

Invited Review

PPAR γ and GLUT-4 expression as developmental regulators/markers for preadipocyte differentiation into an adipocyte

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Abstract

In this document, we have integrated knowledge about two major cellular markers found in cells of the adipocyte lineage (an adipogenic marker and a metabolic marker). This review provides information as to how differentiation of a cell (such as an adipofibroblast, fibroblast or preadipocyte) to become a viable (and new) adipocyte is under different regulation than that experienced by an immature adipocyte that is just beginning to accumulate lipid. The differentiation, prior to lipid-filling, involves PPAR γ . Subsequently, lipid-filling of the adipocyte relies on a late subset of genes and, depending on depot specificity, involves GLUT-4 or any number of other metabolic markers.

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1. Introduction

Small, monogastric animals such as rats, and even larger domestic animals (pigs and cattle), have generated substantial knowledge about adipocyte development and regulation [1–3]. However, due to the presence of the rumen and rumen bacterial conversion of feedstuffs [4–9], mechanisms involving individual adipocyte development or regulation in ruminant animals is not as clear cut. To complicate matters, adipose tissue and its constituent adipocytes are under dynamic physiological regulation [10–12] that appears to be both animal-specific and depot-specific [1,2,4–7,9,13–20]. This complicated (animal and depot) regulation raises the complexity of our overall understanding of both adipose tissue development and metabolism.

In order for any adipocyte to assimilate lipid, however, a mesodermal cell such as a fibroblast, preadipocyte, or adipofibroblast stops proliferating and begins to express genes indicative of the differentiated adipocyte phenotype [1]. Cellular proliferation and the subsequent differentiation “switch” are components of adipogenesis [1]. Alternatively, adipogenesis is stopped and lipid metabolism begins when the differentiated cell (now called an adipocyte) begins to accumulate visible lipid in its cytoplasm [1]. Little is currently known about the appropriate extrinsic and intrinsic regulation of adipogenesis of meat animal-derived cells destined to become adipocytes [1]. For lipid synthesis, there is a requirement of a source of a three-carbon unit (needed to form the α -glycerol phosphate for final triglyceride storage), and intracellular free fatty acids to form the storage triglyceride [4,6–9,15,19–21]. Numerous articles have been published with regards to the regulation of carbohydrate and lipid metabolism in animals [4,6–9,15,20,22,23].

In general, while fatty acid synthesis/storage occurs in adipose tissue of all meat animals, fatty acid storage from dietary triglycerides is primarily driven by what is biologically available to the cell in a depot-specific manner. This appears particularly evident in ruminants when evaluating the bioavailability of carbon sources, since the subcutaneous adipose depot is quite differen-

tially sensitive to acetate, rather than glucose [4,15,20]. While there is not much of glucose or insulin available to adipocytes in ruminants, the insulin/glucose mechanism is operable in some adipose depots [16] and some bovine adipocytes appear to remain responsive to both insulin and glucose [13,19]. Few papers looking at the cellular/molecular regulation of adipogenesis (or lipid metabolism) are available for ruminants and much of the work that has been done is with fetal (or very young) animals, or with other species.

2. Adipogenesis versus lipid metabolism: an overview of the involvement of PPAR γ and GLUT-4

Although they may be expressed under different cellular mechanisms and pathways, these two adipogenic and metabolic regulators, respectively, are jointly linked in the differentiation of most adipose-type cells. PPAR γ has been identified as an important adipogenic regulator/switch. PPAR γ plays an important role in converting adipofibroblasts, fibroblasts, or preadipocytes into differentiated adipocytes. Remarkably, expression and activation of PPAR γ induces adipose conversion of porcine [24], bovine [25] and human [26] satellite cells as well. Once a cell is transformed into a lipid-assimilating adipocyte, in most adipose depots GLUT-4 plays a major role in the energetic/metabolic functions of the adipocyte by allowing glucose transportation into the cell, after it has been signaled by insulin. PPAR γ may regulate some (early) aspects of GLUT-4, which also links adipogenesis to subsequent events of lipid metabolism. In this review we have summarized literature in this area to highlight the recent significance of PPAR γ versus GLUT-4 in adipocyte development, building on several excellent papers published previously [10–12,27–44].

