Adipogenesis

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SUMMARY

Adipose tissue is an important site for lipid storage, energy homeostasis, and whole-body insulin sensitivity. It is important to understand the mechanisms involved in adipose tissue development and function, which can be regulated by the endocrine actions of various peptide and steroid hormones. Recent studies have revealed that white and brown adipocytes can be derived from distinct precursor cells. This review will focus on transcriptional control of adipogenesis and its regulation by several endocrine hormones. The general functions and cellular origins of adipose tissue and how the modulation of adipocyte development pertains to metabolic disease states will also be considered.

Outline

- 1 Introduction
- 2 WAT versus BAT
- 3 Negative effectors of adipogenesis
- 4 Endocrine control of adipogenesis
- 5 Adipogenesis and metabolic disease states
- 6 Concluding remarks

References

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1 INTRODUCTION

1.1 Adipose Tissue: A Dynamic Organ

Studies over the last two decades have established adipose tissue as a dynamic organ that carries out several important physiological processes. There are two distinct classifications of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT accounts for the majority of fat present in adult humans and is a critical site for energy homeostasis, insulin signaling, and endocrine action. BAT is predominantly responsible for nonshivering thermogenesis, which is mediated by uncoupling protein-1 (UCP1) in the mitochondria (Farmer 2008). Brown fat has been well established as an important fat depot in postnatal babies and in a variety of mammals. To great surprise within the scientific community, several recent studies using ¹⁸F-fluorodeoxyglucose positron emission tomography and computed tomography suggest that BAT depots are also present in the thoracic and supraclavicular regions of adult humans (Nedergaard et al. 2007; Cypess et al. 2009). Additional studies suggest that this adipose tissue may be metabolically active BAT because of the presence of UCP1, an important protein used to classify BAT (Zingaretti et al. 2009). Recent studies revealed surprising evidence suggesting that brown adipocytes are detectable in subcutaneous WAT in mice (Barbatelli et al. 2010). These observations have increased interest in the potential use of brown adipocytes in correcting WAT pathologies.

Adipose tissue comprises a variety of cell types, including endothelial cells, blood cells, fibroblasts, pericytes, preadipocytes, macrophages, and other immune cells (Geloen et al. 1989). However, the predominant cells present in adipose tissue are mature adipocytes. Brown and white adipocytes require key transcription factors that are necessary to promote preadipocyte differentiation into mature adipocytes. As shown in Figure 1, these transcriptional regulators include critical factors like peroxisome proliferator-activated receptor γ (PPAR γ) and other transcription factor families, including CCAAT/enhancer-binding proteins (C/EBPs), signal transducers and activators of transcription (STATs), and Kruppel-like factor (KLF) proteins. The specific roles of these transcription factors will be discussed in a section on positive regulators of adipogenesis.

1.2 The Cellular Origins of Adipocytes

Our understanding of the origins of adipocytes has changed substantially in the last five years. In the recent past it was largely accepted that both brown and white adipocytes arose from resident mesenchymal progenitor cells that were present in adipose tissue. This idea was supported by evidence indicating that both brown and white adipocytes require PPAR γ for development. However, the notion that brown and white cells arise from a similar origin is outdated. For example, it has been shown that brown adipocytes present in BAT depots can arise from Myf5-expressing myogenic



Figure 1. Transcription factors that are positive and negative effectors of adipocyte development. Many transcription factors are induced during adipocyte differentiation. Some of these, like members of the AP-1 family, are induced during clonal expansion. Other transcription factors, like PPARγ, promote adipocyte differentiation. Adipocyte development is also influenced by several transcription factor families that have negative effects on adipogenesis.

precursor cells (Seale et al. 2008). The transcriptional regulator PRDM16 has been shown to control the development of brown adipocytes in classic BAT depots (Seale et al. 2007, 2008; Kajimura et al. 2008). However, brown fat-like adipocytes are found interspersed in WAT in both humans and rodents. As shown in Figure 2, the development of these cells is enhanced by β3-adrenergic stimulation, chronic activation of PPARy, or adaptation to cold response (Huttunen et al. 1981; Cousin et al. 1992; Ghorbani and Himms-Hagen 1997; Guerra et al. 1998; Himms-Hagen et al. 2000; Xue et al. 2005; Petrovic et al. 2010). These brown fat-like cells present in WAT depots have been referred to as brite cells (brown in white), beige cells, adaptive brown fat cells, and recruitable brown fat cells (Enerback 2009; Ishibashi and Seale 2010; Petrovic et al. 2010). It was originally reported that PRDM16 was present only in BAT, but not WAT (Seale et al. 2007). However, this study only examined the epididymal WAT depot. It is now known that PRDM16 is highly expressed in subcutaneous WAT, and transgenic expression of PRDM16 in all adipose tissues results in a transformation of subcutaneous WAT to a brown fat-like phenotype (Seale et al. 2011). As reviewed in Figure 2, these results suggest that brown cells in BAT and brown fat-like cells in WAT have different cellular origins, and the transcriptional modulator PRDM16 plays an important role in both of these cell types to regulate genes required for thermogenesis (Seale et al. 2011). To make the situation more complex, there is also evidence to indicate that bone marrow progenitor-derived adipocytes and adipocyte progenitors can arise from hematopoietic cells via the myeloid lineage (Majka et al. 2010). Collectively, these recent studies suggest that our understanding of the origin of adipocytes is rapidly changing, and additional studies will be necessary to clarify our understanding of this emerging area of biology.

2 WAT VERSUS BAT

2.1 Adipocyte Morphology

The morphological differences between brown and white adipocytes can be readily observed via light or electron microscope. White adipocytes are generally spherical cells with an average diameter in a young animal of less than 10 μ m. However, this size can increase to as much as 100 μ m, particularly in the epididymal fat depots (Cinti 2005). The organelles of white adipocytes are typically subjected to a confined arrangement because of the presence of a unilocular lipid droplet that occupies the majority of the cytosol. The nucleus is usually compressed between the fat and plasma membrane. There is a sparse distribution of mitochondria in white adipocytes and other cellular organelles, such as the Golgi and smooth and rough endoplasmic reticulum.

Brown adipose tissue is innervated by the sympathetic nervous system and is well vascularized. Brown adipocytes are typically ellipsoid in shape and range from $15-50 \mu m$ in size and contain multilocular lipid droplets (Cinti 2005). The nucleus of these cells is relatively centralized within the



Figure 2. White and brown adipocytes are derived from distinct precursor cells and have distinct morphological characteristics. White adipocytes can be derived from mesenchymal precursor cells. Brown adipocytes are derived from Myf5-expressing precursor cells. Mature brown adipocytes contain multilocular lipid droplets and are mito-chondria rich. PRDM16 is present in both brown and white adipocytes and is an important factor in transforming white adipocytes to beige adipocytes.

cytoplasm and all other classical organelles are present. The hallmark components of brown adipocytes are their abundant distribution of cristae-rich mitochondria and the largely exclusive expression of the proton transporter UCP1 (Cannon et al. 1982). When UCP1 is ectopically expressed in subcutaneous WAT, the population of cells characteristic of brown adipocytes is increased (Rossmeisl et al. 2002). Recent studies have indicated that UCP1 can be present in WAT (Barbatelli et al. 2010), but as previously indicated, this is likely caused by the presence of brown fat-like adipocytes in WAT.

