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Adipogenesis and Obesity: Rounding Out the Big Picture

Review

Bruce M. Spiegelman* and Jeffrey S. Flier†
*Dana-Farber Cancer Institute and the Department of Cell Biology
Harvard Medical School
44 Binney Street
Boston, Massachusetts 02115
†Division of Endocrinology
Beth Israel Deaconess Medical Center and the Department of Medicine
Harvard Medical School
330 Brookline Avenue
RN 325
Boston, Massachusetts 02215

Introduction

The ability to assure continuous availability of energy despite highly variable energy supplies in the environment is a major determinant of the survival of all species. For higher organisms including mammals, a solution to this problem involved development of the capacity to efficiently store excess energy as triglycerides in adipose cells, from which stored energy could be rapidly released for use at other sites. In addition, physiological mechanisms arose to enhance survival by stabilizing adipose energy stores during prolonged periods of deficient or excess nutritional availability. The outlines of such physiological systems are simple: with prolonged nutritional deprivation, falling energy stores are sensed, leading to increased food-seeking behavior and decreased energy expenditure; with prolonged nutritional abundance, food intake is reduced and energy expenditure is increased to avoid excessive energy storage, i.e. obesity. The systems involved in such adaptations are referred to as systems for regulated energy balance.

A physiological system for regulation of energy balance and avoidance of obesity would be expected to have several requirements. The first is a mechanism by which information on the level of energy stores in adipose tissue can be sensed, and the resulting information relayed to regulatory sites elsewhere in the body. Although a number of candidate mechanisms have been described for a feedback signaling system reflecting the amount of adipose energy stores (Schwartz et al., 1994), the existence of such a mechanism was made certain by the cloning of the *ob* gene and identification of its encoded protein leptin (Zhang et al., 1994; see below). The second requirement is a mechanism whereby such a signal can be received and integrated at one or more regulatory sites, most likely in the central nervous system. The hypothalamus has been known to be a key brain region for regulation of metabolism and energy expenditure, and a variety of chemical or physical lesions in this region can produce dramatic syndromes of hypo- or hyperphagia accompanied by severe metabolic disturbance (Bray et al., 1990). Identification of the leptin receptor as the product of another obesity gene (*db*; Tartaglia et al., 1995), and demonstration of its expression in the hypothalamus (Chen et al., 1996; Lee et al., 1996), provides a key link between peripheral signals and the central pathways involved in the regulation of energy balance. The third requirement is a mechanism by which signals from the periphery and higher brain centers can act to influence the major physiologic determinants of energy balance: regulation of energy intake, and regulation of energy expenditure. Hunger and satiety are the result of complex neural events that involve actions by and interactions between an expanding array of neuropeptides and neurotransmitters. Energy expenditure is the consequence of myriad metabolic events at the cellular level, and can be divided into those energy consuming processes that are related to the support of cellular functions and physical work on the one hand, and those, on the other, that exist solely for the purpose of regulating energy balance, such as so-called adaptive thermogenesis. The latter, at least in rodents, involves the function of brown adipose tissue (BAT; Lowell and Flier, 1996). Unlike white adipose tissue, which is designed for energy storage, BAT is designed for the regulated expenditure of energy as heat. Neural signals that emanate from the hypothalamus and are transmitted by the autonomic nervous system regulate this highly innervated tissue, which employs the uncoupling protein (UCP), a 32 kDa protein of the inner mitochondrial membrane to uncouple mitochondrial respiration (Jezek et al., 1994).

Despite this highly regulated physiological system, it is evident that obesity is currently an exceptionally common problem in humans, and the search for causal factors has been intensifying. The last several years have produced breakthroughs in two obesity-related areas of investigation: the identification of genetic loci at which mutations cause obesity in rodents, and the identification of transcription factors that regulate the differentiation, pattern of gene expression, and lipid content of fat cells. This review is intended, first, to provide an up-to-date perspective on advances in these two areas. The second goal of this review will be to describe recent work that may begin to link these previously distinct areas of investigation, that is, to explain how a disequilibrium in energy homeostasis caused by obesity genes may ultimately result in changes in fat cell number and lipid content.

Obesity Genes

The identification of five genes responsible for distinct syndromes of spontaneous monogenic obesity in mice (Table 1) has advanced our knowledge dramatically, and together with insights obtained through induced mutations, a preliminary view of a "wiring diagram" for the control of body weight is beginning to take shape. The pace of progress has been extremely rapid, and this discussion will therefore be selective.

The ob *and* db *Genes*

The identification of the *ob* (Zhang et al., 1994) and *db* (Tartaglia et al., 1995) genes over the past 18 months has provided the first genetic framework on which future understanding of body weight regulation and obesity

Table 1. Monogenic Obesity Syndromes in Mice

Gene	Chromosome/ Inheritance	Gene Product	Where Expressed	Mode of Action	Human Disease Analog
ob	6/AR	Leptin	White adipose tissue	Absent signal of energy stores	No coding mutants found; ?? linkages in some groups
db	4/AR	Leptin receptor	Hypothalamus; choroid plexus; elsewhere	Absent reception of above signal	No coding mutants found (n = 5)
tub	7/AR	Hypothalamic protein	Hypothalamus	?? role in hypothalamic signal pathway	???
A^y	2/AD	Agouti	Ubiquitously in mutants, skin in normal mice	?? antagonize melanocortin signal in CNS or other sites	???
fat	8/AR	Carboxypeptidase E	Endocrine/neuroendocrine tissues	?? altered CNS neuropeptide, such as MCH	? 1 patient with similar syndrome (O'Rahilly et al., 1995)

AD, autosomal dominant; AR, autosomal recessive.

