Hormonal Signaling and Transcriptional Control of Adipocyte Differentiation\textsuperscript{1,2}

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ABSTRACT Recent advances regarding the biology of adipose tissue have identified the adipocyte as an important mediator in many physiologic and pathologic processes regarding energy metabolism. Consideration for a central role of adipose tissue in the development of obesity, cardiovascular disease and noninsulin-dependent diabetes mellitus has resulted in new incentives toward understanding the complexities of adipocyte differentiation. Current knowledge of this process includes a cascade of transcriptional events that culminate in the expression of peroxisome proliferator-activated receptor-\(\gamma\) (PPAR\(\gamma\)) and CCAAT/enhancer binding protein-\(\alpha\) (C/EBP\(\alpha\)). These prominent adipogenic transcription factors have been shown to regulate, directly or indirectly, the gene expression necessary for the development of the mature adipocyte. Hormonal and nutritional signaling that impinges on these trans-acting factors provides a molecular link between lipids and lipid-related compounds and the gene expression important for glucose and lipid homeostasis. Knowledge concerning the transcriptional events mediating adipocyte differentiation provides a basis for understanding the physiologic processes associated with adipose tissue as well as for the development of therapeutic interventions in obesity and its related disorders. J. Nutr. 130: 3116S-3121S, 2000.

KEY WORDS: • energy metabolism • adipose tissue • transcription • gene expression
• peroxisome proliferator-activated receptor-\(\gamma\)

Pleiotropic functions of the adipocyte

Recent advances in our understanding of energy metabolism have shed new light on adipocyte function. For many years, it has been postulated that when energy intake exceeds energy expenditure, the excess metabolic fuel is stored passively in the adipocyte in much the same way that an oil can store its contents. An active role for the adipocyte in energy metabolism was demonstrated with the discovery of leptin, a secretory product that originates almost exclusively from the adipocyte and serves a hormonal function in mediating satiety through receptors located in the hypothalamus [reviewed in Flier (1998)]. These findings gave new incentive to adipose tissue research that has resulted in new discoveries and reconsideration of previously ascribed functions, positioning the adipocyte near the center of physiologic processes mediating energy metabolism as well as a plethora of potentially unrelated functions.

As illustrated in Figure 1, adipocyte functions can be grouped generally into three categories with potentially overlapping modalities. Processes associated with lipid metabolism are best exemplified by the storage and release of fatty acids for vitally important processes such as myocardial contraction during times of need. Secretion of glycerol and fatty acids from the adipocyte also plays an important role in hepatic and peripheral glucose metabolism. Moreover, adipose tissue as well as heart and skeletal muscle are the only known tissues to express and regulate the insulin-dependent glucose transporter, Glut4, which facilitates the entry of glucose into these cells and out of circulation postprandially. Emerging data suggest that the adipocyte also plays an important role in numerous processes through its secretory products and endocrine functions. In this regard, leptin has a wide spectrum of biological activities, independent of satiety, including effects on fertility, reproduction and hematopoiesis. In addition to this hormone, adipose tissue secretes a variety of peptides, cytokines and complement factors whose various functions are linked inseparably to the adipocyte as a source for their production [reviewed in Gregoire et al. (1998)].

Although the adipocyte is vitally important to energy homeostasis, adipose tissue may also play a central role in many of the pathologies associated with obesity and its related disorders (Fig. 2). Genetic mutations that alter the release of leptin from the adipocyte or suppress its interaction with receptors in the hypothalamus are well-known causes of obesity in mice. Cytokines and lipids released from adipose tissue...
may lead to a decrease in glucose utilization in skeletal muscle and enhance glucose production by the liver, both of which contribute to high levels of glucose in the peripheral circulation, a hallmark of noninsulin-dependent diabetes mellitus. Furthermore, cytokines from the adipocyte may play a role in activating various inflammatory responses that are considered to be important mediators of cardiovascular disease. In addition, the development of atherosclerotic lesions is likely to be compounded by hyperlipidemia contributed to by the release of fatty acids from fat-laden adipocytes.