2.2 Adipocyte Physiology

The first study to show that adipose tissue is a bona fide endocrine organ was that which characterized the satiety hormone leptin (Zhang et al. 1994). Adiponectin is another adipocyte-specific hormone (Scherer et al. 1995; Hu et al. 1996; Maeda et al. 1996) that regulates several physiological processes. The most clearly understood are its effects on glucose and lipid metabolism (Berg et al. 2001; Yamauchi et al. 2002). Adiponectin has also been shown to be cardioprotective in mice and humans (Chen et al. 2003; Shibata et al. 2004), and more recent studies indicate it can act on the brain to regulate satiety (Kubota et al. 2007). Another adipocyte-specific hormone was identified as a member of the "found in inflammatory zone" (FIZZ) family (Holcomb et al. 2000). Immunoneutralization studies suggested that this hormone, resistin, might be involved in the pathogenesis of insulin resistance (Steppan et al. 2001). In these studies insulin sensitivity was improved when antiresistin IgG was administered to rodents with obesity/type 2 diabetes mellitus. It is important to note that the distribution pattern of resistin in humans is different from that in mice. Resistin is expressed at low levels in human adipocytes (Nagaev and Smith 2001; Savage et al. 2001; Janke et al. 2002), but the majority of resistin expressed in humans is found in monocytes and macrophages that reside in adipose tissue (Savage et al. 2001; Patel et al. 2003). A highly innovative experiment sought to determine if the resistin derived from human macrophages was sufficient to confer insulin resistance. Researchers engineered a mouse model that was deficient in adipocyte-derived resistin and produced human resistin from a macrophage-specific promoter. These mice are referred to as humanized resistin mice and exhibit a phenotype of insulin resistance and WAT inflammation (Qatanani et al. 2009). These studies indicate that resistin has similar functions in mice and humans despite the differences in expression.

WAT is a key organ that carries out insulin-stimulated glucose uptake, an important physiological process that is mediated by a signal pathway in which insulin activates a cascade that results in glucose transporter-4 (GLUT4) translocation to the plasma membrane to facilitate an increase in the influx of glucose. The details of this mechanism have been well studied worldwide by numerous groups. Brown adipocytes do not take up significant amounts of glucose on insulin stimulation. However, they are responsive to the sympathetic nervous system, and norepinephrine can stimulate the uptake of glucose independent of insulin (Shimizu et al. 1996).

Brown and white adipocytes store and metabolize lipids in different manners. White adipocytes accumulate nutrient-derived triglycerides and release them by lipolysis during periods of fasting, whereas brown adipocytes oxidize their lipid stores in an elegant heat-producing pathway in which the primary player is UCP1. UCP1 is highly expressed in brown adipocytes and is the central mediator in the ability of BAT to produce adaptive heat. The importance of UCP1 was shown in knockout mice, whereby UCP1-deficient mice were unable to survive long-term exposure to cold (Golozoubova et al. 2001). UCP1 is localized at the mitochondrial inner membrane, where it reduces the proton gradient across the membrane by facilitating the flow of protons from the intermembrane space of the mitochondria back into the matrix, thus releasing heat rather than producing ATP. Subthermoneutral temperatures trigger this pathway and stimulate the sympathetic nervous system to release norepinephrine. Norepinephrine acts via β -adrenergic receptors to play a role in the activation of thermogenesis. The physiological importance of the β -adrenergic receptors was shown in mice deficient of all three β-adrenergic receptors. These mice had reduced metabolic rates and susceptibility to severe obesity upon high-fat feeding (Bachman et al. 2002).

2.3 Model Systems of Adipogenesis

A variety of cellular model systems are used to study the molecular pathways of adipogenesis and adipocyte function in vitro. These models can largely be placed in two classes. The first group includes pluripotent fibroblasts that have the ability to differentiate into several cell types, including myocytes, chondrocytes, and adipocytes. This class includes the 10T1/2, BALB/c-3T3, RCJ3.1, and CHEF/18 fibroblast cell lines. The second group of model systems comprises fibroblastlike preadipocytes that are committed to differentiating into adipocytes and includes 3T3-L1, 3T3-F422A, 1246, Ob1771, TA1, and 30A5 preadipocytes. This latter class represents the cells used most frequently to study adipogenesis.

10T1/2 fibroblasts, established in 1973 from 14- to 17-day-old C3H mouse embryos (Reznikoff et al. 1973), can generate committed preadipocytes, premyocytes, or prechondrocytes when treated with 5-azacytidine, an inhibitor of DNA methylation (Taylor and Jones 1979). Bone morphogenetic protein-4 (BMP4) is capable of inducing 10T1/2 cells to convert into committed preadipocytes that can develop into mature fat cells when provided with differentiation media (Tang et al. 2004). Ectopic expression of important proadipogenic transcription factors, including PPAR γ (Brun et al. 1996), members of the C/EBP family (Freytag et al. 1994; Yeh et al. 1995; Wu et al. 1996), and STAT5 (Floyd and Stephens 2003), can also confer adipocyte differentiation in these cells.

3T3-F442A and 3T3-L1 are the two most extensively characterized and used preadipocyte cell lines. Both of these lines were derived from disaggregated 17- to 19day-old Swiss 3T3 mouse embryos (Green and Meuth 1974; Green and Kehinde 1976). However, unlike 3T3-L1 cells, 3T3-F442A preadipocytes do not require glucocorticoid-supplemented differentiation cocktail to induce adipogenesis (Rubin et al. 1978; Spiegelman and Ginty 1983; Moustaid et al. 1990). It is commonly believed that 3T3-F442A precursors are at a more advanced stage of commitment than 3T3-L1's. Implantation of 3T3-F44A, but not 3T3-L1, preadipocytes into athymic mice results in the generation of ectopic fat that is histologically (Green and Kehinde 1979) and biochemically (Mandrup et al. 1997) indistinguishable from adipose tissue. The 3T3-F442A and 3T3-L1 cell lines are preferred because they develop a homogeneous population of mature adipocytes that are morphologically (Novikoff et al. 1980) and biochemically (MacDougald and Lane 1995) similar to adipocytes in situ.

2.4 Positive Effectors of Adipogenesis

The development of fully differentiated mature adipocytes from precursor cells is an elegant progression of the sequential activation of a battery of transcription factors. In white adipocytes this sequence commences with the activation of members of the activating protein-1 (AP-1) family of transcription factors, and continues with the induction and expression of PPAR γ , a critical proadipogenic transcription factor (see Fig. 1). Other transcription factors facilitate adipocyte maturation, including STATs, members of the KLF family of proteins, sterol response element– binding protein-1 (SREBP-1), and members of the C/EBP family. There are also potent negative repressors of adipocyte differentiation, including preadipocyte factor-1 (Pref-1) and members of the GATA and Wnt families.