can be built. The products of the *ob* and *db* genes constitute a hormone-receptor pair (leptin and the leptin receptor, respectively) that provides molecular identity to one system through which the status of energy stores is signaled to the brain. Total inability to produce leptin (*ob*/*ob*) or respond to it (*db*/*db*) result in profound, early onset obesity with persistently excessive food intake, inappropriately decreased energy expenditure, severe insulin resistance, and genetic background–dependent diabetes. Repletion of *ob*/*ob* mice with recombinant leptin is remarkably effective at reversing all aspects of the syndrome so far studied over a period of days to weeks (Halaas et al., 1995; Pellymounter et al., 1995; Campfield et al., 1995; Chehab et al., 1996). Weight loss following leptin treatment of *ob*/*ob* mice results from both reduction in food intake and increased energy expenditure (Schwartz et al., 1996; Halaas et al., 1995; Pellymounter et al., 1995). Energy expenditure is mediated by both increased physical activity and activity-independent thermogenesis, which in part appears to involve activation of brown adipose tissue (Collins et al., 1996). Reversal of insulin resistance and diabetes in *ob*/*ob* mice by leptin is in part a consequence of reduced food intake and body weight, but the rapid time course and dose response for such effects suggests that additional actions of leptin may be involved, acting either directly through peripheral sites, or more likely, through the brain (Schwartz et al., 1996). Although administration of exogenous leptin can limit the obesity that occurs when certain strains of normal mice are exposed to high fat diets (diet-induced obesity) (Campfield et al., 1995), the doses required are high compared to those that are effective in *ob*/*ob* mice, and it is not yet established whether physiological resistance of normal mice to obesity caused by high fat feeding or other environmental stimuli is mediated by increased levels of endogenous leptin. High levels of circulating leptin and what is now being referred to as "leptin resistance" are found in the vast majority of obese humans so far reported (Considine et al., 1995; Maffei et al., 1995).

How Does This System Work?

In the fed state, leptin is expressed and secreted by adipocytes in proportion to their triglyceride stores, and circulates in the blood where its levels correlate to a remarkable degree with the extent of obesity (e.g. Frederich et al., 1995a; Maffei et al., 1995; Considine et al., 1995). It is likely that an unidentified intrinsic factor such as an intracellular metabolite or another signal whose abundance is linked to adipose stores is the major determinant of leptin expression. In addition, a number of factors that are extrinsic to the adipocyte can regulate leptin expression both in vitro and in vivo, over a period of hours, in some cases changing leptin expression out of proportion to changing fat stores. These include insulin (MacDougald et al., 1995a), glucocorticoids (Slieker et al., 1996), beta adrenergic ligands (Mantzoros et al., 1996), and cytokines (Grunfeld et al., 1996). Although the putative role of leptin as a long term integrative signal of energy stores suggested that leptin levels would change slowly, it appears that short term changes in leptin levels may have important consequences as well.

The functional analysis of the leptin promoter is at an early stage; functional C/EBP binding sites are present (He et al., 1995; Hwang et al., 1996). Thiazolidinediones reduce leptin mRNA (Zhang et al., 1996), consistent with involvement of PPARγ on the leptin promoter (Kallen and Lazar, 1996). Cloning and identification of the leptin receptor DNA reveal it to be a member of the class I cytokine receptor family that signals, at least in part, through the Jak/STAT pathway (Ghilardi et al., 1996). The leptin receptor gene is complex, producing multiple splice variants expressed at very low levels in a tissue specific manner (Lee et al., 1996). The *db* allele encodes a mutation in the non-coding region that alters splicing and results in deletion of most of the intracellular, signaling domain (Chen et al., 1996; Lee et al., 1996). The predominant site in which this "signaling" form of the receptor is expressed appears to be the hypothalamus, but the mRNA for this form is also seen at lower abundance elsewhere as detected by RT-PCR. Missense mutation of the leptin receptor gene has also been described in the Zucker *fa*/*fa* rat (Phillips et al., 1996; Chua et al., 1996). One or more short forms of the receptor that are unlikely to have signaling potential are heavily expressed in several tissues including the choroid plexus, where the receptors may mediate uptake across the blood–cerebral spinal fluid barrier. The possibilities of blood–brain barrier transport or direct access to sites in the brain that reside outside the blood brain barrier have not been excluded. What appears to be a saturable system for transport of leptin from blood to brain has

been described in vivo in rats (Banks et al 1996), and in normal humans, levels of leptin in cerebral spinal fluid are approximately 2–5% of serum levels (Schwartz et al., 1996). Initial reports suggest that the ratio of cerebral spinal fluid to plasma leptin shows characteristics of saturability when leptin levels in plasma rise to the level seen in obesity (Schwartz et al., 1996; Caro et al., 1996), and this could be one of the factors that might limit the ability of peripheral leptin to effectively signal the state of adiposity to the central nervous system.

Leptin Targets

Identifying targets for leptin action in the brain is a matter of great interest, both from scientific and pharmaceutical perspectives. One target for leptin action is the level of expression of the hypothalamic neuropeptide Y (NPY) (Dryden et al., 1994). This 36 amino acid peptide is expressed widely throughout the nervous system. Expression of NPY mRNA in the arcuate nucleus of the hypothalamus is increased in *ob*/*ob* and *db*/*db* mice and in response to fasting in normal rats (Marks et al., 1992). Repetitive administration of NPY into the central nervous system produces hyperphagia and obesity (Stanley et al., 1986) decreased energy expenditure with suppression of BAT activity (Billington et al., 1991), and hyperinsulinemia (Zarjevski et al., 1993), all features of obesity in *ob*/*ob* and *db*/*db* mice. On the basis of its pattern of nutritionally regulated expression in the arcuate nucleus, and the consequences of its direct administration into the brain, NPY has been proposed to be a key effector of the hypothalamic regulation of nutritional homeostasis; it's overexpression may be an important link between leptin deficiency and the development of obesity. Since leptin treatment of *ob*/*ob* mice suppresses NPY expression in the arcuate nucleus (Stephens et al., 1995; Schwartz et al., 1996), and leptin may act directly on NPY-expressing cells in vitro (Stephens et al., 1995), the NPY neuron is likely to be a key leptin target. The recent identification of a new species of NPY receptor (Y5) that has characteristics expected of a receptor that mediates the action of NPY on food intake (Gerald et al., 1996) is a potentially important advance. However, the precise role of NPY is made less clear by the phenotype of NPY knockout mice, which apparently regulate feeding and body weight normally, and respond to exogenous leptin with normal or even excessive inhibition of food intake (Erickson et al., 1996). Whether absence of NPY will alter the phenotype of mice with various forms of obesity will presumably soon be known through physiological studies and genetic crosses. Whatever the results of such experiments, it already seems clear that suppression of arcuate NPY cannot be the sole mechanism for leptin action to regulate body weight, and one or more other central leptin targets must exist. One recent candidate for an alternative leptin target is melanin concentrating hormone, a neuropeptide that is expressed predominantly in the hypothalamus, is overexpressed in *ob*/*ob* and starved normal mice, and causes hyperphagia after injection into the lateral cerebral ventricles of rats (Qu et al., 1996). Neuropeptides that inhibit food intake, such as corticotropin releasing hormone (Arase et al., 1988) are also potential targets of leptin. Our understanding of how these and other central neuropeptides interact, with each other and with classical neurotransmitters that regulate feeding behavior such as serotonin and norepinephrine, is in an embryonic stage and will be an important subject for future research.