The increasing incidence, health implications and economic cost of obesity, atherosclerosis and Type II diabetes have established these diseases as some of the most serious health issues in the United States. Consequently, all aspects of adipocyte biology, including adipogenesis, have recently become the targets of intense scientific investigation (Must et al. 1999). Knowledge concerning the sequence of transcriptional events during adipogenesis provides a basis for understanding the physiologic processes associated with adipose tissue as well as for the development of therapeutic intervention in adipocyte-related disease.

Transcription factors regulating adipocyte differentiation

It is estimated that the structural and functional morphogenesis associated with adipocyte differentiation involves changes in the expression levels of ~300 proteins. The directional change of many of these proteins has been reviewed comprehensively (Cornelius et al. 1994). Many of these changes occur at the level of gene expression through a series of molecular events involving several transcription factor families that exhibit diverse modes of activation and function. The following represent only the more prominent trans-acting factors that are currently considered to play a regulatory role in the process of adipogenesis.

Peroxisome proliferator–activated receptors. The peroxisome proliferator–activated receptors (PPARs) are a subset of the nuclear hormone receptors whose transcriptional activities are modulated by ligand-receptor interactions (reviewed in Brun and Spiegelman (1997)). The three known PPAR family members, PPARα, PPARγ and PPARδ, bind similar peroxisome proliferator response elements, but exhibit different transactivating functions, which are mediated in part by tissue distribution, ligand specificity and coactivator recruitment. Through utilization of different start sites and alternate splicing, the PPARγ gene gives rise to two isoforms, γ1 and γ2. PPARγ2 is highly enriched in adipose tissue and generally mediates gene expression regarding fatty acid metabolism. The notion that PPARγ plays a major role in regulating adipogenesis is supported by the fact that thiazolidinediones (TZD), which are high affinity, synthetic ligands for PPARγ, are potent inducers of adipocyte differentiation. Furthermore, ectopic expression of PPARγ in multiple nonprogenitor cells lines under adipogenic conditions results in consistent and potent induction of adipocyte differentiation. Gene ablation studies reporting an absence of white adipose tissue have positioned PPARγ and its obligate heterodimeric partner, retinoid X receptor α (RXRα), as prominent transcription factors in regulating the gene expression leading to adipogenesis (Barak et al. 1999, Kubota et al. 1999, Rosen et al. 1999).

CCAAT/enhancer-binding proteins. The CCAAT/enhancer-binding proteins (C/EBPs) belong to a large family of leucine zipper transcription factors, which function through homo- and heterodimeric complexes with C/EBP family members. Three of these family members, C/EBPα, C/EBPβ and C/EBPδ, are expressed in both white and brown adipose tissue and have been studied and reviewed extensively for their roles in regulating adipogenesis (Darlington et al. 1998, Lane et al. 1996). Ectopic expression of C/EBPα or C/EBPβ induces adipogenesis in nonprogenitor fibroblasts, whereas antisense expression of C/EBPα inhibits differentiation of cultured preadipocytes. Gene ablation studies that target C/EBPα or combined C/EBPβ and C/EBPδ demonstrate a reduced propensity for adipogenesis, with deficient animals developing markedly less adipose tissue compared with wild-type littermates. Collectively, these data demonstrate an important role for C/EBPα, polyunsaturated fatty acids; RXR, retinoid X receptor; SREBP, sterol regulatory element binding proteins; STAT, signal transducers and activators of transcription; TZD, thiazolidinediones.

FIGURE 2 Central role of adipose tissue in obesity related disorders. The effect of hormones, cytokines, e.g., tumor necrosis factor α (TNFα), and free fatty acids (FFA) released from the adipocyte play a role in positioning adipose tissue as a central mediator of obesity, noninsulin-dependent diabetes mellitus and cardiovascular disease.
family members during the development of adipocyte differentiation, in vitro and in vivo.

