Nutritional regulation of gene expression and animal development

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Summary

This paper deals with regulation of animal development, with emphasis on nutritional regulation of gene expression. Regulation of muscle and adipose development, in particular, the roles of myostatin and PPARγ are discussed, as is the endocrine role of adipocytes. The importance of the acid labile subunit (ALS) in the transition from autocrine and paracrine regulation to effective endocrine regulation by the insulin–like growth factor system, and of regulation of placental glucose transport, are also highlighted. These topics are presented as a series of short reviews related to on–going investigations in the laboratories of the authors.

Introduction

Genes produce specific mRNAs which are templates for the production of peptides. These peptides regulate development of cells, accretion of cellular products, or may themselves be products we utilise. Improvements in efficiency of animal production and consumer satisfaction requires an ability to develop desirable animal characteristics. Advances in animal genetics have enhanced our capacity to achieve this objective; however, extrinsic factors such as nutrient supply during development may also have important epigenetic roles to play in determining the suitability of end–products. This review discusses regulation of gene and peptide expression in animal development. The reader is also referred to reviews by Chesters (1998), Hesketh et al. (1998) and Reue (1998) on molecular methods and their application in nutrition.

Placental nutrient transport capacity (R.A. Ehrhardt)

Placental size and transport capacity determine fetal nutrient supply

Consequences of prenatal nutrition for neonatal well–being, postnatal development, and disease in later life are becoming increasingly recognised. Fetal nutrient restriction caused by a variety of nutritional perturbations affects not only fetal growth but also relative growth and development of specific organs and tissues.

Placental size and capacity to transport nutrients to the fetus is an important determinants of fetal nutrient supply. Fetal growth even in well–nourished ewes is constrained during late pregnancy, with much of this constraint imposed by the placenta (Bell 1984). Further evidence of the importance of the placenta in determining fetal nutrient supply comes from studies where placental size and/or transport capacity have been manipulated experimentally (reviewed by Owens et al. 1989). These studies have shown that placental size and the size–independent capacity of the placenta to supply nutrients are critical determinants of fetal growth. Intravenous supplementation of glucose to the fetus during late pregnancy in well–fed ewes increases fetal size, providing additional evidence that the placenta limits fetal nutrient supply (Stevens et al. 1990).

Glucose is a primary limiting nutrient for fetal growth. Its delivery to the fetus is explained by facilitated diffusion kinetics (Widdas 1952) and is dependent on facilitative glucose transport proteins (GLUTs) in the placenta. The insulin–independent GLUT
protein isoforms, GLUT1 and GLUT3, are present in the placentae of various species including sheep (Ehrhardt and Bell 1997). Physiological studies of placental glucose transport capacity are performed extensively in sheep due to the wealth of information available on maternal and fetal glucose metabolism and have provided well characterised models of placental glucose transport. There is increasing evidence that the concentration and relative proportions of these GLUT isoforms has important influences on placental glucose transport capacity and hence fetal growth during late pregnancy.

**Developmental regulation of placental glucose transporters**

The glucose requirement of the sheep conceptus increases six fold from mid to late pregnancy (Bell et al. 1986) and is partially met by an increasing transplacental glucose gradient and, but more significantly, by a six fold increase in placental glucose transport capacity (Molina et al. 1991). The sheep placenta reaches maximum dry mass and DNA content at mid–pregnancy (d 75–80 post coitus) and remains constant to term (Ehrhardt and Bell 1995). Therefore, increased glucose transport capacity cannot be accounted for by alterations in placental mass, but can largely be explained by a more than threefold increase in GLUT protein concentration as estimated by cytochalasin B binding capacity (Ehrhardt and Bell 1997).

