Control of Adipocyte Growth and Adipocyte Control of Animal Growth

Harry J. Mersmann*

Adipocyte Growth and Differentiation

Metabolism. Historically, adipose tissue was considered to be a metabolically inactive energy storage depot for intake in excess of energy utilization. Considerable conceptual change has occurred over the years. Investigators began to decipher the metabolic pathways in adipocyte de novo fatty acid biosynthesis about 35 years ago; information about cattle, pigs and sheep appeared slightly later (Vernon, 1981; Mersmann, 1986; Smith and Smith, 1995). Information about the biosynthesis of the main storage lipid, triacylglycerol (i.e., triglyceride) began to be published about 25 years ago, including some data regarding cattle, pigs, and sheep (Vernon and Clegg, 1985; Mersmann, 1986; Rule, 1995). Investigations regarding the degradation of adipocyte lipid began to appear in the biomedical literature in the 1960s and slightly later in cattle, pigs and sheep (Mersmann, 1986,1990). Aspects of pharmacological control and receptor biology in the agricultural species became known in the 1980s and 1990s (Carey, 1995; Etherton et al., 1995; Mills and Mersmann, 1995).

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The literature cited is to a large extent review articles and is not comprehensive, but is intended to provide the interested reader with access to the literature in these areas of considerable experimental activity.

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Isolated cells as research tools. Isolated adipocytes were first prepared in the early 1960s, providing a major tool to study metabolism in vitro, under controlled conditions (Rodbell, 1964); these cells provide no information about differentiation. A clonal cell line (3T3-L1) was derived from mouse cells; these cells multiply in culture as fibroblast-like cells, and differentiate upon introduction of appropriate adipogenic factors such as insulin or glucocorticoids (Green and Kehinde, 1975). Many of the current concepts about adipocyte growth and differentiation were formulated from investigations of clonal cell lines.

Another valuable tool in vitro is the use of adipocyte precursor cells isolated from adipose tissue, and grown and differentiated in culture. These stromal-vascular (S/V) cells are more complex than clonal cell lines because multiple cell types are present and the degree of differentiation is less than that of most clonal cell lines (Bjorntorp et al., 1980; Hausman et al, 1984; Suryawan and Hu, 1995). However, S/V cells may be more representative of the physiology in vivo than clonal cell lines that have undergone changes over the many years of passage, and S/V cells may be isolated from cattle, pigs and sheep (Suryawan and Hu, 1995).

Clonal and S/V cell cultures allow the quantification of the effects of individual hormones and growth factors on cell multiplication and differentiation, including the filling of adipocytes with lipid. Cell culture allows investigation of the effects of concentration, interactions between factors, and temporal relationships. Many growth factors modify hyperplasia and/or differentiation (Figure 1); insulin, glucocorticoids and cAMP are among the most active adipogenic factors (Hausman et al., 1993; Suryawan and Hu, 1995).

Utility. Investigation of the metabolism of the adipocyte has led to an understanding of most of the endocrine and nutritional controls modulating the growth and development of adipocytes, including the temporal patterns of development. These studies allowed mechanisms to be formulated and tested for the modification of carcass composition by β–adrenergic agonists, somatotropin, and sex steroids. Cell culture allowed the experimental study of cell hyperplasia and the control of differentiation by growth factors. Understanding how these influences affect the growing animal is the ultimate goal of all this research,

however, the complexity of the intact animal precludes details of mechanism in most studies in vivo.

Role of Adipocyte Gene Function in Control of Growth and Differentiation

Transcription Factors. The temporal pattern during differentiation is the result of coordinated sequential transcription of various genes to produce the RNA messages that guide the synthesis of the coded proteins. Transcription factors are individual cellular proteins that bind to specific DNA sequences (response elements) of a particular gene and modify the rate of transcription of the mRNA for that gene. Increased mRNA usually results in increased translation into protein.