3. Peroxisome proliferator activated receptors (PPARs)

Peroxisome proliferator activated receptors (PPARs) are a class of ligand-dependant nuclear receptor

transcription factors associated with gene expression [40–43,45–47] and, generally, as transcriptional regulators of subsequent lipid metabolism [44]. Three homologous PPARs: PPAR α , PPAR β/δ , and PPAR γ [38,42,44,45,48–50] have been described thus far and are differentially expressed, both spatially and temporally [43,49]. PPAR α is found in a variety of tissues including liver, heart, and skeletal muscle, whereby it functions in fatty acid oxidation [41,43–45,49,50], and has anti-inflammatory activity [43]. PPAR β/δ is found more ubiquitously [41,43,44,49] and, in the prenatal organism, aids in the formation of organs through the regulation of cytotrophoblast migration [43], as well as influencing stem cell differentiation [43]. The third, PPAR γ , provides a dynamic and specific regulation during the differentiation of an adipocyte precursor cell (fibroblast, adipofibroblast or preadipocyte) into a fully developed adipocyte [27,39,41,43–45,48]. It is this ability of PPAR γ to induce differentiation that will be the main focus for this section of the review.

3.1. PPAR γ

PPAR γ has been implicated in many biological processes such as cellular conversion/differentiation, insulin sensitivity, type-2 diabetes, atherosclerosis, and cancer [32]. Four mRNA isoforms of PPAR γ (1–4) arise from differential promoter action and alternate gene splicing [39,44]. Both PPAR γ 1 and PPAR γ 2 are expressed in adipocytes and differ by only 30 amino acid residues in their N-termini [31,32,39,40,42,44,47,48,51], but PPAR γ 2 is the form that functions in adipocyte differentiation through gene regulation [32,39,40,42,44]. The third isoform, PPAR γ 3, has been localized to macrophages, adipose tissue, and the large intestine [42,44]. It is now known that the mRNA variants PPAR γ 3 and PPAR γ 4 yield translated proteins that are identical to PPAR γ 1; thus only two receptors result (PPAR γ 1 and PPAR γ 2) from the effects of differential promoter action and alternate gene splicing [44]. The complete tissue distribution of PPAR γ 4 remains to be elucidated [44].

3.1.1. Biochemistry (structure) of PPAR γ

Similar to other nuclear receptors, PPAR γ has a modular structure with several distinct domains [52]. The N-terminus contains the DNA binding domain in the form of two zinc finger motifs, which bind to the regulatory region of DNA PPAR response elements (PPREs) when activated [31,32,42,44,53]. The N-terminus also contains a ligand-independent transcriptional-activation function [44]. The C-terminus contains the ligand-

binding domain and promotes the heterodimerization of PPAR γ with the retinoid receptor [38,42–44]. The ligand-binding domain is a secondary structure of α -helices and a β -sheet, which can bind natural and synthetic ligands [52].

3.1.2. Physiology/function of PPAR γ

Most studies of PPAR γ proteins during preadipocyte differentiation *in vitro* have utilized Western blots of whole cell proteins, whereas Western blots of nuclear proteins [54,55] or immunocytochemical localization of PPAR γ protein have been utilized in very few studies [56–60]. Immunolocalization studies are essential to study functionality since nuclear localization of PPAR γ protein follows PPAR γ activation by PPAR γ ligands [61–63]. Nuclear localization of PPAR γ can not be assumed since cytosolic and nuclear localization of PPAR γ protein have been reported in culture studies of various cell types [64–66], including 3T3-L1 preadipocytes [67]. Immunocytochemistry for PPAR γ , C/EBP α , and C/EBP δ proteins during preadipocyte differentiation was coupled with immunocytochemistry for AD-3, an early preadipocyte marker. Double staining for lipid and these proteins in fetal stromal vascular (S-V) cell cultures indicated that reactivity for nuclear PPAR γ protein was developmentally linked with lipid accretion in differentiating preadipocytes [56]. Therefore, regulation of adipogenesis may ultimately depend, in part, on PPAR γ protein expression and nuclear localization.

In 3T3-L1 cells in which both PPAR γ 1 and PPAR γ 2 expression was inhibited, ectopic expression of PPAR γ 2 induced adipogenesis whereas ectopic expression of PPAR γ 1 did not [27]. It has also been shown that PPAR γ 1 and PPAR γ 2 were able to stimulate adipogenesis in PPAR γ -/- fibroblasts [27]. *In vivo* work done with PPAR γ knockout mice support the assertion – that PPAR γ 2 is not required for adipocyte differentiation [27]. The ability of PPAR γ to regulate gene expression is through the binding of small lipophilic ligands, forming heterodimers with retinoic X receptors (RXR), and then through the binding of specific PPREs in enhancer regions of target genes [40,42,44,49,68–70].