During the latter half of pregnancy, there is a decline in the relative proportion of GLUT1 and an increase in the relative proportion of GLUT3 in the sheep placenta (Figure 1). In the ovine maternal–fetal trophoblast tissue barrier critical for maternal–fetal glucose exchange, GLUT3 is located on the apical, maternal surface while GLUT1 predominates on the basolateral, fetal surface (Das et al. 1999). Much GLUT1 is also found in epithelial cells lining the uterine glands in the maternal portion of the sheep placenta (Das et al. 1999). GLUT3 (Km=1.8mM) is a more efficient transporter than GLUT1 (Km=2–5mM) and these properties and their unique localisation pattern suggest a polarised process whereby GLUT3 traps glucose efficiently, due to its low Km, into cells surrounding the fetal vasculature and from which it exits into fetal blood via GLUT1 (Das et al. 1999). Increased relative proportion of GLUT3 during late pregnancy may help direct maternal glucose towards the fetal vasculature at the expense of consumption by the maternal component of the placentome (Ehrhardt and Bell 1997; Das et al. 1999).

**Nutritional regulation of placental glucose transporters**

Effects of chronic physiological alterations to maternal glycemia for varying duration on placental GLUT protein expression have been examined in pregnant ewes rendered hypoglycaemic by continuous insulin infusion or hyperglycaemic by continuous glucose infusion (Das et al. 1998, 1999). Hyperglycaemia markedly increased placental GLUT1 after 48 h with no change in GLUT3, followed by a persistent decline in concentration of both GLUT isoforms after 10 d treatment. Hypoglycemia reduced placental GLUT1 at 48 h which remained depressed for up to 40 d treatment. GLUT3 remained unaffected by hypoglycemia throughout periods of up to 40d. Time–dependent declines in both GLUT1 and GLUT3 during maternal hyperglycaemia may be a mechanism to protect the fetus from glucose toxicity. During prolonged maternal hypoglycemia there is a decline in GLUT1 relative to GLUT3 which may act to partition glucose towards the growing conceptus.

The notion that relative increases in placental GLUT3 could act to partition maternal glucose towards the fetus during maternal hypoglycemia is supported by effects of chronic moderate maternal undernutrition on placental GLUT expression during late pregnancy (Ehrhardt et al. 1998). Twin–pregnant ewes were fed to maintain zero energy balance in maternal tissues (control) or at 55% intake of controls (underfed) for 14 d during late pregnancy. Underfeeding reduced the maternal–fetal glucose gradient while markedly increasing placental glucose transport capacity as measured by compartmental modeling of maternal–fetal 3–O methyl glucose kinetics (Ehrhardt et al. 1996). Increased placental glucose transport due to underfeeding was associated with a marked increase in number of cytochalasin B binding sites and a more modest increase in GLUT3 protein concentration (Ehrhardt et al. 1998). In fact, concentration of cytochalasin B binding sites explained 81% of the variance in placental glucose transport capacity, hence GLUT concentration was shown to be an important determinant of placental glucose transport capacity.

**Figure 1** Proportion of cytochalasin B binding sites (pmol/mg microsomal membrane protein) accounted for by GLUT1 and total cytochalasin B binding sites at d 75, 110 and 140 of pregnancy in the sheep placenta. Means that are significantly different (P<0.01) for GLUT1 are denoted by different letters (a,b) as are those for total sites (by d,e,f).
In summary, there is developmental and nutritionally time-dependent physiological regulation of placental GLUT expression. Placental GLUTs appear to buffer the fetus from the deleterious effects of alterations in maternal glycemia. When faced with a diminished maternal glucose supply, the placenta adapts to fetal glucose demands by increasing glucose transport capacity, in part by increasing the proportion of placental GLUT3. These findings demonstrate that the placenta plays an active role in the partitioning of maternal glucose and underscore the importance of this organ in determining fetal nutrient supply, with consequences for neonatal survival and normal postnatal growth and development.

The circulating IGF system
(Y.R. Boisclair)

There is a transition from autocrine and paracrine regulation towards endocrine regulation as extrinsic factors become increasingly important during fetal and neonatal development. Recent advances in understanding the IGF system detailed below have further elucidated this concept and provided new insight into regulation of the IGF system.

In most mammals, including sheep, the circulating IGF system comprises IGF–I and IGF–II (IGFs) and six IGFBPs (IGFBPs 1 to 6) (Baxter 1994; Ooi and Boisclair 1999). These IGFBPs bind IGFs with high affinity, resulting in little free IGFs in circulation. During most of development in sheep, IGFBP–2, –3 and –4 are easily detected, with IGFBP–3 many times more abundant than other IGFBPs (Carr et al. 1995).