2.4.1 AP-1

AP-1 is a large group of transcription factors that include v-Jun, c-Jun, JunB, JunD, v-Fos, c-Fos, FosB, Fra1, Fra2, and activating transcription factors (ATF2, ATF3/LRF1, and

B-ATF). On activation the AP-1 proteins form homodimers or heterodimers and interact with DNA to regulate genes involved in differentiation and proliferation of various cell types. In addition, AP-1 can be a part of cellular proapoptotic machinery (Colotta et al. 1992; Johnson et al. 1996). In regards to adipocyte development, in vitro studies performed with 3T3-L1 and 3T3-F442A preadipocytes have found the induction of mRNA expression of c-Fos, c-Jun, Fos-B, and Fra1 at the onset of adipocyte differentiation. AP-1 modulates the expression of aP2, particularly via interaction between c-Fos and the FSE2 regulatory sequence of the aP2 promoter (Distel et al. 1987). Additionally, mice that have fat-specific expression of A-ZIP/F, a dominantnegative protein that hinders bZIP-DNA interaction and disrupts C/EBP and Jun transcriptional activity, show impaired WAT development and have significantly less BAT (Moitra et al. 1998).

2.4.2 KLFs

This family of transcription factors has strong homology, particularly at the zinc finger–DNA interaction domains, with the *Drosophila* embryonic pattern regulator protein Kruppel (Schuh et al. 1986). To date there are 17 members of the C2H2 zinc finger Kruppel-like family of transcription factors, and they are implicated in development, cellular differentiation, and proliferation (reviewed in Dang et al. 2000). Members of this family can assume opposing transcriptional roles, some being activators of gene transcription while others act as repressors of transcription. KLFs 4, 5, 6, and 15 have been shown to be positive effectors of adipogenesis.

KLF5 expression is induced during the early stages of adipocyte differentiation and has been shown to be necessary for adipocyte differentiation in vitro and in vivo (Oishi et al. 2005). KLF5 small interfering RNA (siRNA) expression in 3T3-L1 preadipocytes results in impaired adipocyte differentiation and a substantial decrease in lipid accumulation. Also, $KLF5^{+/-}$ heterozygote mice have a reduction in fat pad size (Oishi et al. 2005). KLF15 is also essential for adipogenesis (Mori et al. 2005). KLF15 is highly induced during the differentiation of 3T3-L1 cells, and dominantnegative mutations and RNA interference knockdowns result in attenuated adipocyte differentiation (Mori et al. 2005). These studies also showed that ectopic expression of KLF15 can confer adipogenesis by inducing activation of PPAR γ in NIH-3T3 cells. KLF15 plays another key role in adipocyte physiology, as studies have shown an interaction of KLF15 with the GLUT4 promoter, indicating a link between adipocyte insulin sensitivity and KLF15 (Gray et al. 2002). KLF4 is also highly expressed in white adipose tissue, but it is expressed at much lower levels in vitro in mature

3T3-L1 adipocytes. Further analysis revealed that the enrichment of KLF4 in adipose tissue can be attributed to its high expression in preadipocytes of the stromal vascular fraction of WAT (Soukas et al. 2001). These findings led to the hypothesis that KLF4 may also be a key regulator of adipogenesis. KLF4 mRNA is induced early in 3T3-L1 differentiation, and siRNA knockdown results in marked decreases in lipid accumulation (Birsoy et al. 2008). KLF6 has also been identified as a positive effector of adipogenesis. KLF6 is induced during 3T3-L1 adipogenesis (Inuzuka et al. 1999), and studies suggest that KLF6 promotes adipocyte differentiation by repressing delta-like protein-1 (DLK-1) gene expression in 3T3-L1 preadipocytes (Li et al. 2005). Taken together, these studies show that KLF4, KLF5, KLF6, and KLF15 are transcription factors that promote adipocyte development.

2.4.3 C/EBPs

C/EBPs comprise another family of basic leucine zipper transcription factors. There are six C/EBP isoforms, all of which possess a highly conserved bZIP domain that serves as a point of interaction for homodimerization or heterodimerization with other family members (Vinson et al. 1989). The most common nomenclature for the members is C/EBPs α , β , δ , γ , ε , and ζ . In vivo and in vitro experiments have shown that C/EBPs α , β , and δ promote adipocyte differentiation. McKnight and colleagues presented the first set of data to show the orchestrated induction of these family members during adipogenesis. C/EBPB is primarily induced by methylisobutylxanthine, and C/EBPô is highly induced by dexamethasone in the very early stages of adipogenesis. C/EBPB and C/EBP8 work in concert to trans-activate C/EBPa (Cao et al. 1991). Loss-of-function experiments in 3T3-L1 and NIH-3T3 cell lines confirmed the importance of C/EBP β and - δ , as inhibition of either of these C/EBPs resulted in the attenuation of adipogenesis (Yeh et al. 1995). Interestingly, ectopic expression studies with C/EBP α showed that it was sufficient to induce adipogenesis in precursor cell lines that are susceptible to adipogenesis, but also in nonprecursor fibroblasts (Freytag et al. 1994; Yeh et al. 1995). In vivo studies indicate that C/EBPβand/or $-\delta$ -deficient mice show defective adipose tissue development (Tanaka et al. 1997). C/EBPa-deficient mice have a phenotype of substantially low lipid accumulation (Wang et al. 1995; Flodby et al. 1996).

2.4.4 SREBP-1

This basic helix-loop-helix regulatory molecule was originally termed ADD-1, the adipocyte determination and differentiation factor-1 (Tontonoz et al. 1993). ADD-1 was identified in a rat adipocyte cDNA expression library screen as a factor that binds to an E-box domain, a DNA recognition site for basic helix-loop-helix proteins. Northern blot analysis showed a substantial increase in ADD-1 mRNA expression during adipocyte differentiation in three well-established models of adipocyte development: 3T3-F442A, 3T3-L1, and 10T1/2 cells. The human homolog of the same protein can bind the sterol response element in the promoter region of the low-density lipoprotein receptor, thus labeling it sterol response element-binding protein-1 (SREBP-1) (Yokoyama et al. 1993). To date there are three isoforms of SREBP: SREBP-1a, SREBP-1c, and SREBP-2. SREBP-1a and SREBP-1c originate from the same gene through utilization of different promoters (Hua et al. 1995). SREBP-1a is the more potent transcription factor because of a longer NH3-terminal DNA-binding domain (Shimano et al. 1997a). SREBP-1c is the predominantly expressed isoform in white adipose tissue (Shimomura et al. 1997) and a chief regulator of lipogenic gene transcription. SREBP-2 is transcribed from an alternate gene and primarily regulates the expression of genes involved in cholesterol biosynthesis (Horton et al. 1998).