PPAR γ can also repress gene transcription by negatively interfering with the NF- κ B, STAT and AP-1 signaling pathways independently of DNA binding [49]. PPAR γ functions to initiate the differentiation program through a loss of DNA binding activity of a transcriptional regulator of many genes involved in cell growth, E2F/DP, as well as through changes in expression of cyclin-dependant kinase inhibitors [53]. PPAR γ plays a crucial role in the function of many fat cell-specific genes such as aP2 and PEPCK [31,48]. PPAR γ was

once thought to be constitutively active and capable of recruiting co-activators even in the absence of ligands [44]. PPAR γ , when bound with RXR, can silence the transcription of genes [41,44]. PPAR γ 2 is capable of inducing adipogenesis under stringent conditions [71], and thus PPAR γ has been termed the ultimate “thrifty gene” [44] and has been thought to regulate adipogenesis by imparting insulin sensitivity to fat cells [72]. In the absence of exogenous ligands, PPAR γ co-localizes with co-activators or co-repressors on PPAR target gene promoters, facilitating constitutive expression of some genes (e.g. aP2) while repressing expression of others [73]. Although not fully defined, the addition of PPAR γ ligands leads to transcriptional changes in target genes by causing conformational changes in PPAR γ , as well as co-repressor release and co-activator recruitment [73].

In the rodent model, differentially expressed gene profiles during 3T3-L1 preadipocyte differentiation have been well established and demonstrate that many genes are up- or down-regulated through the course of differentiation [74,75]. Thus, gene families like PPAR, sterol regulatory element binding protein (SREBP) and CCAAT enhancer binding protein (C/EBP) have been reported to play important roles for preadipocyte differentiation [76,77] (Fig. 1). These genes encode transcriptional factors which regulate gene expression in lipid and fatty acid metabolism, however, the interactions among differentially expressed genes are as yet still unclear. PPAR γ is expressed in adipocytes as well as preadipocytes with different results [48]. In the adipocyte, PPAR γ , as well as the C/EBP α , induces the expression of late-stage-differentiation genes. Adipocyte-specific proteins include adipsin, aP2, lipoprotein lipase, and GLUT-4 and will remain elevated for the life of the cell [32,48,78]. Indeed, increases of PPAR γ in adipose tissue result in increases in GLUT-4 [32]. It is also suggested that PPAR γ is capable of direct modulation of the insulin-signaling pathway through the up-regulation of several factors (such as the insulin receptor substrate 1 and 2 and the p85 subunit of PI3) of the signaling cascade for GLUT-4 [44], allowing GLUT-4 to function within the cell. Inhibition of adipogenesis results in a decrease of C/EBP α and PPAR γ [79].

3.1.3. Regulation of PPAR γ by C/EBPs and other ligands

The three members of the C/EBP family, α , β , and δ , are expressed at specific times during adipogenesis, underscoring the regulatory role needed during the differentiation process [45]. In rodent cell lines, expression of C/EBP β and C/EBP δ occurs 24–48 h after adipogenic conversion [34,45,78]. These upstream

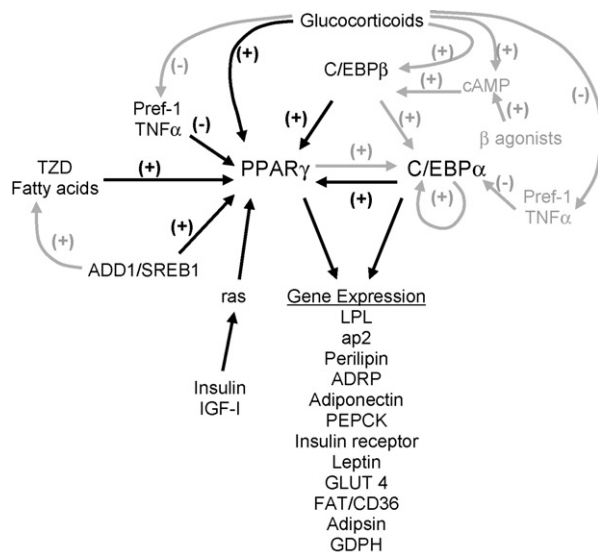


Fig. 1. Signaling factors that both directly and indirectly regulate PPAR γ . Cells to become viable adipocytes stop proliferating and begin to accumulate lipid. Lipid accumulation is dependent on cellular uptake of fatty acids and glucose, which is converted to make the glycerol backbone of storage triglycerides. When is the earliest that one may determine if a committed cell such as an adipofibroblast has become determined and differentiated to form an adipocyte? While numerous cellular markers have been identified in adipocytes, is there a specific cell-associated marker that (if expressed and identified) concretely proves that differentiation has taken place? For example, is it the marker expressed when the cell switches into adipocyte mode (traditionally thought as being no longer capable of proliferating), or is it the marker expressed when the cell accumulates discernable lipid? Our report suggests that PPAR signaling events are required prior to GLUT-4 recycling and subsequent glucose transport, as affected by insulin. Note that PPAR γ is an indirect regulator of itself through its action on C/EBP α . Black arrows = direct regulation; gray arrows = indirect regulation.