An additional level of complexity in the circulating IGF system is seen when comparing fetal and postnatal plasma by neutral gel filtration chromatography (Baxter 1994; Ooi and Boisclair 1999). In fetal plasma, most of the IGFs is recovered in fractions eluting at ~ 50 kDa which correspond to binary complexes of IGFBP:IGF. After birth, however, most of the IGFs occurs in ternary complexes of ~ 150 kDa consisting of one molecule each of IGF–I or –II, IGFBP–3 and a serum protein called the acid labile subunit [ALS] (Baxter 1994). This shift from 50 to 150 kDa complexes also occurs in the sheep during the perinatal period (Butler and Gluckman 1986).

ALS and the actions of endocrine IGFs

ALS is the driving force behind the formation of the 150 kDa complex, and we hypothesise that ALS is absolutely critical to development of a fully operational endocrine IGF system after birth. A comprehensive view of the biology of ALS can only be obtained by reviewing research in humans and rodents. These studies support the following roles for ALS:

1. Increasing serum IGFs. IGFs are cleared rapidly when they circulate in free form [half-life \( t_{1/2} = 10 \text{ min} \) (Guler et al. 1989; Hodgkinson et al. 1987)]. Association with any IGFBP prolongs their \( t_{1/2} \) to 20–90 min (Zapf et al. 1995). Only after ALS combines with the IGFBP–3:IGF complexes does the \( t_{1/2} \) of IGFs increase to 12–14 h (Guler et al. 1989; Hodgkinson et al. 1987; Zapf et al. 1995).

2. Promoting the endocrine role of IGFs. This increase in \( t_{1/2} \) allows liver-produced IGF–I to accumulate in circulation and to mediate the indirect action of GH (Daughaday and Rotwein 1989; Etherton and Bauman 1998). Increasing serum IGF–I in the form of ternary complexes is essential to its endocrine actions. For example, GH therapy is more effective than IGF–I in GH-deficient rodents, presumably because only GH induces ALS and the formation of the 150 kDa complex (Gargosky et al. 1994).

3. Determining the availability of IGFs to cells. By retaining most IGFs in ternary complexes, ALS prevents IGFs from activating the insulin receptor. IGFs circulate at ~ 1000 fold the concentration of insulin and bind with low affinity to the insulin receptor (Baxter 1994). Binary complexes alone cannot prevent this activation (Zapf et al. 1995). A necessary correlate of this model is regulated release of IGFs from ternary complexes via proteolytic attack or disruption by proteoglycans (Baxter 1990; Blat et al. 1994).

Cloning of the sheep ALS gene

The only work relating to ALS in livestock has been analysis of plasma by gel filtration chromatography (Butler and Gluckman, 1986; Hodgkinson et al. 1987). Direct study of ALS in farm animals has been hindered by lack of reagents: human or mouse cDNAs do not hybridise to ALS mRNA of sheep, pigs or cattle and existing RIAs are specific for human or rodent ALS.

Our initial step to study the biology of ALS in ruminants was to clone the sheep ALS gene (Rhoads et al. 1998). The sheep ALS gene covers ~3.0 kb of chromosomal DNA and is composed of two exons separated by a 977 bp intron. The first exon encodes the first five amino acids while the second exon encodes the remainder of protein. This organisation is identical to that of rat and mouse ALS genes (Boisclair et al. 1996; Delhanty and Baxter 1997b). The sheep ALS cDNA is 80 and 75% identical to human and mouse cDNAs, respectively. The sheep cDNA encodes a protein with a signal peptide of 32 amino acids and a mature protein of 579 amino acids. The mature sheep protein is 76% identical to human ALS and 72% identical to mouse ALS. Sheep ALS also contains 18–20 leucine–rich domains of 24 amino acids each which are thought to confer to ALS its ability to associate with the IGFBP–3:IGF binary complexes.
Regulation of ALS gene expression in the sheep

We first examined the expression pattern of the sheep ALS gene by Northern blotting (Figure 2). In adult sheep, ALS mRNA was detected only in liver, similar to findings in rodents and primates (Dai and Baxter 1994; Boisclair et al. 1996; Delhanty and Baxter 1997a). In the rat, ALS mRNA was also identified in proximal tubule epithelium of the kidney by in situ hybridisation (Chin et al. 1994), but we see no evidence of this by Northern analysis of the sheep kidney. Overall, the present and previous studies indicate that liver is the main site of synthesis of circulating ALS in mammals.