In vitro evidence suggested that ADD-1/SREBP-1 is a critical factor in adipocyte development. Ectopic expression of ADD-1/SREBP-1 in NIH-3T3 fibroblasts in the presence of hormonal induction cocktail resulted in differentiation that was accompanied by increased expression of adipocyte-specific genes and lipid accumulation (Kim and Spiegelman 1996). Ectopic expression of a dominant-negative ADD-1 that contained a point mutation within the DNA-binding domain caused profound inhibition of differentiation and repression of adipocyte marker genes (87).

In vivo studies exploring the involvement of SREBP-1 in adipogenesis are less persuasive. Transgenic mice with adipose tissue–specific knockout of SREBP-1 had minimal inhibition of adipose tissue development, with no overt effects on the expression of adipocyte marker genes (Shimano et al. 1997b). Ectopic expression of a constitutively active SREBP-1 c surprisingly conferred lipodystrophy in mice (Shimomura et al. 1998). Other in vivo studies with a double-mutant leptin-deficient ob/ob mice and SREBP-1^{-/-} mice suggested that SREBP-1 was not needed for adipose tissue expansion (Yahagi et al. 2002). Overall, the data suggest that although there is in vitro evidence to show SREBP-1 is required for adipocyte differentiation, the in vivo studies consistently show that SREBP-1 expression is not critical for adipose tissue development and/or expansion.

2.4.5 STATs

There are seven STAT proteins, designated STATs 1, 2, 3, 4, 5A, 5B, and 6, which have unique tissue distributions and

regulate the expression of tissue-specific genes (Darnell 1997). STAT expression and modulation of gene expression can be cell specific, and transgenic knockout studies have shown critical roles for every member of the STAT family (Darnell 1997). The STATs are primarily activated by cytokines and hormones. Ligand binding initiates a cascade that results in STAT tyrosine phosphorylation, dimerization, and translocation to the nucleus, where STATs modulate transcription (Schindler and Darnell 1995; Darnell 1997). Several groups have established that STATs 1, 3, 5A, 5B, and 6 are expressed in adipocytes. STATs 1, 5A, and 5B are substantially induced during 3T3-L1 adipocyte differentiation (Stephens et al. 1996). Similar induction patterns of STATs 5A and 5B occur in human subcutaneous preadipocytes (Harp et al. 2001). The studies in mouse and human cells revealed similar induction of STATs 3, 5A, and 5B, but there was a difference in STAT1 induction in mouse and human cells. However, it is unlikely that STAT1 plays a critical role in adipocyte development, because STAT1knockout mice do not have any apparent body weight abnormalities (Meraz et al. 1996).

Although the relevance for the differential expression of STAT1 during the adipogenic program remains unclear, there are several studies that demonstrate a role for STATs 3, 5A, and 5B in human and murine adipogenesis. STAT3 expression increases during the proliferative phase of 3T3-L1 adipogenesis (Deng et al. 2000), and its expression is tightly regulated by protein inhibitor of activated STAT3 (PIAS3) (Deng et al. 2006). Inhibition of adipogenesis has been observed with both AG490, a Janus kinase 2 (JAK2) inhibitor, and STAT3 siRNAs (Zhang et al. 2011). Additionally, ectopic expression of a dominant-negative STAT3 suppresses adipocyte differentiation (Wang et al. 2010). Mice lacking STAT3 in adipose tissue were generated using the aP2 promoter and had higher body weights and increased adipocyte size compared with wild-type littermates (Cernkovich et al. 2008). Taken together, these studies suggest a possible role for STAT3 in adipogenesis and body weight homeostasis. However, additional studies are necessary to further clarify the contribution of STAT3 in adipocyte development and physiology.

The involvement of STAT5 in adipogenesis has been widely studied. Ectopic expression of C/EBP β and - δ in nonprecursor cells has been shown to induce adipogenesis (Wu et al. 1996) in a manner that promotes an increase in STAT5A and STAT5B expression (Stephens et al. 1999). PPAR γ has also been shown to up-regulate the expression of both STAT5 proteins during adipogenesis (Stewart et al. 1999). In 3T3-F442A preadipocytes, the modulating effect of growth hormone (GH) on adipogenesis is attenuated by STAT5 antisense oligonucleotides (Yarwood et al. 1999), and constitutively active STAT5 can drive adipogenesis in pression studies and transgenic knockout experiments have confirmed the physiological relevance of STAT5 proteins in adipogenesis. Ectopic expression of STAT5A induces adipogenesis in 3T3-L1 preadipocytes (Nanbu-Wakao et al. 2002) and in two nonprecursor fibroblast cell lines: BALB/c and NIH-3T3 cells (Floyd and Stephens 2003). Of note, STAT5B was unable to display similar proadipogenic properties in these nonprecursor cells (Floyd and Stephens 2003). Studies have shown that the GH-activated STAT5 proteins can induce PPARy expression in 3T3-L1 cells and C3H10T1/2 cells (Kawai et al. 2007), suggesting a mechanism by which STAT5 proteins are able to promote adipocyte differentiation. Interestingly, transgenic knockout experiments have shown that disruption in either STA-T5A or STAT5B or both genes resulted in abnormal adipose tissue, and mice lacking both STAT5 proteins had fat pads one-fifth the normal size (Teglund et al. 1998). To date there are no studies on tissue-specific knockout of STAT5 genes in adipocytes, and the phenotype of the STAT5-null mice could be attributed to developmental effects of STAT5 that are independent of direct effects on preadipocyte differentiation. However, recent observations have shown that ectopic STAT5A expression can confer the adipogenic capabilities of Swiss 3T3 fibroblasts in athymic mice (Stewart et al. 2011). In addition to showing a direct role of STAT5A in preadipocyte differentiation in vivo, these studies also show the usefulness of athymic mice in studying the role of transcription factors in adipose tissue development. In summary, the importance of the STAT5 proteins in adipogenesis has been shown in vitro and in the whole animal.

this model system (Shang and Waters 2003). Ectopic ex-

2.4.6 PPARy

There are many proadipogenic transcription factors that have been characterized, but none are as critical as PPARy, the master regulator of adipocyte differentiation and gene expression. Of note, the majority of identified repressors and activators of adipogenesis have been shown to modulate PPARy expression and/or activity. Studies using in vitro models of adipogenesis have consistently shown that PPARy mRNA is induced by several transcription factors, including C/EBPβ, C/EBPδ, EBF1, and KLF5. Repressors of adipogenesis such as GATA2, KLF2, and CHOP have been shown to attenuate PPARy expression (reviewed in Rosen et al. 2009). PPAR γ is a member of a superfamily of hormone nuclear receptors and has been shown to be crucial and sufficient for adipocyte development in vitro and in vivo (Tontonoz et al. 1994; Hu et al. 1995; Rosen et al. 1999). In 1995 the antidiabetic thiazolidinedione drugs were shown to be high-affinity PPARy ligands (Lehmann et al. 1995). This study was the first to indicate that PPAR γ