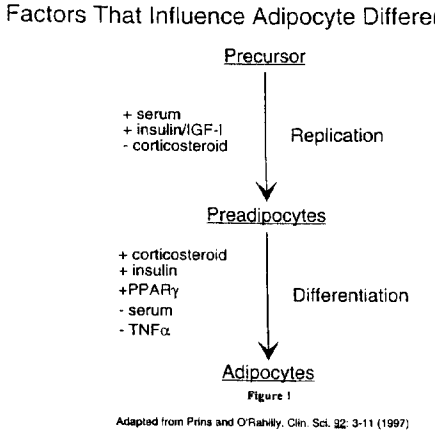
The role of various transcription factors in adipocyte differentiation evolved from observations of the temporal pattern of the factors during differentiation; overexpression of factors in transgenic mice; transfection of the coding sequence for the factor into cells leading to overexpression of the factor; and removal of the factor in knockout mice. The extensive research on adipocyte differentiation has been reviewed many times (e.g., MacDougald and Lane, 1995; Brun et al., 1996, 1997; Kovacs and Graves, 1996; Lemberger et al., 1996; Schoonjans et al., 1996a, 1996b; Spiegelman and Flier, 1996; Hwang et al., 1997; Loftus and Lane, 1997; Mandrup and Lane, 1997.

Ectopic expression of some differentiation-regulating proteins (e.g., the transcription factor peroxisome proliferator-activated receptor γ = PPARγ) can induce adipogenesis, sometimes even in nonadipogenic tissues (Tontonoz et al., 1994). However, the exact physiological role of each transcription factor is not clear because the high degree of homology within both the CCAAT enhancer binding protein (C/EBP) and PPAR families of transcription factors allows functional replacement of a factor by one of its homologs, particularly when overexpressed.

Descriptions of the details of temporal patterns and interactions between transcription factors vary among laboratories because of the use of different clonal cell lines and different culture conditions. Individual species probably operate with variations of the general temporal sequence, utilize alternative members of a transcription factor family, or replace one transcription factor family with another to achieve the same general effect.

The C/EBP family of transcription factors has been implicated for a number of years in adipocyte differentiation (Cao et al., 1991; McDougald and Lane, 1995). The C/EBP proteins form homodimers that bind to a DNA sequence that is CCAAT, or some closely related sequence; binding of a C/EBP protein to such a sequence or response element changes the rate of transcription of the gene. For example, C/EBPa can bind to the DNA for αP2 (adipocyte fatty acid binding protein) to increase the mRNA concentration, resulting in increased αP2 protein synthesis. The

Figure 1

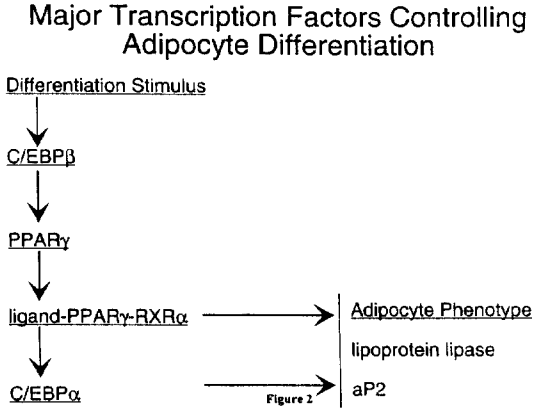


Adapted from Prins and O'Rahilly, Clin. Sci. 92: 3-11 (1997)

C/EBPa is antimitotic and may play a role in the cessation of hyperplasia in differentiating adipocytes (McDougald and Lane, 1995).

Figure 2 depicts a simplified description of the temporal pattern of adipocyte differentiation, beginning with stimulation of the adipocyte precursor cell with the proper adipogenic factors (e.g., insulin, glucocorticoids, cAMP). The cell then begins to increase the transcripts for C/EBPβ (and C/EBPδ), leading to increased translation and synthesis of the protein(s). The increased C/EBPβ protein directly or indirectly impedes hyperplasia, and directly acts as a transcription factor for PPARγ, the predominant adipocyte PPAR. The PPARγ can form a heterodimer with the retinoid X receptor α (RXRα), and after binding of an appropriate ligand (a specific prostaglandin or perhaps a fatty acid),

Figure 2



act as a transcription factor for some adipocyte genes. For example, ligand-PPARγ -RXRα is a transcription factor for the lipoprotein lipase gene, resulting in increased lipoprotein lipase mRNA and protein. The PPARγ can also act as a transcription factor for C/EBPα to increase its transcription and translation. The C/EBPα can positively regulate its own production and has been implicated in maintenance of the adipocyte phenotype. Some proteins that char-

characterize the phenotype of the adipocyte have response elements for C/EBPα to regulate the transcription rate, e.g., glucose transporter 4.

