic genes but differentiate normally, suggesting that PGC-1α is important for the thermogenic function of brown fat but is dispensable for induction of lipid accumulation (Uldry et al., 2006).

Several other factors modulate brown versus white differentiation via regulation of expression or activity of PGC-1α (Table S2) (Hansen and Kristiansen, 2006). FOXC2, CREB, SIRT3, SRC-1, and p/CIP, as well as intracellular messengers, such as nitric oxide and p38 MAP kinase, are known to positively regulate PGC-1α gene transcription; whereas repressors of transcription, such as pRB, necdin, p107, TIF2, SHP, and LXR, suppress expression of PGC-1α. In addition, the translational inhibitor 4E-BP1 specifically represses translation of PGC-1α mRNA, thereby inhibiting brown adipogenesis. Activity of PGC-1α can also be regulated posttranslationally. For example, p38 MAP kinase can directly phosphorylate PGC-1α protein and enhance its transcriptional activity in brown fat. Ultimately, docking of PGC-1α to transcription factors, such as PPARγ, thyroid hormone receptor, NRF-1, and PPARδ, stimulates a conformational change in PGC-1α that permits binding of other nuclear activators, such as SRC-1 and CBP/p300, leading to a great increase of transcription of target genes. Conversely, the p160 myb-binding proteins are able to suppress the activity of PGC-1α via a p38MAP kinase-dependent pathway.

There are also important PGC-1α-independent mechanisms involved in the controls of UCP-1 expression and/or mitochondrial biogenesis. For example, the nuclear corepressor RIP140 is expressed at high levels in WAT where it suppresses oxidative metabolism and mitochondrial biogenesis (Powelka et al., 2006). RIP140 knockout mice are lean and show significantly increased mitochondrial oxidative phosyphorylation and UCP-1 expression in WAT depots (Leonardsson et al., 2004). In contrast, the cell death-inducing factor, Cidea, is expressed at high levels in BAT, where it directly interacts with UCP-1 and suppresses its activity. Cidea null mice are resistant to obesity and have a higher metabolic rate in BAT and higher body temperature when exposed to cold as compared with wild-type mice (Zhou et al., 2003).

Hormonal/Environmental Control of Fat Cell Differentiation

In addition to the many hormones that are known to induce both white and brown adipocyte differentiation, brown and white adipocyte development and function are also influenced by many extrinsic factors. The extracellular matrix plays important differential roles in brown and white adipocyte differentiation. Thus, mice lacking the membrane-type 1 matrix metalloproteinase display a selective absence of WAT, but not BAT (Chun et al., 2006). In rodents, the autonomic nervous system has opposing roles in the recruitment/development of BAT and WAT. The differentiation of brown preadipocytes is strongly stimulated by adrenergic agents such as norepinephrine, whereas both proliferation and differentiation of white preadipocytes increases following sympathetic denervation (Cousin et al., 1993). Adrenergic stimulators promote proliferation and differentiation of brown preadipocytes, protect mature brown adipocytes from apoptosis, and enhance the thermogenic capacity of BAT via induction of UCP-1 gene expression (Cannon and Nedergaard, 2004). Cold exposure and feeding increase BAT activity and UCP-1 expression via norepinephrine released from the sympathetic nervous system. Other stimuli can induce UCP-1 expression, including thyroid hormone, insulin, thiazolidinediones, retinoic acid, cAMP analogs, and β-adrenergic agonists (Diehl and Hoek, 1999). Bile acids can increase whole-body energy expenditure by promoting intracellular thyroid hormone activation in BAT and muscle (Watanabe et al., 2006). Conversely, glucocorticoids inhibit *UCP-1* gene expression in response to adrenergic stimulation.

Plasticity and Regenerative Capacity of Adipose Tissue

As in all tissues, cells in adipose tissue have a natural life cycle. Although the precise rate of turnover of fat cells is not know, recent studies have demonstrated that white fat pads may contain dead or dying adipocytes that are often surrounded by macrophages that phagocytose the lipid droplets and ultimately form multinucleate giant cells (Cinti et al., 2005). Adipocyte necrosis increases in obese humans and mice up to 30-fold. Apoptosis of adipocytes and preadipocytes can also be increased by treatment of cells with cytokines such as TNFα. Genetically engineered mice in which caspase 8 activation in fat is made drug inducible exhibit massive apoptosis of adipocytes (Pajvani et al., 2005). Interestingly, within weeks after cessation of the drug treatment, there is a remarkable regrowth of fat to nearly normal levels, indicating the potential for preadipocytes to regenerate new adipocytes.

Plasticity of BAT and WAT remains a controversial issue. In both rodents and humans, BAT depots are replaced by WAT during aging. Conversely, brown adipocytes are observed in classical white fat depots, and their number increases dramatically during cold adaptation or after hormonal and/or pharmacological treatments, such as β3 adrenoceptor agonist, retinoic acid, thiazolidinediones, and leptin administration (Diehl and Hoek, 1999). Although these findings may represent transformation of white adipose to brown and vice versa, based on the data reviewed above, it seems more likely that the UCP-1-expressing brown adipocytes observed in WAT under these conditions come from recruitment and differentiation of mesenchymal progenitor cells or brown preadipocytes within the WAT. Indeed, the stromovascular fraction is a heterogeneous cell population and appears to contain pluripotent cells capable of differentiating into multiple cell lineages, including cells of mesodermal lineage (adipocytes, osteoblasts, chondrocytes, myocytes), cells of endodermal lineage (endothelial cells and hepatocytes) (Fraser et al., 2006), and cells of neuroectodermal lineage (neuronal cells and insulin-producing cells) (Timper et al., 2006). In rodents, stromovascular fractions derived from subcutaneous inguinal WAT exhibit a greater potential to differentiate into multiple lineages than those derived from intra-abdominal fat (Prunet-Marcassus et al., 2005).

Perspective

The adipose organ is complex, with multiple compartments composed of cells with different functions that most likely arise from different developmental lineages. Although the field of adipocyte biology has made great progress, we still lack markers capable of distinguishing MSC from preadipocytes that can define various stages in the differentiation process or that can identify preadipocytes with different developmental potentials. Although it is likely that brown and white preadipocytes are already determined in their lineage, controlling the numbers of each of these cell populations and their depot-specific differentiation could provide new treatments for obesity and its complications. With this approach, we could truly track the current epidemic of obesity to its developmental origins.

Supplemental Data

Supplemental Data include two tables and Supplemental References and can be found with this article online at http://www.cell.com/cgi/content/full/131/2/242/DC1/.

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