was a target for this antidiabetic class of drugs. PPAR γ proteins are expressed in two forms, PPARy1 and PPARy2, which are produced by a combination of differential promoter usage and alternative splicing (Fajas et al. 1997). PPARy1 is expressed at low levels in multiple tissues, whereas PPAR γ 2 is highly expressed in fat cells and differs from PPARy1 by an amino-terminal extension of 30 amino acids (Tontonoz et al. 1994). A key regulatory role of PPARy in fat cell differentiation was observed by gain-offunction experiments that revealed that ectopic expression and activation of PPARy in fibroblasts or myocytes promoted adipogenesis (Tontonoz et al. 1994; Hu et al. 1995). Recent studies have shown that PPAR γ 2, but not PPAR γ 1, profoundly affects adipogenesis (Ren et al. 2002) and that increases of only PPARy2 were observed in adipocytes of morbidly obese individuals (Sewter et al. 2002). Although these studies suggest differential roles of the PPARy isoforms, a study in PPARy-null cells showed that both PPARy1 and PPARy2 can stimulate robust adipogenesis (Mueller et al. 2003). However, PPARy2 shows an enhanced ability to confer adipogenesis because of its increased sensitivity to ligands and increased capacity to bind components of the DRIP/TRAP complex (Mueller et al. 2003).

In vivo studies have shown that PPAR γ deletion in mice results in placental dysfunction and embryonic lethality (Barak et al. 1999). To overcome this developmental limitation, fat-specific knockout mice and mice in which placental dysfunction was averted were engineered to study the proadipogenic capabilities of PPARy. The use of these mouse model systems has shown that PPAR γ is critical for fat cell development (Rosen et al. 1999), and mice with adipose tissue-specific loss of PPARy display decreased fat pad size and insulin resistance in adipose tissue and liver (He et al. 2003). Although PPARy is required for adipogenesis, there is evidence to suggest that this nuclear receptor may not be needed to maintain the differentiated state of the cell after adipogenesis (Liao et al. 2007). Of note, PPARy heterozygote mice show enhanced insulin sensitivity (Miles et al. 2000), suggesting the amount of protein present is highly important. The importance of PPAR γ in human adipose tissue has also been established, and subjects with mutations in the PPAR γ gene can develop severe insulin resistance and lipodystrophy (Barroso et al. 1999; Doney et al. 2004; Monajemi et al. 2007). Taken together, these studies show the requirement for PPARy in adipocyte differentiation and whole-body insulin sensitivity.

The expression and activity of PPAR γ can be modulated by various pathways, and several studies in the last decade have focused on the posttranslational regulation of this nuclear receptor. In particular, the ubiquitin-proteasome system has emerged as an important regulator of PPAR γ (Hauser et al. 2000; Dennis et al. 2001; Floyd and Stephens

2002). Both the AF-1 and ligand-binding domains (LBDs) of PPAR γ are targeted to the proteasome for degradation, but only the LBD is conjugated to ubiquitin (Kilroy et al. 2009). Of note, a fully functional ubiquitin system is required for PPARy activation (Kilroy et al. 2009). Overall, these studies indicate that the ubiquitin-proteasome pathway is an integral determinant of PPAR γ activity and that this nuclear receptor is targeted to the proteasome for degradation via ubiquitin-independent and ubiquitin-dependent mechanisms. Small ubiquitinlike modifier (SUMO), a protein structurally homologous to ubiquitin, is another important regulator of PPARy. SUMOylation can occur in the AF-1 domain at Lys-107 of PPAR₂ or Lys-77 of PPARy1 to control stability and activity (Floyd and Stephens 2004; Ohshima et al. 2004; Yamashita et al. 2004; Pascual et al. 2005). PPARy can also be SUMOylated in a ligand-dependent manner in the LBD at Lys-365 (Pascual et al. 2005). Another regulatory covalent modification of PPARy is phosphorylation at Ser-112 by mitogen-activated protein kinases (MAPKs) such as p44/p42 (ERKs 1 and 2) and c-Jun amino-terminal kinase (JNK), which results in transcriptional inactivation of PPARy (Camp and Tafuri 1997). Although it is widely accepted that phosphorylation of PPARy2 inhibits its activity, a point mutation at Ser-112 does not disrupt the ability of PPARy2 to confer adipogenesis (Rangwala et al. 2003). However, in vivo studies have shown that PPARy2 phosphorylation at this residue modulates insulin sensitivity in the setting of diet-induced obesity (Rangwala et al. 2003). Recent studies have revealed that CDK5 phosphorylates PPARy at Ser-273 to attenuate PPARy trans-activation of important adipocyte genes, including adiponectin (Choi et al. 2010). The tightly controlled regulation of PPARy by ubiquitylation, SUMOylation, and phosphorylation indicates the importance of modulating the expression and activity of this master regulator of adipogenesis.

3 NEGATIVE EFFECTORS OF ADIPOGENESIS

3.1 Wnt Signaling

The Wnt family is a group of >16 secreted glycoproteins that has pleiotropic effects on cell-fate specification, proliferation, and differentiation (reviewed in Cadigan and Nusse 1997). Wnts signal in an autocrine or paracrine manner through the Frizzled receptors, or low-density lipoprotein receptor-related protein (reviewed in Bejsovec 2000). Upon binding its Frizzled receptor, Wnt activates the canonical pathway that inhibits glycogen synthase kinase-3 (GSK3) and results in an increase of cytosolic β catenin, then translocates to the nucleus, where it binds to the T-cell factor/lymphoid-enhancing factor (TCF/LEF) transcription factors to regulate the expression of Wnt target genes (reviewed in Cadigan and Nusse 1997). Microarray analyses revealed that Wnt10b and -5a are repressed during adipogenesis (Ross et al. 2000, 2002). As shown in Figure 1, Wnt signaling inhibits fat cell differentiation by reducing the expression of PPAR γ and C/EBP α in 3T3-L1 and 3T3-F442A cells (Ross et al. 2000; Bennett et al. 2002; Moldes et al. 2003). Of note, activation of the Wnt pathway attenuates the expression of brown adipocyte marker genes UCP1 and PPARy coactivator-1 α (PGC-1 α), while the expression of other adipocyte genes is maintained (Kang et al. 2005). To further examine the effects of Wnts on adipogenesis, researchers engineered transgenic mice that express ectopic Wnt10b using the aP2 promoter. These mice had a 50% reduction in WAT and severely reduced BAT. Interestingly, this inhibition of adipose tissue development did not result in lipodystrophic diabetes, and these mice showed enhanced insulin sensitivity (Longo et al. 2004).