Other transcription factors, such as C/EBPδ or adipocyte determination and differentiation- dependent factor 1 (ADD1), enhance adipocyte differentiation, but when overexpressed, do not by themselves induce differentiation; they appear to increase the activity of other factors or modulate the transcription of other factors, such as PPARγ (Kim and Spiegelman, 1996; Hwang et al., 1997). Retinoic acid is an inhibitor of adipogenesis via its interaction with retinoic acid receptor (not RXRα, the protein that binds to PPARγ to form the active heterodimer); the inhibition appears to involve decreased transcription of C/EBPβ (Schwarz et al., 1997).

Utility. Understanding which transcription factors provide the principal controls for adipocyte differentiation in a particular species will allow the manipulation of key transcription factors, resulting in positive or negative control of adipocyte growth. Perhaps the most amenable area will be to provide the cell with a specific positive ligand to enhance the function of a transcription factor, or a negative ligand to impede the binding of the normal ligand. Delivery will have to be at the correct time and to the desired tissue. Conceivably, these approaches could be used not only to inhibit deposition of fat , but to stimulate fat deposition at critical periods of the life cycle to produce animals of desired body composition. More complex technologies involve transgenic animals, genetic selection for or against a cell specific transcription factor (PPARγ might be adipocyte-specific enough to fit this definition), or gene therapy approaches.

Role of Fatty Acids in Adipocyte Growth and Differentiation

Fatty acids and genes. More than two decades ago, researchers found that high-fat diets suppress rates of fatty acid (FA) synthesis in mammalian liver and/or adipose tissue. Polyunsaturated FA, which are usually more potent than saturated FA, decrease the steady-state concentrations of mRNA for several enzymes involved in FA biosynthesis (Clarke and Jump, 1996; Goodridge et al., 1996). Although a high-fat diet suppresses de novo synthesis of FA, the adipocyte concomitantly undergoes hypertrophy, using dietary FA to synthesize triacylglycerol.

Adipocyte differentiation is modified by FA. In Ob 1771 clonal cells, FA increase the steady-state concentration of transcripts for adipocyte fatty acid binding protein and lipoprotein lipase; transcript concentrations are dependent on FA concentration and chain length (Amri et al., 1991, 1994).

Many of the effects of FA on adipocyte differentiation may be mediated by PPARγ via the FA- PPARγ -RXRα complex (Chawla et al., 1994; Vidal-Puig et al., 1997). Perhaps the PPARγ 2 isoform is more important than PPARγ 1 (Tontonoz et al., 1994), but tissue and individual variations occur in isoform distribution (Mukherjee et al., 1997). Ligand-induced PPARγ stimulation of differentiation of human S/V cells isolated from different adipose tissue depots suggests a mechanism for differential adipose tissue depot growth within a species and even between species (Adams et al., 1997). There are distinct ligands for the members of the PPAR family (Krey et al., 1997), including distinctions between the FA specificity for PPARα and PPARγ (Kliewer et al., 1997).

Different PPAR may control the tissue-specific response of lipoprotein lipase transcripts. The PPARα is prevalent in liver, and PPARγ is prevalent in adipocytes. In rats, fenofibrate, a preferential activator of PPARα increased lipoprotein lipase activity in liver but not in adipose tissue, whereas BRL 49,653, a preferential activator of PPARγ increased lipoprotein lipase activity in adipose tissue but not liver (Schoonjans et al., 1996b). The PPAR mechanism may not account for regulation of some hepatic lipid metabolism genes by polyunsaturated FA (Clarke and Jump, 1996); nevertheless, it provides a working mechanism for continued investigation, particularly in the adipocyte.