signaling molecules regulate the initial expression of two key adipogenic transcription factors and a unified pathway of adipogenesis: C/EBP α and PPAR γ [34,36,39,42,45,51,78,80]. C/EBP α and PPAR γ are initially expressed in low levels, which are then able to induce each other's expression in a positive feedback loop [33]. C/EBP α helps in maintaining PPAR γ levels in the differentiated adipocyte [33,42,81] but, through loss of function studies, it has been determined that PPAR γ is essential for adipogenesis *in vivo* and *in vitro* and that cells lacking PPAR γ show marked reductions in C/EBP α [34]. Sterol responsive element binding proteins (SREBP) have also been implicated in the transcriptional regulation of PPAR γ [27,39,42]. Fig. 1 summarizes both intrinsic and extrinsic factors that influence PPAR γ . Recently Salma et al. [82] demonstrated that C/EBP β and δ proteins bind to the PPAR γ and C/EBP α promoters within 1–4 h of adipogenic stimula-

tion in 3T3-L1 cultures. Furthermore, they observed that binding of specific C/EBP proteins to adipocyte genes *in vivo* occurs rapidly after induction of C/EBP proteins and does not correlate with the onset of target gene expression [82]. Remarkably, they reported that several hours later C/EBP β and δ proteins similarly bind to promoters of target genes such as adiponectin, resistin and leptin [82].

The regulatory cascade that characterizes 3T3-L1 preadipocyte differentiation involves the transient expression of C/EBP β and δ , which then activates the expression of PPAR γ [83,84], which, upon ligand activation, induces C/EBP α expression concurrent with differentiation [83,84]. Studies of primary cultures of stromal-vascular (S-V) cells and fetal adipose tissue indicate very different temporal patterns of C/EBP isoforms and PPAR expression during adipogenesis. For instance, PPAR γ and C/EBP α , β , and δ proteins and mRNA are detectable either in preconfluent cultures or within the first day of culture in human, pig and rat S-V cell cultures [56,85–91]. Furthermore, PPAR γ and C/EBP β mRNA were detectable before induction of differentiation in bovine S-V cell cultures [92,93]. Moreover, either no subsequent “peaks” of C/EBP α , β , and δ expression [89,92,93] or concurrent peaks of C/EBP α , β and δ expression are observed [85,86,91]. These studies also show that C/EBP β and δ are expressed before and throughout adipocyte differentiation in S-V cultures in contrast to transient expression of C/EBP β and δ observed in 3T3-L1 studies. Fetal studies also indicate unexpected temporal patterns of expression since C/EBP α and PPAR γ protein expression [94] preceded both adipogenesis and C/EBP β and δ protein expression in fetal porcine adipose tissue [95]. Furthermore, fetal hypophysectomy induced PPAR γ protein expression, adipocyte hypertrophy and lipogenesis with no change in C/EBP β protein levels [94]. Additionally, the development of fetal rat brown adipose tissue (BAT) is characterized by early expression of both C/EBP β and C/EBP α [96]. Early expression of C/EBP isoforms in S-V cultures may simply reflect the expression of these proteins *in vivo*. Alternatively, conditioning of S-V cells *in vivo* by blood borne factors, like hormones or PPAR γ activators, could account for expression of these proteins at the onset of culture.

Thus, the temporal relationships between C/EBP isoform and PPAR γ expression clearly distinguish preadipocyte differentiation in primary S-V cultures from 3T3-L1 preadipocyte differentiation. However, expression of C/EBP δ protein precedes lipid accretion and expression of PPAR γ and C/EBP α proteins in S-V cell cultures [56,85,86,91,97] as in 3T3-L1 preadipocyte

cultures [83]. Furthermore, studies of S-V cell cultures are consistent with the concept that cross-regulation between PPAR γ and C/EBP α controls adipogenesis or maintains the differentiated state [84] since adipocytes express both proteins in S-V cultures, regardless of conditions. Activation and expression of PPAR γ controls adipogenesis in S-V cell cultures derived from both bovine perirenal [98] and subcutaneous [99] adipose tissue depots. Therefore, expression of PPAR γ protein may rescue a proportion or all C/EBP α reactive cells in S-V cell cultures regardless of species and depot. Further studies are necessary to determine the molecular aspects of PPAR γ and C/EBP α cross-regulation of preadipocyte differentiation, and the control of PPAR γ and C/EBP α in dedifferentiated adipocytes (like those documented for S-V cells or 3T3-L1 cells) is unknown.