**ADD1/SREBP-1c.** Sterol regulatory element binding proteins (SREBP) are known to modulate transcription of numerous genes encoding proteins that function in both cholesterol and fatty acid metabolism [reviewed in Brown and Goldstein (1997)]. The SREBP family consists of three proteins, designated SREBP-1a, -1c and -2, which are encoded by two independent genes. In humans and mice, SREBP-1a and SREBP-1c are produced from a single gene through the use of alternate transcription start sites. Adipocyte determination-and differentiation-dependent factor 1 (ADD1), cloned independently from a rat adipocyte cDNA library (Tontonoz et al. 1993), is homologous to human SREBP-1c. Although all three SREBP are capable of activating similar gene expression, regulation of fatty acid biosynthesis is mediated primarily by SREBP-1a and ADD1/SREBP-1c. In vivo, adipose tissue expresses predominantly ADD1/SREBP-1c over other forms of SREBP, and ectopic expression of a constitutively active form of ADD1/SREBP-1c enhances adipocyte gene expression in nonprogenitor NIH-3T3 fibroblasts under adipogenic conditions. In addition, expression of a dominant negative form of this SREBP isoform represses 3T3-L1 preadipocyte differentiation (Kim and Spiegelman 1996). Although ablation of the SREBP-1 gene (mice lacking both SREBP-1a and SREBP-1c) has been reported to have little effect on white adipose tissue mass, redundancy of function (i.e., SREBP-2 expression) has not been ruled out (Shimano et al. 1997).

**STATs.** Signal transducers and activators of transcription (STATs) comprise a family of cytoplasmic proteins that are activated by and mediate gene expression in response to extracellular effectors that target receptors with intrinsic kinase activity or receptors to which Janus kinases (JAK) are bound [reviewed in Darnell (1997)]. Ligand-mediated dimerization of the receptor results in phosphorylation of the associated kinase, which subsequently phosphorylates the cytoplasmic tail of the receptor that serves as a docking site for STAT recruitment. The receptor-bound STAT is phosphorylated, then dimerizes with other STAT proteins and translocates to the nucleus to mediate specific gene expression. The expression of three members of this family, STAT1, STAT5A and STAT5B, is upregulated during differentiation of cultured preadipocytes (Stephens et al. 1996). Although the function of STATs during adipocyte differentiation is unclear, gene ablation of STAT5A and STAT5B produces animals with markedly less white adipose tissue compared with wild-type littermates, demonstrating a significant role for these proteins during adipogenesis (Teglund et al. 1998).

**Cascade of transcriptional events mediating adipogenesis**

Much of our knowledge concerning the sequence of transcriptional events mediating adipogenesis has evolved from cultured cell lines (e.g., 3T3-L1) that differentiate from determined, fibroblast-like cells into functionally mature adipocytes resembling those found in white adipose tissue in vivo. A summary of the molecular process of adipocyte differentiation, focusing only on transcriptional events, is depicted in Figure 3. The 3T3-L1 preadipocyte cell line can be “induced to differentiate” with the addition of mitogens and hormonal agents (e.g., insulin, glucocorticoids and agents that lead to an increase in cAMP); this initiates a cascade of transcriptional events that collectively account for the expression of most proteins mediating adipocyte function. Immediately after exposure to exogenous mediators, the gene expression of C/EBPβ and C/EBPδ significantly and transiently increases (Fig. 2), marking an event that is likely to distinguish a preadipocyte from a nonadipogenic precursor cell (Cao et al. 1991). The activity of these C/EBPs then mediates the expression of PPARγ (Clarke et al. 1997, Wu et al. 1995), which forms functional heterodimer with RXRα. Although yet unproven, a consensus sequence in the C/EBPα promoter suggests that C/EBPβ and C/EBPδ may also regulate the expression of C/EBPα (Christy et al. 1991, Lin et al. 1993). Once activated, PPARγ and C/EBPα appear to cross-regulate each other, thus maintaining their gene expression despite the ensuing decay of C/EBPβ and C/EBPδ (Schwarz et al. 1997, Shao and Lazar 1999). Although data suggest that C/EBPα may also have the capacity to autoregulate its own expression, recent studies have demonstrated that ectopic expression of PPARγ is not capable of activating endogenous PPARγ gene expression in C/EBPα-deficient fibroblasts (Wu et al. 1999).