The developmental expression of the ALS gene had previously been examined only in rats. The ALS gene is expressed at very low levels after birth, is induced at ~3 weeks of age and becomes maximal by 42 d of age, and circulating levels of ALS exhibit a similar developmental pattern (Chin et al. 1994; Baxter and Dai 1994). To determine the onset of expression in sheep, total RNA was extracted from livers of normal lambs before and after birth. Expression was not detected at 100 d of fetal life or at birth, but had increased sharply by 12 d of postnatal age [Figure 2] (Rhoads et al. 1998). We now know that high expression starts as early as d 7 of postnatal life. Therefore, ALS gene expression in the sheep occurs earlier than in rats. This difference likely reflects the greater degree of maturity at birth in sheep than in rodents. This early expression of the ALS gene agrees with the occurrence of IGFs in complexes of 50 kDa prior to birth, and in complexes of 150 kDa one week after birth (Butler and Gluckman 1986).

Finally, a group of neonatal lambs was used to study the effect of nutrition on ALS gene expression. Lambs were fed milk replacer at restricted or ad libitum levels from birth until sacrifice at ~ 35 d of age. The restricted level supported average growth of 150 g/d, and the ad libitum level, growth of 337 g/d (Greenwood et al. 1998). ALS was expressed at higher levels in the livers of lambs fed ad libitum (Figure 2). In contrast, fasting or protein deficiency do not alter the abundance

![Figure 2](image-url)

*Figure 2*  Northern analysis of ALS gene expression in the sheep. Total RNA (15 µg/lane) was electrophoresed on agarose/formaldehyde gels, blotted onto a nylon membrane and hybridised to an [³²P]dCTP-labeled sheep cDNA probe. The signal corresponding to the 2.2 kb ALS mRNA is indicated by an arrow.

**Top Panel: Spatial expression.** Total RNA was extracted from various adult sheep tissues.

**Middle Panel: Developmental expression.** Livers were obtained from lambs at the indicated times of fetal and postnatal life.

**Bottom Panel: Nutritional regulation.** Neonatal lambs were fed a milk replacer at restricted or at ad libitum levels until ~ 35 days of age. Livers were then obtained and analysed for ALS gene expression.
of ALS mRNA in the liver of juvenile rats (Dai and Baxter 1994; Takenaka et al. 1996). Therefore, the nutritional regulation of ALS mRNA in neonatal lambs may reflect a species difference or a developmental delay associated with slower growth.

**Future investigations on ALS**

The effects of various factors (development, nutrition, physiological states, etc.) on circulating IGF and IGFBPs have been studied extensively in ruminants. We are interested in the possibility that these factors could also regulate the expression and circulating levels of ALS. Changing the levels of ALS by altering the fraction of IGFs present in the slowly turning–over 150 kDa complexes could contribute to the effects of these factors on circulating IGFs levels. The characterization of the ovine ALS gene, as well as the development of sheep specific reagents, will allow us to test this hypothesis.

**Muscle development (D. E. Gerrard)**

Two factors limiting postnatal muscle growth are the number of myofibres established in utero, and potential for generation of new myonuclei from muscle satellite cells to augment muscle fibre hypertrophy. There is much information on in vitro regulation of myogenesis, yet few existing hypotheses have been tested in vivo in livestock species. This section provides an overview of developments associated with understanding myogenesis and myonuclei accumulation, in particular, the emerging role of myostatin in modulating myogenesis. Although not reviewed here, reported effects on fetal development of administering growth hormone to pregnant sows (Kelley et al. 1995) or steroids to pregnant ewes (Gill et al. 1998), and of culture (Maxfield et al. 1998a) or temporary exposure of embryos to an advanced uterine environment (Maxfield et al. 1998b) are also of potential importance for meat production. However, recognition that genetic selection for rapid growth and musculature results in modification of muscle fibre types, with important consequences for meat quality (reviewed by Klont et al. 1998), must not be overlooked during genetic or epigenetic programs aimed at meeting consumer demands.