While C/EBP α and PPAR γ have a close relationship in regulating cell conversion to a viable lipid-assimilating adipocyte, as well as maintenance of the differentiated state of the adipocyte, other endogenous ligands of PPAR γ have recently been identified which may play a role in transdifferentiation of cells of the adipose lineage or in expression of the differentiated phenotype. In a recent review, Kim and Surh [100] illustrated ability of known ligands of PPAR γ to “dock and lock” to PPAR γ . Such activity increases biological activity of PPAR γ at the level of activation of the molecule and subsequent gene transcription. These types of molecules need further definition in their overall availability to exert effects in adipocytes.

3.1.4. Insulin

The presence of insulin is essential for adipocyte differentiation in most depots and in most animals [28,101]. However, preadipocytes express few active insulin receptors, and the effect of insulin on differentiation has been shown to occur through cross-activation of the IGF-I receptor by insulin at pharmacological levels [28,51]. Indeed, as the cell progresses through the differentiation process, IGF-IR mRNA is down-regulated, whereas insulin receptor mRNA is up-regulated [28,102]. Insulin and IGF-I activate several distinct downstream signal transduction pathways, such as ras [51]. Activated ras induces adipogenesis, whereas the reduction of ras diminishes normal differentiation [51]. Paradoxically, ras is a potent activator of the MAP kinase pathway, which can inhibit adipogenesis. Thus, it appears that the timing of ras activation in the differentiation process may prove to be critical in determining whether it will act in a positive or a negative manner [51]. Another downstream effector of insulin action, protein kinase B (PKB, also known as Akt), is a possible mediator of adipogenesis.

Expression of a constitutively activated allele of PKB in 3T3-L1 cells induces their spontaneous differentiation [51]. Thus, insulin is a major stimulator of adipogenesis through the activation of insulin receptor (IR), IR substrate-1 (IRS-1), and the downstream effectors phosphatidylinositol 3-kinase (PI-3K) and Akt [103]. Akt may help regulate adipogenesis and metabolic homeostasis by modulation of a transcription factor activity, as is the case for FOXO1 [103].

3.1.5. Cortisol

Cortisol operates through the glucocorticoid receptor, a nuclear hormone receptor in the same superfamily as PPAR γ , whose transcriptional targets remain largely unclear [51]. In cultured cells, corticosteroids have been shown to induce higher C/EBP δ levels; however, cells that over-express C/EBP δ still require corticosteroids, indicating a more complex role for this compound [51]. Another role of corticosteroids is the reduction of the expression of preadipocyte factor-1 (pref-1), a negative regulator of adipogenesis that is found in preadipocytes [51]. That glucocorticoids induce the expression of C/EBP δ during the differentiation of 3T3-L1 cells suggests that the major role of these steroid hormones in committed adipofibroblasts is to augment the levels of C/EBP δ in order to induce PPAR γ expression [45]. Although the mediation of DEX induced PPAR γ expression by C/EBP β is clearly evident in 3T3-L1 studies, it is less so in studies of S-V cell cultures [51]. For instance, DEX has no essential role in adipogenesis in rat S-V cultures [89], and DEX does not enhance C/EBP β expression in pig S-V cultures [91]. However, a comprehensive study of human S-V cultures indicated that DEX induced a very early increase in C/EBP β protein [86]. In pig S-V cultures, DEX increases an early and concurrent increase in C/EBP α and PPAR γ expression, whereas early major increases in C/EBP β and δ expression were independent of DEX [91]. Furthermore, DEX plus insulin increased PPAR γ -expressing cell numbers and maintained C/EBP α reactive cell number [56,91].