3.2 GATA Factors

There are six members of this zinc finger domain family of transcription factors; these are designated GATA1 to -6. These transcription factors bind to specific (A/T)GA-TA(A/G) DNA consensus sequences to regulate cellular differentiation and proliferation (Weiss and Orkin 1995). Of the six factors, only GATA2 and -3 are expressed in preadipocytes that are present in WAT but not in BAT (Tong et al. 2000). The expression of these transcription factors is repressed during adipocyte differentiation, which suggests that GATA2 and -3 exert antiadipogenic effects on preadipocytes (see Fig. 2). In support of this hypothesis, GATA2-deficient embryonic stem cells exhibit an enhanced differentiation potential and ectopic expression of GATA2 partially reduces fat cell development by directly binding to a PPARy promoter region to repress basal activity (Tong et al. 2000). GATA2 and -3 can also associate with C/EBP α and $-\beta$ to disrupt their transcriptional activity (Tong et al. 2005), suggesting that GATA can attenuate adipogenesis via multiple pathways. GATA proteins can be regulated posttranslationally by acetylation, SUMOylation, and phosphorylation (Boyes et al. 1998; Chun et al. 2003; Menghini et al. 2005). Akt phosphorylation of GATA results in cytosolic sequestering and inhibition of nuclear translocation (Menghini et al. 2005). Friend of GATA (FOG) and carboxy-terminal binding proteins (CTBPs) act together with GATA2 as coregulators of adipocyte proliferation and differentiation (Jack and Crossley 2010).

3.3 KLFs

As indicated above, KLFs 4, 5, 6, and 15 promote adipogenesis. However, other members of the KLF family can inhibit fat cell differentiation. Studies have shown that KLF2 and KLF7 are negative regulators of fat cell differentiation. KLF2 mRNA is highly expressed in preadipocytes present in adipose tissue, and its expression is decreased during adipogenesis. Ectopic expression of KLF2 in 3T3-L1 preadipocytes significantly disrupts lipid accumulation by repressing PPAR γ 2 gene expression (Banerjee et al. 2003) and partially restoring Pref-1 expression (Wu et al. 2005). The effect of KLF2 on adipocyte development is also evident in mouse embryonic fibroblasts derived from KLF2^{-/-} mouse embryos that show enhanced lipid accumulation (Wu et al. 2005). Ectopic expression of KLF7, another negative regulator of adipogenesis in human preadipocytes, significantly inhibits adipocyte development and expression of PPAR γ and C/EBP α (Kawamura et al. 2006).

3.4 Pref-1

Pref-1 was identified in a 3T3-L1 cDNA library for its enriched expression in preadipocytes (Smas and Sul 1993). DLK-1 is the human homolog of Pref-1 and was originally identified by purification from fetal circulation as fetal antigen-1 (Jensen et al. 1994). Pref-1 is a transmembrane protein that belongs to a family of EGF-repeat-containing proteins and shares structural homology with the Notch/ Delta/Serrate proteins (reviewed in Sul 2009). Pref-1 is activated by proteolytic cleavage to regulate cell-fate specification (Smas et al. 1997). This protein is abundantly expressed in preadipocytes, but levels substantially diminish during adipocyte development (Smas et al. 1998). Constitutive expression of Pref-1 reduces PPAR γ and C/EBP α expression and inhibits the differentiation of 3T3-L1 preadipocytes (Smas and Sul 1993). Pref-1-deficient mice show growth retardation and accelerated adiposity (Moon et al. 2002). Transgenic mice with aP2-mediated ectopic expression of Pref-1 in adipose tissue have severe lipoatrophy, impaired glucose tolerance, and hypertriglyceridemia (Lee et al. 2003).

4 ENDOCRINE CONTROL OF ADIPOGENESIS

4.1 Thyroid Hormone

The thyroid hormones, triiodothyronine (T3) and thyroxine (T4), are controlled by a well-studied classic negative-feedback loop. T3, the biologically active form of the hormone, has pleiotropic effects on various physiological processes and is a key regulator of metabolism and development. Thyroid hormone action is mediated through a family of nuclear thyroid hormone receptors that can repress transcription in a ligand-independent manner. An association between thyroid hormone and adipose tissue development has been established since 1888, when a study K. Sarjeant and J.M. Stephens

on myxedema suggested that obesity was a prerequisite for a diagnosis of hypothyroidism (reviewed in Mariash 2003). Rodent hyperthyroidism induces adipocyte hyperplasia, whereas hypothyroidism impedes adipose tissue development (Levacher et al. 1984).

It is well-known that T3 induces brown adipocyte differentiation. T3-supplemented medium is commonly used to stimulate differentiation of cultured brown adipocytes (Klaus et al. 1994). The effect of thyroid hormone on lipogenesis has been observed in Sprague-Dawley rats treated with T3 (Oppenheimer et al. 1991), suggesting that thyroid hormone can induce the expression of lipogenic genes. Studies investigating the involvement of thyroid hormones in adipose tissue development are controversial. To date, no mechanisms of thyroid hormone action in adipogenesis have been documented. Nonetheless, it is clear that thyroid hormones are capable of regulating adipocyte development and modulating the expression of genes required for lipogenesis.

4.2 Steroid Hormones

Steroid hormones regulate the distribution and development of fat in mammals. Hence it is not surprising that adipocytes express abundant levels of steroid hormone receptors. The actions of steroid receptors are mediated by genomic responses and events that are independent of transcription. This section will focus on the effects of estrogen (E2), androgens, and glucocorticoids on adipocyte differentiation. The receptor-mediated effects of estrogen occur via two receptors, ER- α and ER- β , to regulate metabolism and adipose tissue distribution (Wade et al. 1985; Bjorntorp 1991). Potential gene targets of E2 can be estimated by the expression and distribution of its receptors. Studies investigating the expression patterns of ER- α and ER- β showed that they are expressed in rat and human preadipocytes, mature adipocytes, and other cell types present in adipose tissue, including macrophages (Price and O'Brien 1993; Crandall et al. 1998; Dieudonne et al. 2004). Various studies have shown that E2 regulates adipocyte differentiation; however, the results are contradictory. Estrogen has been reported to induce the differentiation of 3T3-L1, female rat subcutaneous fat, and human preadipocytes (Dieudonne et al. 2000; Anderson et al. 2001). However, another study showed that estrogen inhibits 3T3-L1 preadipocyte differentiation (Lea-Currie et al. 1999). In vivo studies have yielded more consistent observations, as both male and female ER- α -deficient mice have increased WAT accumulation and insulin resistance (Heine et al. 2000). Studies using E2-deficient rodent models also supported the inhibitory effect of this hormone on adiposity. Aromatase-deficient mice (ArKO) develop increased fat

pad mass, hyperinsulinemia, hyperlipidemia, and liver steatosis (Jones et al. 2000). Mice deficient of follicle-stimulating hormone receptor show increased development of visceral fat (Danilovich et al. 2000). Collectively, the majority of studies suggest that estrogen attenuates adipocyte development.