Additional early events are known to be important for the activation of PPARγ and C/EBPα gene expression. For example, exposure of preadipocytes to mitogens and hormonal agents that induce differentiation also leads to an early up-regulation of ADD1/SREBP-1c gene expression (Ericsson et al. 1997). It has been postulated that this SREBP family member also plays a role in upregulating PPARγ gene expression (Fajas et al. 1999). Moreover, evidence suggests that ADD1/SREBP-1c may be involved in gene expression that leads to the production of endogenous PPARγ ligands required for transcriptional activity (Kim et al. 1999). Other studies have demonstrated that undifferentiated preadipocytes express a number of inhibitory proteins that must be repressed or functionally inactivated to allow the differentiation process to occur. Exposure of preadipocytes to differentiating agents leads to repression of AP-2α and SP1 transcriptional activity, events that are necessary for PPARγ and C/EBPα gene expression (Jiang et al. 1998b, Tang et al. 1999). Inhibitory molecules may function, in vivo, to maintain the preadipocyte phenotype until hormonal and nutritional conditions are supportive for adipocyte differentiation. Similar paradigms are now considered dogma in cell proliferation pathways in which numer-
ous proteins serve check-point functions regulating commitment to cell cycle progression.

After their expression, PPARγ and C/EBPα are considered to play a prominent role in regulating the gene expression of proteins necessary for the development of the functional mature adipocyte. To date, however, only a limited number of the exhaustive list of genes encoding for proteins mediating adipocyte function are known to contain active consensus sequences for either PPARγ or C/EBPα. It is conceivable that these potent adipogenic transcription factors can modulate indirectly the expression of other genes through the activation of intermediary trans-acting factors. In this regard, recent evidence indicates that the differentiation-dependent induction of STAT1, STAT5A and STAT5B is regulated downstream of PPARγ in the differentiation paradigm (Fig. 2) (Stephens et al. 1999). Although complete adipocyte differentiation requires the expression of STAT5, the precise function that indirectly links the activity of PPARγ to adipocyte gene expression has yet to be determined. Although future studies will undoubtedly identify other unknown transcription factors downstream of PPARγ and/or C/EBPα, activation of STAT expression represents the only known regulation of trans-acting factors by either of these adipogenic mediators.

Any property of the mature adipocyte is likely to require the expression of numerous genes that collectively account for that specific function. With this in mind, PPARγ and C/EBPα have been shown to transactivate subsets of genes as a function of either trans-acting factor alone or one requiring the cooperative efforts of both. An example of this is also noted when expressing PPARγ in NIH-3T3 fibroblasts (Wu et al. 1999) or in NIH-3T3 fibroblasts that are defective for C/EBPα expression (El-Jack et al. 1999). Under potent adipogenic conditions, including PPARγ ligand supplement, fibroblasts in either case formed characteristic lipid droplets and expressed many genes associated with adipocyte differentiation. However, these fibroblasts are not responsive to insulin regarding glucose uptake (Fig. 3). Rescue of this defect with coexpression of C/EBPα clearly demonstrates synergy among these adipogenic transcription factors within a program of events involving many proteins necessary for the complex process of insulin sensitivity. Although the precise defect remains under investigation, positive cooperation between C/EBPα and PPARγ appears to be obligatory for this important aspect of adipocyte function.