Role of Leptin: Obesity vs Starvation?

The discovery of leptin and its receptor as genetic loci carrying mutations that cause severe obesity in rodents suggested to most observers that one physiological role of leptin is to prevent obesity during exposure to nutritional excess (i.e. a negative feedback "adipostatic" function). However, it is not clear that prevention of obesity is leptin's most important role or the problem that the leptin system evolved to address. What other role might leptin be playing? In addition to rising as energy stores increase, leptin levels were noted to fall with weight loss (Frederich et al., 1995; Maffei et al., 1995; Considine et al., 1995). Since an effective adaptation to starvation would offer a survival advantage under the conditions of intermittent food supplies that is likely to have confronted mammals through evolution, it is possible that leptin evolved to provide a signal to inform the brain that energy stores are sufficient, and, in parallel, to entrain the brain's response to starvation. Recent experiments support this hypothesis (Ahima et al., 1996). Several changes in neuroendocrine status accompany fasting or limitation of food intake. These adaptations, which include limitation of reproductive competence, reduced thyroid hormone levels, and activation of the pituitary-adrenal "stress" axis are prevented or blunted when leptin levels are replaced exogenously during a fast (Ahima et al., 1996). The dose of leptin required for these neuroendocrine actions is lower than the dose required to produce weight loss in normal rodents. In addition, except for *ob*/*ob* mice, which are very sensitive to leptin, obesity in most rodent models (Frederich et al., 1995, 1995a; Maffei et al., 1995) and in most human obesity (Considine et al., 1995) is associated with high levels of leptin and what is being referred to as "leptin resistance." It is not yet clear whether "leptin resistance" reflects widespread disease due to dysfunction in pathways downstream of leptin, or the inherent physiologic limitations of the putative role of leptin as a signal to prevent obesity. This question will only be answered by careful studies of leptin physiology in lean and obese animals and humans under a variety of nutritional conditions and genetic analysis of the pathways downstream of leptin in lean and obese individuals.

Agouti, Fat, Tub

Whereas identification of *ob* and *db* established a defined physiological loop, the identity of the agouti, fat and tub mutations has yet to elucidate the physiological system that is perturbed by these mutant alleles to produce obesity. The obesity in these models develops more slowly than is seen with *ob* or *db*, although in each case an action of the mutant gene product in the central nervous system is suggested. Lethal yellow (A^y) is an autosomal dominant syndrome of obesity with insulin resistance, diabetes, and yellow coat color that is due to widespread ectopic expression of the agouti protein (Bultman et al., 1992). This gene, which was the first obesity locus to be cloned, encodes a 131 amino acid protein that is normally expressed solely in skin during hair growth, where it influences hair pigmentation and coat color (Manne et al., 1995). It does this by antagonizing the binding of melanocyte stimulating hormone to

one or more melanocortin receptors on the melanocyte (Lu et al., 1994). It has not yet been established at what tissue site or through what receptor mechanism ectopically expressed agouti acts to cause obesity. It is intriguing that melanin concentrating hormone and agouti share an ability to antagonize melanocyte stimulating hormone action in some systems (Rance and Baker, 1979). This parallel suggests that agouti might cause obesity by mimicking an action of melanin concentrating hormone to antagonize melanocyte stimulating hormone or a melanocyte stimulating hormone–like molecule at one or more sites in the brain.

The *fat* mutation produces an autosomal recessive obesity with hyperinsulinemia that was assumed to be compensatory to insulin resistance, as in other obesity models (Coleman and Eicher, 1990). It was found, however, that these mice responded normally to exogenous insulin, and that much of the circulating insulin was an incompletely processed and biologically less active form. The molecular explanation arrived when the *fat* mutation was mapped to the carboxypeptidase E gene, with *fat* being a missense mutation that abolishes enzyme activity in extracts of pancreatic islets and pituitary (Naggert et al., 1995). Precisely how obesity and accompanying infertility result from a defect in prohormone processing is as yet unknown, but speculation has centered on the possibility that defects in processing of hypothalmic neuropeptides may be the answer. This idea is supported by a recent report that processing of hypothalamic neuropeptides is abnormal in *fat*/*fat* mice, with one unexpected result being increased level of the aforementioned neuropeptide melanin concentrating hormone (Rovere et al., 1996). A potential human homolog of this disorder has recently been described in an obese patient with high levels of incompletely processed insulin and proopiomelanocortin (O'Rahilly et al., 1995), but whether the genetic basis for this disorder involves carboxypeptidase E or another prohormone processing enzyme, such as prohormone convertase 1 (PC1), has not yet been established.

The most recent obesity gene to be identified is *tub*, another autosomal recessive obesity of later onset, in which retinal degeneration and neurosensory hearing loss are found (Coleman and Eicher, 1990). The *tub* gene was identified through positional cloning and encodes a novel and highly conserved 505 amino acid protein that is suggested to be cytosolic in location (Noben-Trauth et al., 1996; Kleyn et al., 1996). It is most highly expressed in eye, testis, and brain, where location in hypothalamic nuclei important for regulation of body weight is consistent with tub playing a role somewhere in the pathway for neural regulation of energy balance.

Another approach to identification of genes involved in the obesity phenotype has involved quantitative trait locus mapping, using intercrosses between strains of rodents with high or low susceptibility to diet induced obesity. Some of the identified loci appear to map close to one or more of the identified mouse obesity genes (e.g., West et al., 1995).

Genetic Basis for Human Obesity

Studies in twins, adoptees, and families suggest that from 40% to as much as 80% of the variance in body mass index can be ascribed to genetic factors (Bouchard, 1995). In rare cases, human obesity is the consequence of single gene disorders (e.g., Bardet Biedl, Prader Willi, Ahlstrom, Cohen syndromes), and these syndromes are characterized as well by specific clinical phenotypes that led to their identification. The responsible genes have been mapped, but not yet identified, and do not appear to be human equivalents of any of the identified mouse obesity genes.