Androgens also regulate fat cell metabolism and adipose tissue distribution (Wade et al. 1985; Bjorntorp 1991). Androgens can be found in adipocytes (Deslypere et al. 1985), and there is substantial evidence to show that the androgen receptor (AR) is expressed in rat (De Pergola et al. 1990; Dieudonne et al. 1995) and human (Dieudonne et al. 1998) preadipocytes and adipocytes. Together, these observations indicate that fat cells are targets of androgen action. As observed with estrogen experiments, in vitro studies have shown opposing effects of androgens on the differentiation of preadipocytes. There is evidence that androgens induce rat fat cell development (Xu and Bjorntorp 1987), whereas others report that androgens inhibit adipocyte differentiation (Gordon et al. 1986; McIntosh et al. 1998; Lea-Currie et al. 1999). It was proposed that the two potent androgens, testosterone and dihydrotestosterone, decrease C/EBP α and PPAR γ 2 expression to inhibit differentiation of 3T3-L1 cells (Singh et al. 2006). Additionally, transgenic mice with targeted AR overexpression in mesenchymal stem cells have substantial reductions in both WAT and BAT depots (Semirale et al. 2011). In summary, the majority of studies on androgens and adipocyte development suggest that these steroid hormones have inhibitory effects.

Glucocorticoids are another class of steroid hormones that regulate adipocyte differentiation. The glucocorticoid receptor (GR) is expressed in most cell types, and glucocorticoids bind to GR to mediate effects on metabolism and immune response (Vegiopoulos and Herzig 2007). It is well established that glucocorticoids promote adipogenesis. Dexamethasone, a synthetic glucocorticoid, is a standard component of the hormonal induction cocktail used to induce 3T3-L1 preadipocyte differentiation (Rubin et al. 1978). Today dexamethasone is used to promote adipogenesis in many cell lines, including 10T1/2 (Chapman et al. 1985) and Ob1771 (Gaillard et al. 1991) cells. Mechanistic studies indicate that dexamethasone induces C/ EBP δ (Cao et al. 1991), which induces the master adipogenic regulator PPAR γ (Wu et al. 1996).

4.3 Peptide Hormones

A large variety of peptide hormones can modulate adipogenesis. Both preadipocytes and adipocytes are responsive to growth hormone. GH signals primarily via the JAK-STAT signaling pathway. As indicated earlier in this review, STAT5 proteins promote adipogenesis in vitro and in vivo (Stephens et al. 1996; Teglund et al. 1998; Harp et al. 2001; Nanbu-Wakao et al. 2002). Because GH is a potent activator of STAT5, it is not surprising that most studies show that GH promotes adipocyte development. The GH-STAT5 pathway enhances both the expression and the activity of C/EBP β and - δ and PPAR γ (Kawai et al. 2007), thus proposing multiple pathways through which GH promotes adipogenesis. Earlier studies showed that GH exerts a dual effect on 3T3-F442A preadipocytes, in which there is an initial direct effect on preadipocytes to initiate the adipogenic program that is followed by an indirect effect of GH to induce insulin-like growth factor-1 (IGF-1) to stimulate growth of the committed cells (Green et al. 1985). Although it has been clearly established that GH promotes adipocyte development in clonal cell lines, several studies show an opposing effect of GH action on differentiation of primary cultured preadipocytes. In primary rat and human preadipocytes, GH inhibits differentiation (Wabitsch et al. 1996a, 1996b).

It is assumed that insulin is a positive regulator of fat cell development. However, levels of insulin in the standard hormonal cocktail are supraphysiological and are capable of activating IGF-1 receptor signaling (Smith et al. 1988). Nonetheless, there is evidence that insulin signaling can modulate the expression of genes required for adipocyte development and physiology in the whole animal. Mice that lack the insulin receptor in adipose tissue show reduced fat mass and decreased levels of SREBP-1 and C/EBP α in white adipocytes. Additionally, these mice have abnormally high leptin serum levels (Bluher et al. 2002). These studies suggest that insulin can promote adipogenesis; however, more studies are necessary to clarify the mechanism(s) of action.

4.4 Glycoprotein 130 (gp130) Cytokines

The family of gp130 cytokines regulate a variety of complex biological processes including hematopoiesis, immune response, inflammation, proliferation, differentiation, mammalian reproduction, cardiovascular action, and neuronal survival (reviewed in Heinrich et al. 2003). These cytokines share gp130 as a common signal transducer in their receptor complex and typically activate STAT3, a latent transcription factor. The gp130 family is also referred to as the interleukin-6 (IL-6) family of cytokines and is a group of functionally and structurally related proteins that includes IL-6, IL-11, IL-27, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), novel neurotrophin-1/ B-cell-stimulating factor-3 or cardiotrophin-like cytokine (CLC), and neuropoietin (NP) (reviewed in Fasnacht and Muller 2008). The gp130 cytokines have become a focus of attention in the scientific community because they have been implicated as potential therapeutic targets in obesity treatment (reviewed in Febbraio 2007). Numerous findings in the last decade have highlighted the differential effects of gp130 cytokines on important adipocyte functions. These studies have shown that adipocytes in vitro and adipose tissue in vivo are responsive to gp130 cytokines (Balhoff and Stephens 1998; Stephens et al. 1998; Lagathu et al. 2003; Rotter et al. 2003; Zvonic et al. 2003, 2004; Tenney et al. 2005; White et al. 2008) and exert differential effects on adipogenesis. To date, no gp130 cytokines have been shown to promote adipogenesis, but as shown in Table 1, several cytokines in this family have been shown to inhibit adipocyte development (Keller et al. 1993; Meng et al. 2001; Zvonic et al. 2003, 2004; Sopasakis et al. 2004; Hogan and Stephens 2005; Miyaoka et al. 2006; Song et al. 2007; White et al. 2008).

5 ADIPOGENESIS AND METABOLIC DISEASE STATES

In the past it was widely hypothesized that inhibitors of adipogenesis were potential antiobesity therapeutics. However, evidence from a variety of experiments in mice and humans suggests that inhibitors of adipogenesis are a poor choice for amelioration of metabolic disease states because limiting fat cell expansion is associated with insulin resistance. As proposed more than a decade ago, a failure in adipocyte differentiation can cause type 2 diabetes (Danforth 2000), and this hypothesis is generally recognized and supported by independent lines of investigation in adipocyte biology.

Inflammation in adipose tissue is widely acknowledged as a cause of insulin resistance. The presence of infiltrating immune cells, such as macrophages and T cells, is well documented, and studies in the last decade suggest that these cells are modulated in conditions of obesity and type 2 diabetes. It is well established that the proinflammatory cytokines tumor necrosis factor- α (TNF α) (Torti et al. 1985) and interferon- γ (IFN γ) (Keay and Grossberg 1980) are potent inhibitors of adipocyte differentiation. Of note, it is also known that both of these cytokines induce insulin resistance in adipocytes (Stephens et al. 1992; McGillicuddy et al. 2009). Both preadipocytes and adipocytes express receptors for TNFa and IFNy (Fig. 3). Moreover, TNFa expression is induced in adipose tissue of obese diabetic rodents (Hotamisligil et al. 1993) and humans (Hotamisligil et al. 1995). It is now largely accepted that TNFα expression in adipose tissue comes from macrophages (Weisberg et al. 2003). More recent studies have shown that IFNy is produced from both NK cells (O'Rourke