**Myogenesis and its regulation in vitro and in vivo**

Control of the myoblast determination is characterised by expression of myogenic regulatory factor (MRF) genes (Ludolph and Koniecny 1995) and may influence muscle cell hyperplasia. However, control of myoblast proliferation and differentiation are probably more important in determining the ultimate number of myofibres at completion of myogenesis which occurs during fetal life in livestock. Proliferation represents an increase in the number of myoblasts, whereas differentiation is that point when myoblasts exit the cell cycle and stop replicating, fuse to form myotubes or myofibres, and express muscle specific contractile proteins. Myoblasts lose their capacity to divide after differentiation and the ultimate number of myofibres is likely to depend upon the number of myoblasts generated prior to differentiation. Furthermore, the number of satellites cells located between the basal lamina and the muscle cell membrane is probably a function of the number of myofibres. Potential means of increasing the number of nuclei in developing muscle are to either delay the onset of myoblast differentiation or to stimulate greater myoblast proliferation prior to differentiation. Although the two events are independent (Spizz et al. 1986), they must be considered equally important when studying the possibilities to increase myofibre and myonuclei number.

Growth factors are important in regulating myoblast proliferation and differentiation (reviewed by Florini et al. 1991). Research in this area has been conducted by treating cultured myoblast cells with transforming growth factor–beta (TGF–β), fibroblast growth factor (FGF) and IGF–I and –II. In general, FGF stimulates proliferation and inhibits differentiation, TGF–β inhibits both differentiation and proliferation, and IGFs stimulate differentiation at low concentrations and proliferation at high concentrations. Unfortunately, most in vitro studies overlook maturation into diversified muscle fibres, an important aspect of postnatal muscle growth. Muscle cells in culture seldom express adult phenotypes, hence, in vivo models are required to study latter stages of myogenesis.

Ashmore et al. (1972) demonstrated that myofibre development involves the formation of primary and secondary muscle fibres. Primary fibres or myotubes (arrowheads, Figure 3) develop first and span the entire length of a developing muscle. In cattle, these fibres form early in development, before 60 d post–coitus (pc), and represent a small proportion of the total number of myofibres. In contrast, secondary fibres (arrows, Figure 3) form after primary fibres (after 70 d) and represent the vast majority of the myofibres. In sheep, primary fibres form from d 32 to 38 pc (Wilson et al. 1992) and secondary fibres until about d 115 pc (Maier et al. 1992). The biphasic development of muscle fibres is well documented and represents an ideal model for studying myogenesis in vivo. In the bovine fetus, histological studies of developing semitendinosus muscle reveal that from 90 to 130 d pc, average muscle fibre cross-sectional area decreases from 100 to 25 µm² due to a 10 fold increase in apparent myofibre number (Gerrard and Judge 1993) which results in many newly–formed small myofibres. Muscle DNA concentration changes rapidly during this period (Hanset 1986) with secondary myofibres continuing to develop from fusion of myoblasts, resulting in changes to the gross differentiation state of muscle. These observations illustrate the quantitative importance of secondary myofibres in fetal muscle development, and demonstrate
that this phase of muscle development, in any species, represents an excellent model for studying control of myoblast proliferation and differentiation in vivo.