In most human obesity, it is believed that a limited number of genes interact with the environment to produce the final phenotype, and several strategies have been used to search for such genes. Candidate genes have been chosen for analysis based on the roles that their gene products are thought to play in the regulation of energy balance. In large studies of the *ob* gene (Considine et al., 1995; Maffei et al., 1996), and a limited study of the ob receptor gene (Considine et al., 1996) in obese humans, functionally significant mutations within the coding regions of these genes have not been found. On the other hand, linkage between the *ob* gene has been found in some (Clement et al., 1996; Reed et al., 1996) obese populations, and in an Hispanic population, obesity has been linked to a region of the genome (1p22-p31) (Duggirala et al., 1996) to which the leptin receptor also maps. Linkage of human obesity to human *tub*, *fat*, or *agouti* genes has not yet been reported. Three other candidate genes for which linkages of as yet uncertain significance have been reported are worthy of note. These include the beta 3 adrenergic receptor (Widen et al., 1995), which could be linked to functional impairment in lipolysis in white adipocytes or thermogenesis in BAT; the glucocorticoid receptor (Clement et al., 1996a), which could influence obesity through the known ability of glucocorticoids to act permissively at a number of key steps in energy balance; and the Na^+,K^+ ATPase (Deriaz et al., 1994), whose function in maintenance of ion gradients contributes importantly to energy expenditure.

Control of Adipose Cell Size and Number

When the intake of energy chronically exceeds energy expenditure, most of the excess energy is stored in the form of triglycerides in adipose tissue. Increased adipose tissue mass can arise through an increase in cell size, cell number, or both. Adipose cells are remarkably variable in size, reflecting principally the amount of stored triglyceride. Mild obesity mainly reflects an increased adipose cell size (hypertrophic obesity) while more severe obesity or obesity arising in childhood typically also involves an increased fat cell number (hyperplastic obesity). As a key part of a homeostatic system controlling energy balance, the molecular mechanisms that regulate preadipose cell growth, adipose differentiation, and lipogenesis in fat cells have been subject to extensive scrutiny. Recent years have seen an explosive increase in our understanding of the transcriptional basis of fat cell differentiation and adipose cell gene expression, particularly the identification of transcription factors that can promote adipogenesis in a dominant fashion. Central to our understanding here are PPARγ, the C/EBPs and ADD1/SREBP1.

PPARγ is a member of the PPAR (peroxisome proliferator activated receptor) subfamily of nuclear hormone receptors. PPARγ exists as two isoforms (γ1 and γ2) formed by alternative splicing (Zhu et al., 1995) and differing in their N-termini (Tontonoz et al., 1994a). PPARγ2 is expressed at high levels in adipose tissue, while low levels of PPARγ1 can be found in many other tissues. This receptor is induced very early in adipose cell differentiation, and is present at higher levels in preadipocytes than other fibroblastic cells. PPARγ appears to function as both a direct regulator of many fat-specific genes and also as a "master" regulator that can trigger the entire program of adipogenesis. PPARγ forms a heterodimer with RXRα and has been shown to bind directly to well characterized fat-specific enhancers from the adipocyte P2 (aP2; Tontonoz et al., 1994a) and phosphoenolpyruvate carboxykinase (PEPCK) genes (Tontonoz et al., 1994b). In fact, PPARγ was cloned as a component of a transcription factor, ARF6, that bound to the aP2 enhancer (Tontonoz et al., 1994a), and it was cloned independently through homology screens seeking new members of the PPAR family (e.g., Zhu et al., 1993). As a member of the nuclear receptor family, the ability of PPARγ to increase transcription through its DNA recognition site (DR-1, direct repeat, 1 base spacer) depends on the binding of ligands. Earlier work with PPARs relied on "activators" that presumably functioned by stimulating the intracellular production of ligands. These activators include drugs that caused peroxisome proliferation (hence the name PPAR) such as clofibrate, ETYA, Wy14,643, as well as a variety of other fatty acid derivatives (e.g., Green and Wahli, 1994). Most recently, the first direct ligands for PPARγ have been described including 15-deoxyΔ12,14 prostaglandin J2 (15dPGJ) and the thiazolidinediones, an exciting new class of insulin-sensitizing drugs in clinical development for non-insulin dependent diabetes mellitus (discussed below).

The regulatory role that PPARγ can play in adipogenesis is vividly illustrated through ectopic expression of this receptor. When expressed with viral vectors at or below the levels normally seen in fat cells, fibroblasts are efficiently converted into bona fide adipocytes upon application of PPARγ activators or ligands (Tontonoz et al., 1994c). In fact, using pioglitazone, a thiazolidinedione ligand, greater than 95% of these cells can be differentiated to fat (Brun et al., 1996). This adipogenic response is apparently through the transcriptional activity of PPARγ, since a point mutation in one of the zinc fingers required for DNA binding completely ablated this action (Tontonoz et al., 1994c). The adipogenic activity of PPARγ, like the well established 3T3-L1 and 3T3-F442A preadipocyte cell lines, is markedly enhanced by the presence of insulin (Hu et al., 1995).

Since all of the PPARs (α, δ, and γ) can bind to the same DNA sequences, and all are expressed in at least some fat depots, it is interesting to know whether α and δ (also called NUC-1 or FAAR) play any role in adipogenesis. Unlike PPARγ, PPARα or δ are not expressed preferentially in fat, but there have been suggestions that both of these receptors could play a direct or indirect role in adipogenesis (Yu et al., 1995; Teboul et al., 1995). Most recently, in a direct comparison of the adipogenic potential of PPARs expressed in 3T3 cells, it was shown that PPARα can induce some adipogenesis when stimulated by its strongest activators, while PPARδ could not stimulate detectable fat cell formation (Brun et al., 1996). Since many fibroblastic cells express a low but detectable level of PPARγ, it seems likely that the previous suggestions of an adipogenic response through PPARδ, especially when stimulated with high levels of non-selective PPAR activators, were actually due to the activation of PPARγ. While PPARα stimulated differentiation was less extensive and slower than that stimulated by PPARγ, the same basic program of gene expression was observed. Hence, the possibility exists that PPARα may contribute to the adipogenesis of some depots, in normal or pathological states. As specific high affinity ligands become available for all of the PPARs, a much more critical analysis of their function will be possible.

The C/EBPs and a Transcriptional Pathway for Adipogenesis

PPARγ is not the only transcription factor that is significantly elevated in adipocyte differentiation. C/EBPα, β, δ, and ADD-1 (also called SREBP-1) are all markedly induced during differentiation in culture, although they are not expressed in an adipose-selective fashion in vivo. C/EBPα is increased relatively late in the process of adipogenesis but ectopic expression of high levels of this factor alone can induce the differentiation of many fibroblastic cell lines (Freytag et al., 1994). On the other hand, when expressed at or near the mRNA levels seen in mature fat cells, this factor has only a modest adipogenic action in fibroblasts (Tontonoz et al., 1994c). Precocious expression of C/EBPα can accelerate the endogenous differentiation program of preadipocytes, while antisense mRNA blocks the differentiation markedly (reviewed by MacDougald and Lane, 1995). The important role of C/EBPα in adipose development is clearly shown in a knockout mouse. This genetic disruption causes a major reduction in the lipid content of the fat tissue; however, fat cell differentiation apparently still occurs (Wang et al., 1995). C/EBPα, like PPARγ, directly binds to many fat cell–specific genes (MacDougald and Lane, 1995). Unlike PPARγ, which tends to bind to distal enhancer regions, C/EBP tends to bind at sites clustered in proximal promoter regions.