Utility. If a specific FA can control genes involved with adipocyte differentiation, it is conceivable that a FA could be added to the diet at certain stages of development to increase or decrease adipocyte growth, differentiation, and hypertrophy. One can envision the use of dietary FA composition and concentration to control the deposition of fat in the carcass in both a positive and negative manner.

The Adipocyte as an Endocrine Organ

The concept of the adipocyte as an endocrine organ has emerged recently (Figure 3). Several different hormone-like substances, e.g., insulin-like growth factor I (IGF-I), tumor necrosis factor α (TNFα), and leptin, are released by the adipocyte to act on adjacent cells or to enter the plasma to act on distant targets.

Leptin. This relatively small protein (167 amino acids) is produced and secreted by the adipocyte. Leptin is produced in the later stages of adipocyte differentiation; its mRNA concentration is increased by overexpression of the transcription factor, C/EBPα (Brouse et al., 1996). Leptin was discovered in an obese mouse model, the *ob/ob* mouse (Zhang et al., 1994), that does not produce active leptin. Intraperitoneal or intracerebroventricular injection of *ob/ob* mice with leptin causes a decrease in feed intake, an increase in energy expenditure, and a reduction in obesity. Injection of leptin into nonobese mice also reduces feed intake (Halaas et al., 1997). Plasma leptin is present in free form as well as bound to several different proteins (Houseknecht et al., 1996)

Leptin receptors. Receptors for leptin are present in many tissues (Tartaglia, 1997). These receptors exist in sev-

Figure 3.

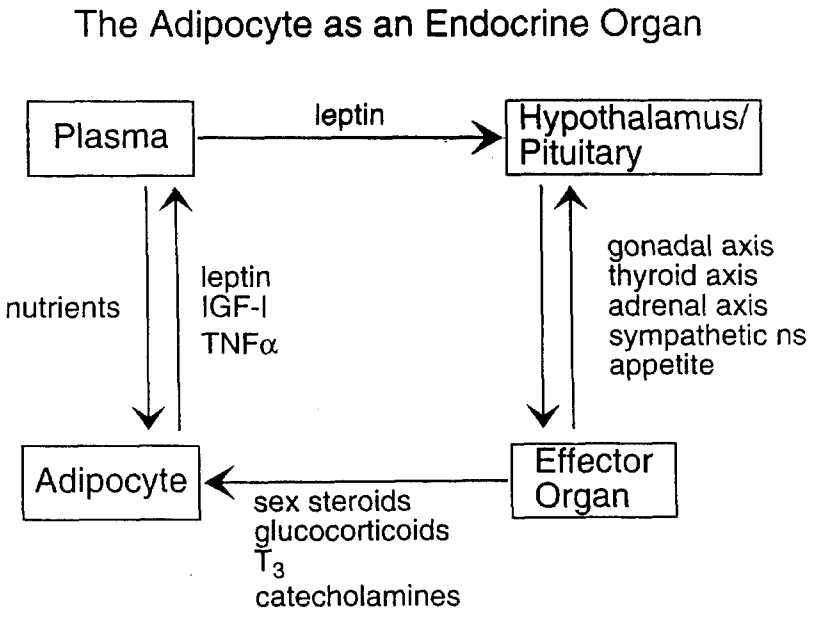


Figure 3
Adapted from Prins and O'Rahilly. Clin. Sci. 92: 3-11 (1997)

several isoforms produced by alternative splicing of the mRNA. Only the long form of the receptor, as present in the hypothalamus, has intracellular signaling capacity. The shorter forms may function in the transport of leptin, including passage across the blood-brain barrier. Receptors for leptin in the hypothalamus, the portion of the brain controlling appetite, are presumably activated by leptin to control feed intake. The obese *db/db* mouse produces leptin, but has a defective leptin receptor, negating activity of the circulating leptin. Injection of these mice with leptin does not decrease feed intake.

