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Review

# Understanding the variegation of fat: Novel regulators of adipocyte differentiation and fat tissue biology☆,☆☆

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ABSTRACT

The differentiation of uncommitted cells into specialized adipocytes occurs through a cascade of transcriptional events culminating in the induction and activation of the nuclear receptor PPARγ, the central coordinator of fat cell function. Since the discovery of PPARγ, two decades ago, our views of how this molecule is activated have been significantly refined. Beyond the cell, we also now know that diverse signals and regulators control PPARγ function in a fat-depot specific manner. The goal of this article is to review the latest in our understanding of the early and late transcriptional events that regulate adipocyte development and their potential impact on energy storage and expenditure in different fat depots. This article is part of a Special Issue entitled: Modulation of Adipose Tissue in Health and Disease.



## 1. Introduction

Obesity is a major risk factor for metabolic disorders such as insulin resistance, diabetes, cardiovascular and liver disease. Excess weight and obesity are caused by an imbalance between energy input and its output, that results in excessive adipose tissue expansion, secondary to hyperplasia of adipocyte precursor cells and hypertrophy [1]. In the past several years, a series of key molecular players that influence adipose tissue mass have been identified and renewed emphasis has been placed on understanding the mechanistic principles that govern the development of these tissues and orchestrate energy homeostasis. Humans and rodents have two major anatomically distinct types of adipose tissues, white and brown. These tissues derive from different cell lineages and exert opposite roles on lipid metabolism. While white fat stores energy, brown fat dissipates it by using lipids as fuel for thermogenesis. Furthermore, white fat is not uniform. Distinct white fat depots exhibit a range of features, including different developmental gene signatures, adipokine repertoire, lipid storage capacity and variable susceptibility to inflammation. Given that these distinctive features influence the onset of the metabolic syndrome, it seems that an in depth understanding of the mechanisms that control different fat tissue types and depots is critical for designing strategies to prevent and treat obesity and its complications.

## 2. Types of adipose tissues and depots

### 2.1. White adipose tissue

White adipose tissue is the primary site of energy storage. Adipocytes, specialized cells devoted to the accumulation of triglycerides, store excess of nutrients as fat so that fatty acids may be released during energy demand in times of scarcity. Morphologically, mature white fat cells are characterized by an unilocular lipid droplet surrounded by a thin cytoplasmic rim containing only a few mitochondria. Fat cells are extremely plastic, able to rapidly expand in size and number, and as a result fat tissue of individuals with high body mass index represents the second largest organ in the body after the skin. Developmentally, fat tissue has been believed to originate from the mesoderm, however recent studies have shown that fat tissue present in the face has a neuro-ectodermal origin [2] and that the vasculature represents the primary source of fat cell precursors [3,4]. Despite similar morphological appearance of white fat tissue in every part of the body, there are major regional differences spanning from distinct gene expression profiles to distinct adipokine production. Microarray molecular analyses have confirmed that both human and mouse white fat tissues from different anatomical locations differ in a large number of expressed genes, including developmental patterning genes [5–7]. It was recently shown that the homeobox gene Hoxc13 is exclusively expressed in gluteal



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subcutaneous fat of both men and women and that its depot-selective expression is maintained in ex vivo cultures of preadipocytes and in differentiated adipocytes. These data suggest that different fat depots may be characterized by defined homeobox codes that identify them in specific locations of the body. Some of the molecular differences present in distinct depots manifest as distinct lipolytic capacity and differential responsiveness to sex hormones. It is now evident that gender-specific fat distribution exists: it has been demonstrated that men have increased propensity to expand their intra-abdominal fat depot, assuming an apple shaped phenotype, while women mostly enlarge the size of depots in the gluteal, hip and femoral area, giving rise to the characteristic pear shaped body conformation. These regional- and gender-specific differences in fat accumulation are particularly relevant given that increased fat in the subcutaneous region is associated with lower risk to develop the metabolic syndrome compared with increased fat deposition in the intra-abdominal area [8].

### 2.2. Brown fat

In response to beta-adrenergic stimuli, brown fat oxidizes lipids for thermogenic purposes. Smaller mammals, such as rodents, need brown fat to maintain their temperature during exposure to cold atmospheric conditions and so do human newborns who have little insulating white fat to protect them from low temperatures right after birth. The view that brown fat existed only in these conditions has undergone a dramatic change. In fact in adult humans, there are substantial amounts of cells containing molecular and functional footprints of brown fat especially in the neck and supraclavicular regions. These areas can be identified by PET scan as regions of high glucose uptake in a variety of patients, including in those exposed to thyroid replacement therapy or to cold temperatures [9–11]. These recent data implicate the existence of "brown fat-like" tissue in adult humans and have revamped the field of brown fat biology.