A dramatically cooperative or synergistic relationship between PPARγ and C/EBPα is observed when both of these factors are expressed in the same cells. Under conditions lacking a PPARγ activator, where neither factor alone promoted significant adipogenesis in fibroblasts, coexpression gave abundant differentiation (Tontonoz et al., 1994c). This cooperation is even more obvious when a cell with little adipogenic potential, such as a determined myoblast, is challenged with these factors. Neither C/EBPα or PPARγ (with activator) alone could give rise to fat differentiation from G8 myoblasts, but coexpression caused the differentiation of at least 70% of these cells into adipocytes (Hu et al., 1995). Thus, while either PPARγ and C/EBPα can promote adipogenesis under very favorable experimental conditions, such as in a very susceptible precursor cell or with the application of high levels of adipogenic hormones or PPARγ ligands, it is likely that these factors cooperate in many, if not most physiological circumstances.

A very interesting role for C/EBPβ in adipogenesis

has recently emerged from the labs of McKnight and Farmer. Ectopic expression of this factor also promotes adipogenesis in fibroblasts (Yeh et al., 1995). Of the two major forms of the C/EBPβ protein, liver-enriched activating protein (LAP) has much more activity than liver enriched inhibitory protein (LIP). Notably, expression of C/EBPβ, which is normally induced very early in adipogenesis, increases the expression of PPARγ (Wu et al., 1995). The differentiation of cells expressing C/EBPβ ectopically is dependent on the addition of a PPAR activator, suggesting that an important role of C/EBPβ in adipogenesis may be to turn on PPARγ expression. C/EBPδ has also been implicated in the regulation of PPARγ; coexpression of both C/EBPβ and δ is necessary to fully induce PPARγ to levels seen in adipocytes (Wu et al., 1996). The promoter for PPARγ2, the isoform expressed most selectively in fat, contains two C/EBP binding sites (Zhu et al., 1995), suggesting a direct regulatory circuit.

Another factor that appears to interact directly or indirectly with PPARγ is ADD1/SREBP1. This protein was cloned independently as a basic helix-loop-helix (bHLH) protein induced very early in adipogenesis (Tontonoz et al., 1993), and a sterol response element (SRE) binding protein (Yokoyama et al., 1993). This factor is quite unusual in that it has dual specificity in DNA binding, interacting with certain E-box sequences and the SRE. Dual specificity is controlled by a single tyrosine residue in the basic DNA binding region, at a site where all other bHLH factors have an arginine (Kim et al., 1995). SREBP1 and its close homolog SREBP2 are posttranscriptionally activated by proteolytic cleavage of a large, transmembrane form of the protein (Wang et al., 1994). When expressed ectopically in fibroblasts ADD1/SREBP1 has two notable activities. Under culture conditions not conducive to adipogenesis, it induces two key genes of fatty acid metabolism, fatty acid synthetase and lipoprotein lipase, without stimulating visible fat cell formation (Kim and Spiegelman, 1996). Under conditions strongly favoring adipogenesis, ADD1/SREBP1 can increase the percentage of fibroblasts that undergo differentiation from 2–3% to 15–20%. This effect is probably due, at least in part, to an influence of PPARγ activity, since cotransfection with ADD1 causes a 3–4 fold increase in the activity of PPARγ through its DNA binding site but has no influence on the activity of C/EBPα through its DNA recognition sequence (Kim and Spiegelman, 1996).

In parallel with these studies, the lab of Brown and Goldstein has implicated ADD1/SREBP1 and SREBP2 in the control of several key genes of cholesterol homeostasis (Yokoyama et al., 1993). This suggests that this family of factors could coordinate the regulation of two of the major lipid pathways, those leading to fatty acids and cholesterol. Since the construction of very low density lipoproteins (VLDL), the parents molecule of IDL and LDL, contains both lipids, it is likely that the dual action of the ADD1/SREBP family reflects the need to coordinate these two separate but related metabolic pathways. In this regard, it will be interesting to know whether the two different pathways in which these transcription factors participate segregate along the lines of E-box versus SRE binding. Also interesting is the possibility that one of these two isoforms is primarily involved in adipogenesis and fatty acid metabolism while the other is primarily a cholesterol regulatory protein. It is notable that ADD1/SREBP1 is regulated dramatically in fat cell differentiation and has been shown to function positively in adipogenesis (Kim and Spiegelman, 1996), but is not observed to be activated through proteolytic cleavage upon cholesterol depletion of hamster liver (Zheng et al., 1995). In contrast, SREBP2 is proteolytically activated upon development of a cholesterol-depleted state, and hence may play the more important role in cholesterol homeostasis in vivo (Zheng et al., 1995). The relative roles of these two proteins is being critically analyzed in several cellular and genetic systems.

The outlines of a regulatory loop involving the key adipogenic factors during differentiation can now be envisioned (Figure 1). C/EBPβ and γ are elevated quite early in this process, probably in response to certain hormones, and may function directly to increase the expression of PPARγ. If the extracellular and intracellular conditions provide the necessary adipogenic hormones and PPARγ ligands, the transcriptional activity of this molecule will begin the cascade of genes that constitute the process of adipogenesis. ADD1/SREBP1 promotes this process by augmenting the transcriptional ability of PPARγ. Since PPARγ activation results in the expression of most or all of the program of differentiation, including C/EBPα, it is reasonable to imagine that PPARγ–C/EBPα cooperation brings about maximal differentiation and a full program of lipogenesis. The ability of PPARγ and C/EBPs to engage in cross-regulation may be sufficient to maintain the differentiated state, once it has been initiated. Of course, maintaining this regulatory loop will require the generation of an endogenous PPARγ ligand. The ability of ADD1/SREBP1 to regulate both key genes of fatty acid metabolism and augment PPARγ activity suggests that this transcription factor may be important in the process of ligand generation.