3.1.6. Fatty acids

The ability of fatty acids to regulate PPAR γ may be a fine-tuning mechanism, due to the rather benign manner of fatty acid transport into the cell. However, several fatty acids, and their derivatives, have been identified as natural ligands for PPAR γ [32,36,40,49], although they bind with low affinity [32]. Polyunsaturated fatty acids (PUFAs), are less potent than saturated FA in increasing adipocyte numbers, but are more effective in inducing preadipocyte differentiation, likely due to PUFAs ability to act as direct ligands for PPAR γ [39]. PPAR γ may

be activated by arachidonic acid metabolites derived from the cyclooxygenase and lipoxygenase pathways, such as 15-deoxy-*D*-12,14-prostaglandin J2 and 15-HETE. Fatty acid derived components of oxidized low density lipoproteins, such as 9-hydroxyoctadecadienoic acid and 13-hydroxyoctadecadienoic acid, can also serve as natural ligands for PPAR γ [49]. Additionally, there appears to be a role for 12/15-lipoxygenase in the generation of endogenous PPAR γ ligands [49]. In work done in porcine adipocytes, oleic acid was demonstrated to increase adipocyte differentiation and RNA transcripts of differentiation related proteins such as PPAR γ , C/EBP α , LPL, and aP2 in a dose dependent manner [104]. In pig preadipocytes, arachidonic acid and *cis*-9, *trans*-11 conjugated linoleic acid increased ADD1 mRNA and protein expression thereby differentiation [105].

3.1.7. Cytokines

Cellular levels of PPAR γ may be controlled by a wide variety of factors, including different inflammatory cytokines [49]. Tumor necrosis factor α (TNF α) is involved in pro-inflammation, apoptosis, lipid metabolism, and insulin resistance [106]. Both mRNA and protein expression of TNF α are increased in obesity [106], and a high level of TNF α suppresses C/EBP α (and thereby GLUT-4 gene activation) [106]. TNF α mRNA is over-expressed in the adipose tissue of most obese animal models [50] and can impair insulin receptor signaling, inhibit lipoprotein lipase (LPL), and stimulate lipolysis in adipocytes, which results in an increased availability of fatty acids for muscles and may cause insulin resistance [50,107]. The cytokine IL-6 is expressed by adipocytes and is positively correlated with obesity and insulin resistance [106]. IL-6 have been shown to suppress gene transcription of IRS-1, GLUT4, and PPAR γ [106]. Like TNF- α , IL-6 has been shown to decrease LPL [107], likely due to the ability of cytokines such as TNF- α and IL-6 to decrease PPAR γ expression in adipocytes [49].

3.1.8. Thiazolidinediones (TZDs)

Thiazolidinediones are a class of synthetic compounds that exhibit a strong insulin sensitizing effect [108] and are ligands for PPAR γ [72] that bind with high affinity [40,49,53]. This family of PPAR γ agonists improves insulin sensitivity by increasing transcription of several genes such as lipoprotein lipase, adipocyte fatty acid-binding protein, acyl-CoA synthase, and fatty acid transport protein [40,72] through its action on PPAR γ . TZDs also are associated with increased expression of the glucose transporters 1 and 4 but not through

direct action on glucose transporter gene promoters but though its action as a PPAR γ ligand [108]. TZDs synergistically activate gene expression leading to an increase in lipid partitioning into adipocytes [40]. Additionally, TZDs have been demonstrated to promote the differentiation of preadipocytes by mimicking the effects of insulin on adipocytes and to regulate glucose homeostasis [72]. It should be noted, though, that not all TZD effects are mediated via PPAR γ [29], and that TZDs do not appear as natural mediators *in vivo*.

4. Glucose transporters (GLUTs)

Glucose transport occurs relatively late in the differentiation program and mid-stage in the metabolism cycle, after the expression of PPAR γ and C/EBP [12], but involves a family of integral membrane proteins [109]. There are four Class I glucose transporters (1–4) [109–112]. GLUT-1 is ubiquitously expressed, is responsible for basal glucose uptake [110,112] and displays modest insulin stimulated redistribution to the plasma membrane [109]. GLUT-2, a low affinity glucose transporter, is expressed in the liver, kidneys, and pancreas [110], whereas GLUT-3, a high affinity glucose transporter, is expressed during fetal development and adult neuronal cells [110,112]. GLUT-4, also a high affinity glucose transporter, is highly expressed in adipose tissue and muscle [109,110,112,113] and is insulin-responsive [81,112]. The focus of this section expands on the papers published previously [110,112–118].