**Nutritional regulation of PPARγ in lipid and glucose homeostasis**

Conclusions drawn from gain of function, tissue distribution and gene ablation studies have established PPARγ as a major component of the transcriptional cascade leading to adipogenesis. Recent investigations have broadened our appreciation of PPARγ as a potential physiologic sensor of lipid levels, linking fatty acids and other lipid-related molecules to glucose and lipid homeostasis (Fig. 4). Ligands for this nuclear hormone receptor include the naturally occurring eicosanoid, 15-deoxy prostaglandin J2 (Forman et al. 1995) and a group of synthetic compounds (the TZDs, e.g., troglitazone, rosiglitazone) that have been used in the treatment of adult onset (Type II) diabetes (Lehmann et al. 1995). Several long-chain polyunsaturated fatty acids (PUFAs) bind to PPARγ in vitro and can stimulate lipid-lowering and enhanced insulin sensitivity effects similar to those reported for synthetic PPAR ligands (Xu et al. 1999). Albeit unproven, it is hypothesized that various PUFAs and/or prostaglandins are likely to function as PPAR ligands in vivo, linking these lipid metabolites to gene expression mediating lipid homeostasis.

Although previous studies have focused primarily on its function in the adipocyte, it is likely that PPARγ also plays a regulatory role in lipid homeostasis in other cell types. Recent evidence demonstrating that PPARγ is expressed significantly in mammary and intestinal epithelia (Lefebvre et al. 1999, Mueller et al. 1998) and two classes of macrophages (Jiang et al. 1998a, Ricote et al. 1998) provides additional support for a general role for PPARγ in lipid homeostasis. This notion is best exemplified by studies examining the development of atherosclerotic lesions within the arterial wall that contain cholesterol-laden macrophages known as foam cells. The uptake of oxidized LDL (oxLDL) through scavenger receptors has been shown to enhance the expression of PPARγ within these macrophages (Nagy et al. 1998, Tontonoz et al. 1998). Results from recent studies have provided evidence leading to the hypothesis that oxidized lipid components of oxLDL, 9-HODE and 13-HODE, can function as endogenous activators and ligands of PPARγ (Fig. 4), thus providing a molecular mechanism linking oxLDL to the transcriptional control of macrophage gene expression. One target of PPARγ in these cells is the scavenger receptor, CD36, which establishes a positive feedback loop that increases the uptake of oxLDL and metabolites that function to enhance PPARγ transcriptional activity (Tontonoz et al. 1998). Although a role for PPARγ in mediating lipid metabolism in macrophages is supported by these findings, it remains to be determined whether activation by oxidized fatty acids plays a significant role in PPARγ-mediated processes in other tissues.

**Differential effects of PPARγ ligands on transcriptional activity**

Discovery of PPARγ in nonadipose tissues alludes to the possibility of diversified function based on tissue-specific expression of coactivators and corepressors and/or the availability of selective ligands that may alter the transcriptional activity and/or targets of PPARγ. In support of this notion, PPARγ-co-activators, PGC-1 and PGC-2, have been reported,
though the effect of troglitazone in enhancing PPARγ activity can be predicted by the increase in αP2 expression, it is clear that the same PPARγ ligand can result in a differential profile of adipogenic gene expression. Others have reported the synthesis of a ligand for PPARγ that binds tightly to the receptor domain and functions as a partial agonist for PPARγ transactivation (Oberfield et al. 1999). Interestingly, this compound was found to be a potent antagonist of adipocyte differentiation. Collectively, these data suggest that the molecule residing in the ligand-binding pocket of PPARγ can have a dramatic effect on the function of this nuclear hormone receptor.

**Prospective**

Although our knowledge concerning the transcriptional control of adipocyte differentiation has advanced significantly over the last few years, many unanswered questions remain. Currently, we position PPARγ and C/EBPα as the most prominent transcription factors mediating adipogenesis. The direct mechanism whereby these trans-acting factors, independently and/or cooperatively, activate gene expression important for adipocyte function and their roles in regulating the expression of intermediary transcription factors will undoubtedly represent a focus for future investigations. The increasing list of adipocyte functions beyond the storage of triglycerides will require an evolving definition of adipocyte differentiation and, consequently, the cascade of transcriptional events mediating adipogenesis. Analysis of the mechanisms whereby hormonal and nutritional signaling impinge on the adipogenic transcription factors will provide an important link between the cellular environment and regulation of gene expression important for glucose and lipid homeostasis.

**LITERATURE CITED**