Several major unanswered questions remain in this scheme. If C/EBPβ is a major inducer of PPARγ in vivo, why is PPARγ not expressed abundantly in other tissues that express high levels of C/EBPβ, such as liver? This suggests the involvement of other important regulators, positive or negative, in fat and non-adipose tissues. A second question relates to the quantitative regulation of C/EBPα in fat. Although expression and activation of PPARγ in fibroblasts is sufficient to give some expression of C/EBPα, it is much lower than the expression in normal adipose tissue, again arguing for the involvement of as yet undescribed molecules. It will also be important to understand the mechanisms of cooperation between PPARγ and C/EBPα in differentiation. How this cooperation occurs at a molecular level is not clear. Since many fat cell–specific genes have binding sites for both proteins, a highly synergistic interaction with the core transcriptional machinery is the simplest explanation. The specificity of the PPARγ–C/EBPα relationship is indicated by the fact that other PPARs do not synergize with C/EBPα in adipogenesis. It is also possible that C/EBPα activates genes whose products control endogenous PPARγ ligand formation. Finally, a direct protein–protein interaction that increases the activity of one or both of these factors has not been ruled out.

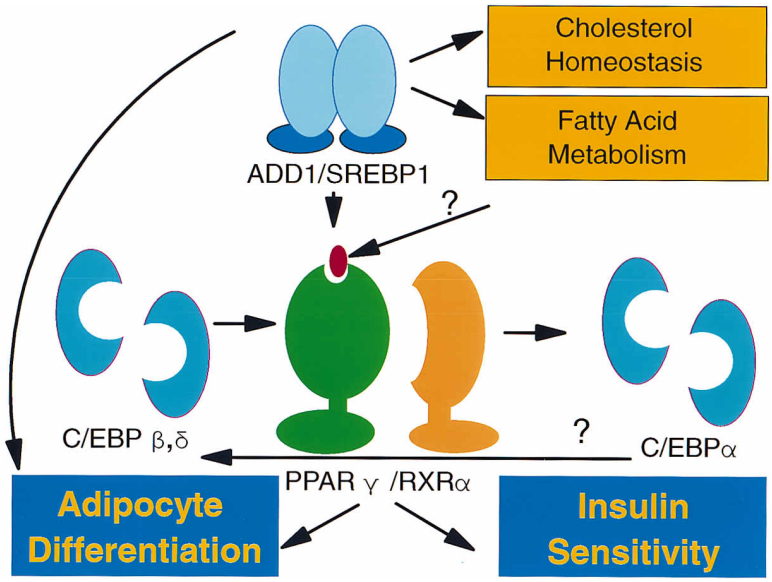

Figure 1. Cascade of Transcription Factors Involved in Adipocyte Differentiation

Hormonal signals initiate a transient increase in expression of C/EBPβ and C/EBPδ. These factors stimulate the expression of PPARγ. ADD1/SREBP1, in addition to regulating genes important in fatty acid metabolism, increases the activity of PPARγ, possibly through the generation of ligand. Activation of PPARγ by ligand allows the differentiation process to proceed. Subsequently, C/EBPα expression is induced. C/EBPα may allow for the continued expression of PPARγ once the levels of C/EBPβ and C/EBPδ have decreased. It is likely that both C/EBPα and PPARγ are important for maintaining the fully differentiated state.

One crucial question that has not yet yielded to current investigation is the transcriptional control of white versus brown adipose differentiation. As described above, white fat cells and brown adipose cells play opposite roles in energy homeostasis although they express a very similar pattern of genes. Since the key component of energy dissipation employed by brown adipose cells is UCP, it seems likely that a hint about the key differentiation factors might come from the study of this gene. Recent data suggests that this promoter, like many genes expressed in white adipocytes, has regulatory sites for the C/EBPs and PPARγ (Yubero et al., 1994; Sears et al., 1996), both of which are expressed in BAT and WAT. Hence, it is likely that other transcription factors or modified versions of these factors must contribute to the critical divergence in the determination of these two cell types.

PPARγ Ligands: Natural and Synthetic

The nuclear receptors are built to receive chemical signals that trigger their activity. Since at least one activity of PPARγ, to promote adipogenesis, is both very dramatic and of considerable physiological importance, there has been an immediate surge of activity to identify ligands, both natural and synthetic.

The identification of a natural ligand for PPARγ was preceded by research showing that many polyunsaturated fatty acids and eicosonoid derivatives, such as ETYA and Wy14,643, were activators of the PPARs (Green and Wahli, 1994). Using transcriptional activation through a reporter gene as an assay, it was found that the prostaglandin D arm of this family had high activity (Forman et al., 1995; Kliewer et al., 1995). 15-dPGJ2 was potent and gave a rapid response, consistent with a direct interaction with this receptor. Indeed, two groups found that this prostaglandin could bind directly to PPARγ, as evidenced by competition for binding with a synthetic thiazolidinedione ligand (see below), with a Ki of ~2 μM. As expected for a direct agonist, it is very effective at promoting adipogenesis when added to cells that express PPARγ.

The better-studied prostaglandin hormones are known to act via cell surface receptors. However, the notion that these lipids can permeate cell membranes and enter the nucleus is feasible. It is not yet known whether 15-dPGJ2 is generated in cells undergoing adipogenesis at levels that would suggest that it could function as a true endogenous ligand for PPARγ.

Thiazolidinediones (TZDs) are an exciting new class of synthetic drugs that increase sensitivity to insulin in patients that are resistant to this hormone. Since insulin resistance is a ubiquitous correlate of obesity and a major component of the form of diabetes (non-insulin dependent diabetes mellitus, NIDDM) commonly associated with obesity, these drugs may have great medical importance. Currently, one member of this class, troglitazone, has reported successful results in a phase III clinical trial to treat NIDDM (Nolan et al., 1994). The connection between the thiazolidinediones and PPARγ was first prompted by the studies of Rolf Kletzien and colleagues. They showed that pioglitazone could specifically induce the aP2 gene in differentiating adipocytes, and this drug appeared to function through the previously identified differentiation control element, ARE6, in the aP2 enhancer (Harris and Kletzien, 1994). After the ARE6 binding factor (ARF6) was cloned and shown to be PPARγ (Tontonoz et al., 1994a), Ibrahimi et al. (1994) found that TZDs could activate PPARs, though they did not examine PPARγ. In fact, the anti-diabetic TZDs BRL49653 and pioglitazone elicit a strong activation of PPARγ, with relatively little activity on PPARα and δ (Lehmann et al., 1995; Forman et al., 1995). Using ^{3}H-BRL49653, this drug was shown to bind directly to PPARγ, while binding to the other PPARs was not detectable. The rank order of potency of the TZDs closely matches the anti-diabetic action, suggesting that PPARγ is the functional receptor in this action (Willson et al., 1996). As expected for an agonistic ligand, these compounds have very powerfully adipogenic action in cells ectopically expressing PPARγ, or in fibroblastic cells that express low but significant amounts of this receptor. It is worth noting that TZDs had been reported to

stimulate the differentiation of preadipocyte cell lines well before the identification of PPARγ as a target (Kletzien et al., 1992).