Brown fat cells are characterized by multilocular lipid content and by a high number of mitochondria. Brown fat burns lipids as fuel and uses UCP1, an inner mitochondrial membrane protein, to uncouple oxidative phosphorylation from ATP production causing the dissipation of energy as heat. The importance of UCP1 in vivo has been demonstrated through knock-out experiments showing that mice lacking UCP1 develop obesity at thermoneutrality [12]. The developmental origin of brown fat tissue was recently uncovered via elegant fate cell mapping analyses. Through these studies it was shown that brown fat has a distinct origin compared to white fat, and that it derives specifically from a population of Myf5+ precursor cells [13]. This muscle-like origin had been previously suspected since the analysis of gene signatures of brown and white fat tissues had shown a closer resemblance of brown fat to muscle than to white fat [14].

### 2.3. Brite/beige fat

In addition to white and brown fat cells, a new type of fat cell, called brite (short for brown in white) or beige, has been recently identified. It has been long established that subcutaneous white fat tissue is quite heterogeneous, with multilocular cells typically found in brown fat, interspersed between classic white unilocular fat cells [15]. These multilocular cells have been observed in white fat cells also in response to treatment with the antidiabetic drugs TZDs [16]. Immunostaining analyses have revealed that, although these "brown-like" cells express the characteristic brown fat marker UCP1, they are Myf5−, suggesting that they derive from a population of cell precursors distinct from that that gives rise to brown fat. To further define the molecular identity of the cells present in regions of browning within white fat tissue, the Spiegelman group embarked in a purification effort to clone and characterize these brite/beige cells. Through this approach, a population of cells, which express low UCP1 levels in the basal state, but can be induced to express it in response to cAMP stimulation, was identified [17]. Further molecular characterization by microarray analysis revealed that these cells express unique gene signatures, suggesting that brite/beige cells are a new and distinct cell type. Among the brite/beige-selective genes, the cell surface markers CD137 and TMEM26 were identified and used to detect brite/beige cells in mouse white fat tissues [17] and were shown to be expressed also in UCP1 positive human fat tissues [17,18]. Interestingly, UCP1 positive depots obtained from adult donors with thyroid cancer showed the coexistence of both classical brown and some brite/beige cell signatures [8], while UCP1 positive biopsies of healthy subject exposed to cold or obtained post-mortem from children mainly expressed beige-selective genes with nearly undetectable levels of classic brown fat markers [17,18].

It is now established that the number of the UCP1 positive cells in white fat tissue seems to be regulated not only by cold temperature, beta-adrenergic stimulation and drugs such as TZDs, but also by hormones and secreted peptides, as demonstrated by the browning effects observed after administration of the myokine irisin [17,19], FGF21 [20] and after treatment with the cardiac natriuretic peptide ANP, activated by p38 [21]. There are currently a number of hypotheses on how brite/beige cells arise in white fat tissue. Both trans-differentiation of white adipose tissue cells [22] and proliferation of brite/beige precursors embedded in white fat in response to various stimuli have been proposed. Recent analyses of transgenic and knock-out mouse models have shown that browning of adipose tissue occurs more often than anticipated, pointing to novel molecular mechanisms regulating browning of white tissue [23–27]. The evidence that certain stimuli not only increase brown fat tissue but also induce browning of white fat and increase energy expenditure has become an area of intense investigation for therapeutic purposes.

## 3. Regulators of adipocyte differentiation and fat tissues function

### 3.1. Terminal differentiation regulators

#### 3.1.1. PPARγ

Adipocyte differentiation in either white or brown fat is under the control of the transcription factor PPARγ. PPARγ's role in adipogenesis was discovered in the early nineties [28], when PPARγ was identified as the transcription factor binding to the fat specific enhancer present in the adipocyte fatty acid binding protein aP2 promoter [29]. In vitro gain of function studies subsequently demonstrated that ectopic expression of PPARγ is sufficient to confer the fat differentiation phenotype and to induce the expression of critical adipose tissue specific genes [28]. PPARγ has the typical nuclear receptor structure, characterized by a DNA binding motif, a ligand binding region and two activation domains, one ligand-independent located at the N-terminus, and the other at the C terminus functioning in response to agonists. PPARγ heterodimerizes with the nuclear receptor RXR and is able to activate transcription by binding to response elements characterized by direct repeats of the sequence 3′-PuGG/TTCA-5′ separated by 1 nucleotide, also called DR1. PPARγ's ligand binding pocket is large and can accommodate a wide array of ligands [30]. In the absence of agonists, PPARγ is bound to corepressors, such as NCor [31]. These repressive complexes are readily displaced after ligand binding, which results in PPARγ to undergo conformational changes to recruit coactivator complexes [32]. Recent advances in technology have permitted the global mapping of PPARγ targets via a systematic approach involving deep sequencing of adipocytes. These studies have confirmed that PPARγ controls lipid storage and allowed the identification of PPARγ targets at a genome-wide level [33–35]. Although PPARγ was discovered almost twenty years ago, its role as a central orchestrator of adipocyte function has remained uncompromised.

Regulation of PPARγ beyond ligand activation has been well documented. Modulation of CDK5-mediated phosphorylation levels of PPARγ at serine 273 by the partial agonists MRL24 has been shown to regulate selective PPARγ target genes, such as adiponectin and adipsin,

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