**Myostatin and muscle development**

Newborn double–muscled (DM) calves have more myofibres than normal muscled (NM) calves (Ashmore et al. 1974). Recently, it was shown that mutations in the myostatin (GDF–8) gene are responsible for the DM syndrome (McPherron and Lee 1997; Grobet et al. 1997; Kambadur et al. 1997). Myostatin belongs to the TGF–β family of growth factors, powerful modulators of myogenesis through inhibition of proliferation and differentiation. Greater myofibre number in DM than NM fetuses before 90 d pc (Gerrard and Judge 1993) coupled with presumed, non–functional myostatin protein in DM genotypes, suggest control of myogenesis by myostatin during embryonic and fetal development is necessary for normal muscle development. Assuming other positive–acting growth factors (such as FGF or IGFs) continue to stimulate proliferation when the inhibitory effects of myostatin are absent, more myonuclei are likely to be realised in developing muscle and, as a result, more myoblasts should participate in differentiation with establishment of more myofibres. To date, however, few data are available regarding the role of myostatin in regulating myogenesis. Recently, we have demonstrated that myostatin is expressed in a time– and tissue–dependent manner in pigs (Ji et al. 1998) and although detailed characterisation of myostatin expression was not performed, we detected increased myostatin expression between 21 to 49 d pc in whole pig embryos. Porcine

![Figure 3](image.png)

**Figure 3** Morphological changes in bovine muscle tissue during development. Arrowheads and arrows represent primary and secondary muscle fibres, respectively, in 60 d (A), 73 d (B), 95 d (C) and 104 d (D) post coital fetal muscle, and neonatal (E) and adult muscle (F).
secondary myofibres start developing at 40–55 d pc indicating myostatin expression is altered by the differentiation state of whole muscles. Further studies have shown that most gene expression is located where active development of myofibres is occurring within muscle, suggesting that myostatin is truly modulating myogenesis in vivo (Ji et al. 1998).

Muscularity of postnatal animals depends on how much and how fast individual myofibres expand in diameter (compare E and F in Figure 3). In order to produce increasing quantities of contractile proteins, myofibres recruit additional nuclei from determined myoblast–like satellite cells which retain capacity to re–enter the cell cycle to support transcriptional needs. Although certain scenarios including rapid muscle hypertrophy (Greenwood et al. 1997) activate these cells, the mechanism(s) of activation remain elusive. The potential role of myostatin in regulation of postnatal muscle growth has been investigated, although not rigorously. Studies on ontogeny of myostatin expression in perinatal and postnatal porcine muscle reveal expression is greatest at 105 d pc, declines post–partum, then increases and plateaus during later growth (~100 kg LW). Moreover, we have shown that expression of myostatin is greater in muscle of pigs weighing only 50% of the mean littermate weight at birth (Ji et al. 1998). Runt pigs have reduced myofibre and myonuclei development (Dwyer et al. 1994), and develop more fat in postnatal life than their heavier contemporaries (Powell and Aberle 1980) as do low birth weight lambs (Greenwood et al. 1998, 1999).

It is tempting to postulate that myostatin expression is responsible for repressing myogenesis and for limiting satellite cell activity during postnatal development. If initial observations hold true, this would be consistent with data regarding myostatin and both the DM syndrome and fetal growth retardation. Unfortunately, data to support this hypothesis do not exist and, hence, further delineation of the role of myostatin during prenatal and postnatal muscle growth is warranted.

Adipose development and function
(K.L. Houseknecht)

Historically, the adipocyte has been viewed exclusively as an energy storage receptacle; stored fuel easily mobilized in the event of energy deficit. More recently, with the discovery of leptin (Zhang et al. 1994), the role of the adipocyte has expanded to that of an endocrine cell. It seems that adipocytes are able to ‘sense’ whole–body endocrine and nutrient status and respond by secreting factors such as leptin which act in an autocrine, paracrine and/or endocrine manner to regulate appetite and energy metabolism. Leptin regulates appetite at the hypothalamic level and contributes to regulation of whole–body energy homeostasis by central and peripheral mechanisms (Houseknecht et al. 1998a). It is also known that adipocytes synthesise and secrete myriad hormones, growth factors, cytokines, fatty acids and glycerol. Synthesis/secretion rates of many of these are highly correlated with adipocyte size, suggesting developmental regulation and sensitivity to changes in whole–body energy stores. Other bioactive factors released from fat cells include TNF–α, IGF–I, adipin, ACRP–30, acylation stimulating protein (ASP), angiotensinogen and plasminogen activator inhibitor I (Spiegelman and Flier 1996). Although the function of many of these factors is not known, elevated TNF–α expression and release from adipocytes is implicated with whole–body insulin resistance associated with obesity. Another major secretory product of adipocytes is fatty acids which are obviously important in energy homeostasis, however, fatty acids and/or their metabolites appear to have hormonal function as well. Dietary fatty acids regulate expression of multiple genes; mechanism(s) by which this transcriptional regulation occurs are only now being elucidated, and may be tied to key transcription factors in adipocyte differentiation.