5. GLUT-4

Expression of PPAR γ is considered an early event in adipogenesis, while GLUT-4 production or insertion into the plasma membranes of adipocytes is considered an early metabolic event that occurs after cell differentiation in monogastric animals and in some, but not necessarily all, of the adipose depots of the ruminant. GLUT-4 is examined, here, in order to demonstrate that metabolic events occur subsequent to the PPAR γ switch. While the subcutaneous adipose depot in ruminants appears to be more sensitive to acetate as a source of triglyceride backbone than use of glucose, GLUT-4 use as a metabolic example might be diminished for ruminants. Other differences are also apparent in animals. Differences in size, adipocyte number [119], mRNA and protein levels [120], response to cytokines [121] and hormones [28], and changes in other circulating factors have also been documented for different animals. One report notes that the subcutaneous depot is less metabolically active than the visceral depot [122], and yet also appears to accu-

mulate more fat stores in response to TZDs over the visceral depot [123]. Increased fat stores in the visceral depot are linked to insulin resistance and cardiovascular disease [27]. Thus, a more complete re-evaluation of the regulation of adipogenesis is through understanding the regulation involving specific adipose tissue depots cellular ability to acquire insulin responsiveness and thus becoming capable of assimilating glucose.

In vitro models for studying GLUT-4 expression are limited by the small number of cultured cell models that express the GLUT-4 gene [118]. Several studies have demonstrated the presence of separate subpopulations of GLUT-4 containing vesicles different from the constitutively recycling endosome population [109]. This might explain why insulin can stimulate a 2–4-fold increase of several recycling proteins (GLUT-1, IGF-2R) in the plasma membrane, whereas plasma membrane GLUT-4 can increase 10–20-fold [109]. Chronic exposure of 3T3-L1 cells *in vitro* or adipose tissue *in vivo* to insulin, however, has differential effects on GLUT-4 gene expression: rats chronically treated with insulin show increased GLUT-4 adipose tissue mRNA, whereas chronic treatment of 3T3-L1 with insulin resulted in either no change or in a reduction in GLUT-4 mRNA levels [118]. The different responses of GLUT-4 mRNA to chronic insulin treatment suggest GLUT-4 mRNA does not respond directly to insulin [118].

5.1. Biochemistry of GLUT-4

The glucose transporter is a plasma membrane protein of 492 amino acids with 12 hydrophobic transmembrane domains and both amino and carboxyl termini located in the cytosol [110,111,117] with a large intracellular loop between transmembrane domains 6 and 7 [111]. Additionally, there is an extracellular loop predicted between transmembrane domains 1 and 2 that contains a glycosylation site [111]. Glycosylation of GLUT-4 at this site increases the efficiency of glucose transport [111].

5.2. Cycling activity of GLUT-4

In the basal state, GLUT-4 is cycled slowly between the plasma membrane and one or more intracellular compartments, with the vast majority of the transporter residing in vesicular compartments within the cell interior [109]. GLUT-4 is translocated to the plasma membrane in the presence of insulin [110,111] resulting in a 10–20-fold increase in glucose transport [110]. Insulin stimulates glucose uptake by recruiting GLUT-4 from its cytosolic site of sequestration to the plasma membrane [110].

5.3. Regulation of GLUT-4 signaling

About 4 days after the onset of differentiation, 3T3-L1 and F442A cells express high levels of GLUT-4 mRNA similar to the levels found in primary adipocytes or adipose tissue [118]. Unlike primary adipocytes or adipose tissue, though, 3T3-L1 adipocytes also express high levels of GLUT-1 [118]. Chronic exposure of 3T3-L1 cells *in vitro* or adipose tissue *in vivo* to insulin has differential effects on GLUT-4 gene expression: rats treated with insulin showed increased GLUT-4 mRNA in adipose tissue whereas insulin treatment of 3T3-L1 adipocytes resulted in either no change or a reduction in GLUT-4 mRNA levels [118]. These differing responses suggest that GLUT-4 mRNA does not respond directly to insulin action on adipose tissue [118]. The incubation of 3T3-L1 cells in glucose-free medium down-regulates GLUT-4 mRNA approximately 10-fold while up-regulating GLUT-1 mRNA. The addition of glucose to the 3T3-L1 adipocytes restored GLUT-4 mRNA levels. These data are consistent with a metabolic rather than hormonal regulation of GLUT-4 gene expression [118].

5.4. Transcriptional regulation of GLUT-4

Inspection of the GLUT-4 gene has revealed the existence of a binding domain for the MEF2 family of transcription factors belonging to a larger family of MADS-box domain transcription factors [118]. Although these transcription factors have been studied largely during myogenesis, these proteins were shown to bind the GLUT-4 MEF2 binding domain in skeletal muscle, heart, and adipose tissue [118]. Further analysis of the GLUT-4 promoter in 3T3-L1 adipocytes revealed an element responsible for insulin-mediated down-regulation of the GLUT-4 gene [118]. The expression of GLUT-4 is greatly increased during differentiation of the preadipocyte [117]. Despite a lack of a discernable PPRE site or C/EBP binding site in the GLUT-4 gene [116], both PPAR γ and C/EBP α appears to up-regulate GLUT-4 [12,116,117,124]. Additionally, a thyroid hormone response element (TRE) has been described in the promoter region of the GLUT-4 gene [117].