PPARγ and Insulin Resistance

The activity of TZDs to promote adipogenesis in culture and improve insulin sensitivity in vivo may seem paradoxical in that increased adipogenesis is most commonly associated with obesity, a condition tightly linked to insulin resistance. However, this paradox becomes less troubling when one remembers that obesity is fundamentally a disorder of energy balance, with the excess energy stored in fat. Indeed, adipose differentiation itself is associated with the development of a marked increase in insulin sensitivity, correlating with greater expression of the insulin receptor, IRS-1, GLUT-4 and probably many other genes that are fundamental to the metabolic response to insulin. Excessive adipocyte number is only contributory to insulin resistance when it is linked (as it usually is in vivo) with an excessive burden of stored energy.

One can imagine several plausible mechanisms for TZD-improved insulin sensitivity. It is likely that one or more key genes relating to insulin action are capable of being modulated by an increased activity of PPARγ activity, even in fully differentiated fat. A TZD ligand, given at pharmacological doses, would be expected to control such genes and improve the insulin sensitivity of this tissue. Of course, a major question is how can TZDs improve insulin sensitivity of muscle and liver, other tissues that are important targets of insulin. One possibility is that TZDs function in a similar manner described above through the low levels of PPARγ that are expressed in many non-adipose tissues. Alternatively, adipose tissue PPARγ might be the primary target of TZDs but this might result in the generation of signals from fat that affect insulin sensitivity in muscle and liver. Simply by virtue of improving insulin sensitivity in fat, the rate of fatty acid release would be expected to decrease, since the suppression of lipolysis is a major action of insulin. In addition, fatty acids have been shown to be capable of inducing insulin resistance (Svedberg et al., 1991). There may also be protein products of fat that affect systemic insulin sensitivity and are regulated by TZDs. One example of this is known: tumor necrosis factor α (TNFα). As mentioned above, TNFα produced in fat has been implicated in systemic insulin resistance, especially in obesity (reviewed by Hotamisligil et al., 1996). TZDs have been shown to affect 2 arms of the TNF pathway: it reduces TNFα expression in fat tissue in vivo (Hofmann et al., 1994) and it can also block certain actions of TNFα on preadipose and adipose cell cultures (Szalkowski et al., 1995). Whether TZD can specifically interfere with the action of TNFα to inhibit signaling through the tyrosine kinase activity of the insulin receptor has not yet been investigated. A further contributing fact to improved insulin sensitivity might be if TZDs do trigger more, smaller cells. It has been suggested that smaller fat cells tend to have greater insulin sensitivity than large, more lipid-laden cells (Abbott and Foley, 1987). A redistribution of lipid into more cells could increase insulin sensitivity, but since the adipose mass is a less significant sink for glucose disposed than muscle, it is not clear how large an increase in insulin sensitivity this could generate.

Finally, it should be appreciated that these models are not mutually exclusive. It is entirely possible that all of these mechanisms contribute and the quantitative importance of each can only be ascertained through a combination of genetic studies, especially tissue-specific knock-outs of PPARγ, and studies of whole body physiology.

Linking Ingestive Behavior to Adipogenesis

Given the rash of recent insights into obesity genes and adipocyte biology, the task remains to integrate these often disparate new findings, first with each other, and then with knowledge of classical metabolism, endocrinology and nutrition, to create a more complete understanding of the biology of weight regulation and obesity. Although we are still a long way from such a synthesis, the outlines of some new connections can now be seen.

One need not be a rocket scientist to notice that increased food intake tends to be associated with obesity, but it is less obvious by what mechanisms increased ingested nutrients are transformed into more and bigger fat cells. Following the ingestion, digestion, and absorption of nutrients, levels of substrates rise, and these, along with signals emanating from the gut, produce changing levels of hormones that guide the substrates to their sites of storage. Insulin is the preeminent hormone of the fed state: elevated levels prevent excessive rises in blood glucose by promoting glucose storage and use and decreasing glucose production by the liver. Insulin also has a critical role in lipid metabolism, promoting the storage of triglycerides in adipocytes through numerous actions on this cell. Among these are stimulation of glucose uptake and inhibition of lipolysis, which occur very rapidly through glucose transporter protein (GLUT 4) translocation or covalent modification of hormone sensitive lipase, respectively. Insulin also stimulates fatty acid and triglyceride synthesis, through the induction of key lipogenic enzymes such as fatty acid synthetase and glycerophosphate dehydrogenase, and induction of lipoprotein lipase. To this picture of insulin action, we can now add recent insights into adipogenic transcription factors. It is clear that an insulin signal promotes the adipogenic action of PPARγ, since differentiation of cells expressing this molecule is greatly increased by this hormone (Hu et al., 1995). The nature of this signal is not yet characterized. Although an insulin stimulated change in PPAR phosphorylation and function may be predicted based on observations with other members of the steroid receptor superfamily (Bunone et al., 1996), it has also been observed that the abundance of PPARγ isoforms in fat falls with starvation and insulin deficiency diabetes (Vidal-Puig et al., 1996). This could occur through regulation of C/EBPβ and δ, which have themselves been shown to be transiently induced by insulin in cell culture (MacDougald et al., 1995b). Thus, the actions of insulin to transduce the nutritional state of the organism to molecular machinery linked to production of new fat cells may involve changes in both the levels and functional state of PPARγ as well as C/EBPβ and δ. Another positive link between adipogenesis and insulin involves ADD1/SREBP1. This molecule, which promotes adipogenesis and fatty acid metabolism, has been shown to be influenced by the presence