Adipocyte differentiation and adipose tissue growth

Adipocyte differentiation and its regulation, and in vitro and in vivo models to study adipocyte differentiation and growth, were recently reviewed by Gregoire et al. (1998). The origin of adipocytes and molecular events leading to commitment of embryonic stem cells to the adipocyte lineage are not well understood. It is known that adipose tissue formation begins during fetal development from mesodermal cell types. However, the rate and degree to which adipose tissue develops prenatally varies widely among species. Adipocyte function and the ability of preadipocytes to differentiate and lipid–fill is dynamic and changes dramatically with aging. Briefly, precursors committed to the pre–adipocyte lineage maintain the ability to grow, but to differentiate must withdraw from the cell cycle. During differentiation, marked changes in cell morphology occur coincident with orchestrated changes in gene expression, including induction of adipocyte marker genes involved in lipid and carbohydrate metabolism. Adipocyte differentiation involves several transcription factors involved in transcriptional regulation of key adipogenic genes. CCAAT/enhancer binding protein alpha (C/EBPα) and peroxisome proliferator–activated receptor gamma (PPARγ) are pivotal. C/EBPα trans–activates promoters of several adipocyte–specific genes; however, C/EBPα expression is not adipose specific, while PPARγ expression predominates in adipocytes. Another member of the C/EBP family, C/EBPβ, also induces adipocyte differentiation in vitro, is induced early in the differentiation process, and differentiation induced by C/EBPβ is mediated by PPARγ. Diverse lipid–like compounds, prostanooids, non–steroidal anti–inflammatory drugs and (TZD) can also activate PPARγ and stimulate adipogenesis.
PPARγ is a key regulator of adipogenesis. It is a member of the peroxisome proliferator–activated receptor subfamily of nuclear hormone receptors (Schoonjans et al. 1996). The PPARγ gene contains three promoters that yield 3 isoforms, γ1, γ2, and γ3 by alternative promoter usage and splicing (Zhu et al. 1993, 1995; Tontonoz et al. 1994b; Fajas et al. 1998). PPARγ functions by forming heterodimeric complexes with retinoic X receptor α, binding to DR–1 sites on DNA, and subsequently activating transcription (Tontonoz et al. 1994a). The sufficiency of PPARγ to drive adipocyte differentiation was proven by studies demonstrating expression and activation of PPARγ in fibroblasts triggered the adipocyte differentiation program (Tontonoz et al. 1994c; Hu et al. 1995).

**Nutritional regulation of adipocyte gene expression: Role of PPARγ**

PPARγ is also important in regulation of obesity and whole–body insulin action. Regulation of PPARγ can occur at the gene expression, ligand availability (endogenous and pharmacological ligands) and PPARγ activity (phosphorylation status) levels. Research has focused on activation of PPARγ, however, few data exist on regulation of PPARγ gene expression. Limited evidence suggests PPARγ expression is nutritionally regulated in various species. PPARγ has been cloned in pigs (Grindflek et al. 1998; Houseknecht et al. 1998b) and cattle (Sunvold et al. 1997) and the sequences are highly conserved between pigs, cattle, humans and mice. PPARγ1 and γ2 isoforms are highly expressed in porcine adipose tissue, although γ1 predominates (Houseknecht et al. 1998b). To date, γ3 has not been identified in livestock. Porcine PPARγ abundance in subcutaneous adipocytes is regulated by nutrition; expression of porcine γ2 but not γ1 mRNA is reduced by restricted food intake or fasting compared to ad libitum feeding (Houseknecht et al. 1998b). Recently, it was found that dietary fatty acids also regulate γ2 expression in swine; expression in subcutaneous adipocytes is up–regulated by supplementation with 18:2 but not 16:0, 18:0 or ω3 fatty acids (Spurlock et al. 1999).