Components of the fat cell differentiation program responsible for establishing insulin-sensitive glucose transport (GLUT-4) seem to require C/EBP α as well as PPAR γ expression [125]. The subprogram(s) leading to insulin sensitivity cannot be driven by expression of C/EBP α or PPAR γ singly, but instead requires synergy to elicit the development of this physiologically important response [124]. Additionally, forced expression of

PPAR γ can differentiate fibroblasts to a morphologically defined adipocyte, but only those cell lines which also express C/EBP α become insulin-responsive [124].

5.5. Cellular regulation

Activation of the insulin receptor triggers a large increase in the rate of GLUT-4 vesicle exocytosis and a smaller rate of internalization by endocytosis [109]. PI-3 is necessary for insulin stimulated GLUT-4 translocation and glucose uptake [109]. Some studies have demonstrated that increased AMP levels as well as G protein coupled receptors can induce glucose uptake [109,126]. PPAR γ is capable of inducing adipogenesis [71], and may regulate lipogenesis by imparting insulin sensitivity to fat cells in responsive adipose depots [72]. PPAR γ is expressed in adipocytes as well as preadipocytes [48]. Increases of PPAR γ in adipose tissue result in increases in GLUT-4 [32]. In the adipocyte, PPAR γ , as well as the transcription factor CCAAT/enhancer-binding protein α (C/EBP α), induces the expression of late-stage-differentiation genes. Adipocyte-specific proteins include adipin, aP2, lipoprotein lipase, and GLUT-4 and will remain elevated for the life of the cell [32,48,78]. PPAR γ is capable of the direct modulation of the insulin-signaling pathway through the up-regulation of several factors (such as the insulin receptor substrate 1 and 2 and the p85 subunit of PI3) of the signaling cascade for GLUT-4 [44], allowing GLUT-4 to function within the cell. Inhibition of adipogenesis results in a decrease of PPAR γ [79].

6. Impact

According to the USDA National Agricultural Statistical Service, in 2004 there were a bit over 35 million head of beef cattle slaughtered in the US. Of these, approximately 14 million head were on feed in the feedlot (for up to 140 days) at any given time. In the beef industry, fat related carcass traits are the major determinants of value and thus any reduction in feed consumption (which accounts for 60–70% of the total cost in a beef operation), while maintaining marbling fat, results in a profound effect on the cost of beef production [127,128]. The role PPAR γ in the alteration of the variables of adiposity in the ruminant animal has practical application as it leads to the consideration of the identification of developmental switching regulators in the development and growth of adipose tissue in a depot-specific manner. On the other hand, as very little is currently known about the importance of adipose tissue GLUT-4 involvement in lipogenesis in ruminant

animals, the involvement of this marker might be more suited for application in other meat animals, like swine. Indeed, fifty-five million head of swine were slaughtered in 2004 and the potential role that GLUT-4 can play in the delicate balance between the amount of desirable “marbling fat” and the undesirable “back fat” that swine put on readily has economic implications for the swine industry. It is known that there are a number of other metabolic markers [12]; however, we suggest that developmental “switching” of a cell needs to occur prior to beginning metabolic operations. Indeed, we also suggest that identification of the regulatory expression patterns and mode of cellular signaling of fat cells (adipocytes) and their precursor cells (preadipocytes/adipofibroblasts) in adipose tissue may reveal avenues to modulate variables of adiposity, or economically important carcass traits, which has broad applications. Historically, a cell has been termed an adipocyte when a fibroblast, adipofibroblast, or preadipocyte expresses markers for lipid metabolism, even though a reversion of mature adipocytes back into proliferative-competent progeny cells has recently been shown to be a possibility [1]. Conversion of proliferative-competent cells into adipocytes is reliant on PPAR γ expression for the genetic switch. GLUT-4 expression, or the expression of other metabolic markers, is needed for optimal glucose metabolism in monogastric animals, but may be depot-dependent in some species like ruminants. This entire mechanism raises the complexity of our understanding of both adipose tissue development and metabolism. Further knowledge is required to fully understand the mechanisms of the involvement of PPAR γ , GLUT-4, and other cellular markers in establishing the differentiated phenotype of adipocyte-type cells in meat animals.

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