K. Sarjeant and J.M. Stephens

Class	Hormone	Effect	Model system and references
Steroid hormones	Androgen	Controversial	Proadipogenic in primary tissue cultures (Xu et al. 1987) Antiadipogenic in 3T3-L1 cells (McIntosh 1990; Lea-Currie et al. 1999) Inhibits fat development in mice (Semirale et al. 2011)
	Estrogen	Controversial	Proadipogenic in rat preadipocytes (Dieudonne et al. 2000) and human adipose tissue (Anderson et al. 2001)
			Antiadipogenic in 3T3-L1 cells (Lea-Currie et al. 1999) and in various transgenic mouse models (Danilovich et al. 2000; Heine et al. 2000; Jones et al. 2000)
	Glucocorticoid	Proadipogenic	Proadipogenic in 3T3-L1 (Cao et al. 1991), 10T1/2 (Semirale et al. 2011), and Ob1771 cells (Gaillard et al. 1991)
	Thyroid	Proadipogenic	Proadipogenic in HIB 1B brown adipocyte cell line (Klaus et al. 1994) T3 promotes lipogenesis in rat adipose tissue (Oppenheimer et al. 1991)
Peptide hormones	Growth hormone	Controversial	Proadipogenic in 3T3-L1 cells (Green et al. 1985; Kawai et al. 2007) Antiadipogenic in primary rat preadipocytes (Wabitsch et al. 1996b) and in human adipocytes (Wabitsch et al. 1996a)
	Insulin/IGF-1	Proadipogenic	Proadipogenic in 3T3-L1 cells (Green et al. 1985; Smith et al. 1988)
gp130 cytokines	CNTF	None	No effect on 3T3-L1 adipogenesis (Zvonic et al. 2003)
	CT-1	None	No effect on 3T3-L1 adipogenesis (Zvonic et al. 2004)
	IL-11	Inhibitory	Antiadipogenic in 3T3-L1 (Kawashima et al. 1991), human marrow (Keller et al. 1993), and human preadipocyte cells (Meng et al. 2001)
	LTF	Controversial	No effect on 3T3-L1 adipogenesis (Hogan et al. 2005; White et al. 2008) Proadipogenic in 3T3-L1 cells (Aubert et al. 1998)
		x 1.11.	Antiadipogenic in bone marrow stromal cells (Gimble et al. 1994)
	NP	Inhibitory	Antiadipogenic in 313-L1 cells (White et al. 2008)
	OSM	Inhibitory	Antiadipogenic in 313-L1 cells (Miyaoka et al. 2006; White et al. 2008), mouse embryonic fibroblasts (Miyaoka et al. 2006), and human adipose-derived mesenchymal cells (Song et al. 2007)

Table 1. Endocrine modulators of adipogenesis

Abbreviations: IGF-1, insulin-like growth factor-1; CNTF, ciliary neurotrophic factor; CT-1, cardiotrophin-1; IL-11, interleukin-11; LTF, leukemia inhibitory factor; NP, neuropoietin; OSM, oncostatin M.

et al. 2009) and T cells (Rocha et al. 2008; Duffaut et al. 2009; Strissel et al. 2010; Yang et al. 2010) present in adipose tissue. Together, these studies suggest that immune cells in adipose tissue produce TNF α and IFN γ , which can inhibit differentiation of preadipocytes and induce insulin resistance in mature adipocytes. In relation to the Danforth hypothesis, the induction of type 2 diabetes is believed to be mediated by the direct effects of TNF α and IFN γ on inducing insulin resistance in mature adipocytes. However, the ability of these cytokines to induce insulin resistance by inhibiting adipogenesis has not been considered. Nonetheless, there are new model systems that clearly suggest that limitations in adipose tissue expansion are associated with insulin resistance. Mice that are very obese but have unlimited adipose tissue expansion are metabolically healthy and insulin sensitive (Kim et al. 2007). Overall, these studies largely support Danforth's idea and indicate that inhibition of adipogenesis is associated with insulin resistance. With the recent characterizations of metabolically healthy obese individuals, it seems clear that obesity is likely preferable to insulin resistance in terms of overall health. It is also

evident that inhibiting adipogenesis is not a viable therapeutic approach.

The role of macrophages and inflammation is less studied in BAT. There is evidence to suggest minimal macrophage infiltration in BAT, just 1% of cells, as compared with 31% observed in WAT (Fitzgibbons et al. 2011). Other evidence suggests that the presence of anti-inflammatory macrophages in BAT plays a role in heat production (Nguyen et al. 2011). Currently there is no consensus on the amount and function of macrophages and inflammatory mediators in BAT.

6 CONCLUDING REMARKS

The way we view adipocytes has been substantially altered over the last two decades. Emerging research has changed our understanding of the origin of adipocytes, and there is clear evidence that brown and white adipocytes can arise from distinct precursor cells (see Fig. 2). Adipocytes are more than inert energy depots, and adipose tissue is a biologically active organ that carries out important

Adipogenesis



Figure 3. Proinflammatory cytokines produced from immune cells in unhealthy adipose tissue inhibit adipocyte development. The adipocytes in healthy adipose tissue are insulin sensitive, and the tissue does not contain TNF α - and IFN γ -producing cells. The adipocytes in metabolically unhealthy adipose tissue are insulin resistant and lack the normal ability to expand. The presence of infiltrated immune cells that produce TNF α and IFN γ results in inhibition of adipocyte development.

physiological processes including energy homeostasis and whole-body insulin sensitivity. Attenuating adipose tissue expansion can result in pathologies including insulin resistance. Several transcription factor families promote adipocyte differentiation, including members of the C/EBP, STAT, and KLF families (see Fig. 1). However, PPAR γ is the most critical transcription factor required for fat cell development. In fact, most inhibitors of adipogenesis modulate PPAR γ expression and/or activity. Adipocyte development can also be regulated by endocrine factors including T3, glucocorticoids, GH, and members of the gp130 cytokine family (see Table 1). However, the overall effects of these factors on adipogenesis are controversial. Nonetheless, our knowledge of the source and differentiation of adipocytes has greatly expanded in the last decade as transcriptional and endocrine modulators of this fundamental development process have been identified.

Future studies in the area of adipogenesis will include the use of sophisticated methodologies to study the cellular origins of brown and white adipocytes. It is also expected that there will be extensive investigation of adipose tissue– derived stem cells for their use as potential therapeutics, including tissue engineering, as these cells are capable of differentiating into numerous cell types of both mesodermal and nonmesodermal origin. Given the role of BAT in energy expenditure, it is highly likely that approaches to expand BAT mass will be used. Current and future research will also include the generation of highly specific PPAR γ ligands that may not only regulate adipocyte development, but also modulate insulin sensitivity in mature fat cells. There is little doubt, given the endocrine function of adipose tissue and evidence that inhibition of adipocyte development promotes metabolic disease states like type 2 diabetes, that adipocyte development will continue to be a highly active area of investigation.

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K. Sarjeant and J.M. Stephens

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Adipogenesis

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Adipogenesis

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