Mechanisms for differential regulation of PPARγ2 versus γ1 gene expression are poorly understood. Limited data are available which characterise the γ1 and γ2 promoters, however, it is known that γ2 contains a binding site for the C/EBP family of transcription factors (Zhu et al. 1995). A recent report (Saladin et al. 1999) also indicates γ2 expression is turned on earlier in adipocyte differentiation than γ1, again suggesting differential regulation of the γ2 promoter. The newly identified γ3 isoform (Fajas et al. 1998) is expressed exclusively in fat and intestine, however, nothing is known about its regulation. Regulation of PPARγ mRNA alternative splicing events is also poorly defined.

It is tempting to speculate that metabolic and/or endocrine modification by nutritional, physiological and pathological states differentially modulate γ1, γ2 and γ3 gene expression and/or activity. Further research is needed to determine the functional importance of the splice variants and to delineate mechanisms that regulate their expression and activation.

**Nutritional regulation of adipocyte gene expression: Fatty acids as ligands for PPARγ**

Data are beginning to accumulate on the role of specific dietary fatty acids in regulation of adipocyte gene expression. Raclot et al. (1997) reported that consumption of dietary n–3 polyunsaturated fatty acids (n–3 PUFA) down–regulated (40–75%) expression of genes involved in lipid metabolism, adipogenesis and food intake regulation in retroperitoneal but not subcutaneous adipose tissue. Genes regulated by n–3 PUFA in retroperitoneal fat included leptin, C/EBPγ, fatty acid synthase, lipoprotein lipase, hormone sensitive lipase and phosphoenolpyruvate carboxykinase. Regulation of expression of these genes was highly correlated with treatment–induced changes in adipocyte size (Raclot et al. 1997). Regulation of the aP2 fatty acid binding protein by PUFA may also be important as it has been implicated in targeting of fatty acids to regulatory elements in the nucleus (Hertzel and Bernlohr 1998).

Another group of fatty acids recently found to regulate adipocyte gene expression, presumably via activation of PPARγ, are conjugated linoleic acid (CLA), which are geometric and positional isomers of linoleic acid found in ruminant meats and milks with anti–cancer, anti–obesity, anti–atherogenic and anti–diabetic properties. Evidence for anti–diabetic effects and links to PPARγ were provided by Houseknecht et al. (1998c). When a mixture of CLA isomers were fed to Zucker (ZDF) pre–diabetic rats for 14 d, impaired glucose tolerance was normalised and serum triglycerides and insulin were reduced, similar to effects seen with TZD treatment (Houseknecht et al. 1998c). CLA treatment induces expression of the PPARγ–responsive aP2 gene in adipose (Houseknecht et al. 1998c) and expression of uncoupling protein 1 (UCP1) in brown adipose and UCP2 in skeletal muscle (Portocarrero et al. 1999). Additionally, we demonstrated that CLA could transactivate PPARγ response elements in transfection experiments (Houseknecht et al. 1998c). These data suggest that CLA can activate PPARγ, and recent work by these authors indicate that the anti–diabetic effects may be specific to certain CLA isomers (Ryder et al. 1999). Thus, dietary fatty acids (and/or their metabolites) may regulate adipocyte differentiation and gene transcription by acting as ligands for nuclear hormone receptors.

**References**

Ashmore, C.R., Robinson, D.W., Rattray P. and Doerr, L. (1972). Biphasic development of muscle fibers in fetal...
lamb. Experimental Neurology 37, 241–255.


Spiegelman, B.M. (1994a). Adipocyte–specific transcription factor ARF 6 is a heterodimeric complex of two nuclear hormone receptors, PPAR \( \gamma \) and \( \gamma_2 \): genetic mapping and differential expression of the two isoforms. Biochemical and Biophysical Research Communications 239, 857–861.