Mammals with chronic high plasma leptin concentrations may become leptin-resistant, analogous to some diabetics who have very high plasma insulin, but low insulin activity at cells in the periphery. Leptin resistance could result from defects in transport into the brain, the leptin receptor (as in the *db/db* mouse), intracellular signal transduction, the effector mechanism (e.g., neuropeptide Y), or some combination of defects.

The voluminous literature about leptin and leptin receptors, including their proposed role in the control of appetite and obesity, has been reviewed many times (e.g., Campfield et al., 1996; Hamann and Matthaei, 1996; Spiegelman and Flier, 1996; Ahren et al., 1997; Considine, 1997; Flier, 1997; Hwang et al, 1997; Leibel et al., 1997; Tartaglia, 1997).

Factors modulating plasma leptin. Plasma leptin concentration is highly correlated with the amount of adipose tissue in several species, including pigs (e.g., Campfield et al., 1996; Hamann and Matthaei, 1996; Ahren et al., 1997; Considine, 1997; Spurlock et al., 1998). Plasma leptin fluctuates with the fluctuation of the adipose tissue mass. Thus, plasma leptin is envisioned as a signal to the central nervous system (CNS) to decrease feed intake when the animal has adequate fat stores. However, it increases with age, independent of body fat (Li et al., 1997).

Leptin is gender-associated, with greater concentrations in females than males. In both sexes, the plasma concentration is dependent on the amount of body fat; however, the slope of the relationship is greater in females. Adjustment for body fat does not negate the sex difference. Apparently, the sex difference is not dependent on sex steroid hormones (Weigle, 1997).

There is a diurnal variation in plasma leptin, with the peak at approximately 2300 to 0200 hours, preceding the corticosteroid peak (Ahren et al., 1997). Insulin stimulates expression of the mRNA for leptin in adipocytes and increases the secretion of leptin from adipocytes, both in vitro and in vivo. Fasting causes a decrease in leptin concentration, perhaps resulting from the decreased insulin concentration (Campfield et al., 1996; Ahren et al., 1997). In contrast to this evidence for insulin control of leptin, in humans, insulin does not have an acute effect on leptin secretion and there is no leptin abnormality in human glucose intolerance or in diabetes (Ahren et al., 1997). Thus, the physiological relationship between insulin and leptin remains to be elucidated. Beta-adrenergic agonists cause a decrease in leptin concentration (Trayhurn et al., 1996), as does somatostatin (Ahren et al., 1997); these agents provide a potential counterregulatory mechanism to the insulin effects.

Control of appetite. Leptin decreases the expression of the mRNA for neuropeptide Y (NPY), a peptide of the central nervous system (CNS) that stimulates feeding (Loftus et al., 1997; White and Martin, 1997). Thus, it is envisioned that the mechanism for appetite control by leptin is

NPY. However, leptin decreases the mRNAs for several other CNS peptides that stimulate feeding behavior (melanin-concentrating hormone, proopiomelanocortin and galanin), and increases the mRNA for neurotensin, a CNS peptide that inhibits feeding behavior (Sahu, 1998). Appetite control is extremely complex, involving the interaction of many CNS and peripheral factors (Rowland et al., 1996). Consequently, the mechanism for leptin regulation of appetite is perceived as complex and probably quite convoluted.

Additional effects of leptin. The *ob/ob* mouse is sterile; the female has no estrus cycles. Injection of leptin results in these mice gaining the ability to reproduce. Leptin does not appear to be the cause of pubertal development in rats, but acts as a metabolic gate in a permissive fashion to allow the development of puberty (Cheung et al., 1997). A single injection of leptin increases the low rates of oxygen consumption in *ob/ob* mice and shifts the metabolic fuel toward fat, i.e., a lower respiratory quotient (Hwa et al., 1997). Leptin overexpression in rats increases fatty acid oxidation and decreases esterification in pancreatic islets; mRNAs for fatty acid oxidation enzymes and for uncoupling protein-2 (a thermogenic factor) are increased and mRNAs for lipid synthesis enzymes are decreased (Zhou et al., 1997). In several white adipose tissue depots, the mRNA for uncoupling protein-2 is increased. These changes suggest mechanisms to decrease lipid deposition and increase energy utilization. Intracerebroventricular infusion of leptin increases adipocyte apoptosis, providing a potential CNS effect on the amount of body fat (Qian et al., 1998). Because leptin receptors are present in numerous cell types, additional potential peripheral mechanisms will become evident. The physiological role of leptin remains to be sorted out; at the moment, experimental evidence is being accrued to suggest numerous activities, some of these functions will be non-physiological because of experimental design or leptin dosage.

Leptin in agricultural species. Leptin has been cloned from cattle (GenBank sequence file U50365), pigs (Bidwell et al., 1997; Mendiola et al., 1997; Ramsay et al., 1998), and sheep (Dyer et al., 1997a). The sequences are highly homologous to each other and to rodent and human leptin. Porcine adipose tissue leptin mRNA concentration varies with body weight and the amount of fat (Spurlock et al., 1998); leptin mRNA and plasma leptin are greater in genetically obese pigs than in lean pigs (Ramsay et al., 1998). The leptin receptor mRNA is expressed in ovine hypothalamus, anterior pituitary gland and adipose tissue (Dyer et al., 1997 b). Leptin infused into the third ventricle of pigs (near the hypothalamus) causes a reduction in feed intake (Barb et al., 1998).

Other products of the adipocyte. In addition to leptin, the adipocyte produces and secretes IGF-I and IGF-II (Ramsay, 1996). Although the physiological role of the IGFs and their binding proteins in the adipocyte is not totally clear, IGFs are adipogenic factors for adipocytes. The IGFs also have effects in many tissues peripheral to the adipocyte, suggesting the possibility of endocrine function of adipocyte-produced IGF. The adipocyte also secretes TNF-α. This cytokine molecule is a major factor in cachexia, the pathological wasting condition accompanying a number of severe diseases. In wasting, there is a major loss of adipose tissue; TNFα appears to decrease anabolic lipid metabolism and associated enzymes and may increase catabolic lipid metabolism (Argiles et al., 1997). In addition to modification of adipocyte lipid metabolism, TNFα secreted by the adipocyte may act as a signal, like leptin, to indicate the state of adiposity. Obese mammals overexpress TNFα in adipose tissue. Perhaps obesity is a TNFα resistant condition, similar to leptin and insulin resistance.

Utility. It may be envisioned that secretory products of the adipocyte will be used to regulate the growth of mammals at particular times in the life cycle. Exogenous proteinaceous materials or positive or negative ligands to modulate receptors may be administered; antibodies may be used to regulate the concentration of a proteinaceous factor; transgenic animals may be produced to express a particular gene only at a selected time in the life cycle and in specific tissues; and perhaps gene therapy techniques will deliver a desired gene to a specific tissue. For example, leptin or leptin-like activity might be increased to reduce feed intake in situations where ad libitum feeding yields excessive fat production, whereas leptin or leptin-like activity might be decreased (perhaps with an antibody) to elevate feed intake, e.g., cattle on low energy diets or modern pigs with considerable muscle growth potential whose growth may be limited by protein or energy intake.

Conclusions

The progress in regulatory biology has been astounding over the last decade. Many new avenues to modify growth of species raised as a source of protein for humans will become available in the future. In the next decade, the regulation of adipocyte precursor cell division, entrance into the preadipocyte stage, and final differentiation will be better understood. Much will be known about various nutrient-gene interactions. Diets then can be formulated to modulate hyperplasia or hypertrophy of particular tissues, including adipose tissue, at specific stages of growth. Because the requirements for body fat are different at various stages of the life cycle and under some environmental conditions, the amount of body fat will be tailored to the animals' needs. Knowledge of the regulation of appetite along with the peripheral and central nervous system signals will continue to emerge. Thus, signals to increase or decrease appetite, as appropriate in a particular life cycle stage, eventually will be controlled.

Multiple approaches to accomplish such tasks may be envisioned including genetic selection, transgenic animals, gene therapy, active and passive immunization, and use of both small molecular weight positive and negative ligands

and proteinaceous effectors.

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