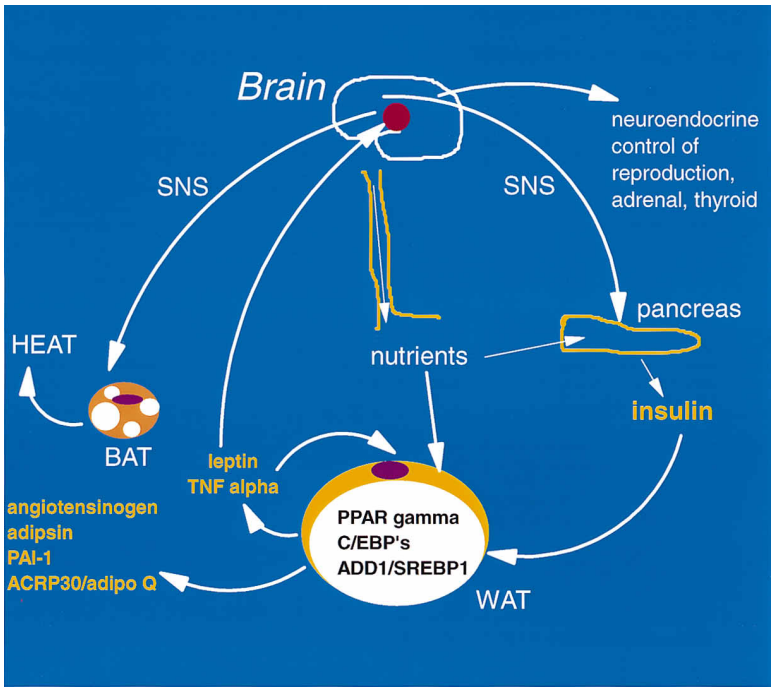

Figure 2. A Homeostatic Cycle for the Control of Energy Balance

The white adipocyte produces leptin as a function of adipose energy stores. Leptin acts through receptors in the hypothalamus to regulate appetite, BAT activity and insulin secretion via sympathetic nervous system (SNS) output, and neuroendocrine function, including reproduction. Ingested nutrients stimulate insulin secretion, and together these may act through several transcription factors to promote adipose differentiation and lipogenesis. Adipocytes secrete proteins in addition to leptin which may have the capacity to act locally and/or systemically to influence energy balance.

of insulin. While ADD1/SREBP1 activity has been shown to be increased by insulin, it is not yet clear whether this occurs by protein modification or at some other level (Streicher et al., 1996). Taken together, it appears that the response to feeding, at least in part via insulin, promotes both energy storage in fat and the adipogenic program itself through PPARγ and perhaps ADD1/SREBP1. Paradoxically, C/EBPα levels have been demonstrated to be negatively regulated by insulin. While this effect cannot explain the positive role of insulin in adipogenesis, it may contribute to the downregulation observed in certain fat cell genes, such as GLUT4, in response to insulin (MacDougald et al., 1995b).

Glucocorticoids also represent a potentially important link between obesity genes and adipogenesis. Many of the monogenic obesity syndromes in rodents develop a hyperglucocorticoid state and reversal of this state with adrenalectomy profoundly reduces obesity and several linked metabolic disorders. It has recently been shown that the induction of PPARγ by C/EBPβ and δ depends almost entirely on the presence of a glucocorticoid (Wu et al., 1996). This induction of PPARγ does not require ongoing protein synthesis, and hence, is likely to represent a direct action of the liganded glucocorticoid receptor on the PPARγ promoter.

Another potentially direct link between diet and adipogenesis involves the natural ligands for PPARγ. Many PPARγ activators and the only identified biological ligand (15-dPGJ2) are eicosanoid derivatives. All eicosanoids are derived from arachidonic or other essential polyunsaturated fatty acids that must be supplied through the diet. It is not clear whether these PPARγ ligands or ligand precursors enter adipose cell precursors directly from the bloodstream or are first stored in a membrane lipid compartment. The mechanisms leading to ligand import or generation of PPARγ ligands in both preadipose cells and mature adipocytes is an important area for future study.

Energy Balance: Elements of a Homeostatic Cycle

It is now clear that the systems that influence food intake and adiposity can be viewed as a cycle, as shown in Figure 2. As discussed above, the key elements are likely to be the fat-produced hormone leptin, the neural system for integration of signals related to energy balance, the pancreatic production of the hormone insulin and the effects of this hormone and eicosonoids on adipogenic transcription factors, particularly PPARγ. However, it would be extremely naive to think that these are the only important players in the fat–brain homeostatic system. Indeed, several other novel proteins secreted by fat that enter the circulation have been described, though their function is not yet known. These include adipsin (Flier et al., 1987), angiotensinogen (Frederich et al., 1992), plasminogen activator inhibitor I (PAI I; Samad and Loskutoff, 1996), and ACRP30/adipoQ (Hu et al., 1995; Scherer et al., 1995). It is highly likely that other fat derived molecules with interesting systemic functions will be described. Similarly, the wiring diagram of neural signaling molecules is very much at an exploratory stage. Although many neuropeptides are known to affect food intake, their relative importance in various nutritional states and the manner in which these neurotransmitters and neuromodulators interact must be determined.

It is fair to ask what implications arise from this new concept of a homeostatic cycle, in terms of our understanding of normal physiology and disease, as well as our ability to intervene therapeutically. First, the multiple levels of feedback control help to explain how most individuals maintain a rather impressive control of body weight. In order to maintain a weight change of less

than 10 Kg over a 10 year period, an adult of 70 Kg must maintain greater than a 98% congruence between energy expenditure and energy intake. Surely this must depend on multiple levels of feedback control involving signals between the regulatory centers in the hypothalamus and adipose mass, food intake, and energy expenditure. Secondly, one might predict that experimental perturbations in various parts of this system would cause unexpected or even paradoxical results. Indeed, it was considered an intriguing mystery for years how monogenic mutations such as *ob* and *db* could cause both increased food intake and decreased thermogenesis. This was only explained when the absence of leptin was shown to cause both of these effects. More recently, mice with a reduction in brown fat through expression of a tissue-specific toxigene were found to develop obesity, with the expected decrease in thermogenesis (Lowell et al., 1993). Completely unexpected, however, was the increase in food intake that these mice exhibit. Since leptin levels are very high rather than deficient in these mice (Frederich et al., 1995), this argues strongly for as yet undiscovered regulatory pathways from brown fat to the brain. Along similar lines, beta 3–selective adrenergic agonists, developed as anti-obesity drugs that would stimulate these fat-selective receptors and increase β-oxidation of fatty acids and energy expenditure acutely reduce food intake and stimulate insulin secretion in mice (Susulic et al., 1995) through as yet unknown mechanisms that are independent of leptin (Mantzoros et al., 1996).

The cyclical nature of energy homeostatic mechanisms also suggest that significant disorders of energy balance could result from primary lesions at sites in these pathways that are not necessarily *directly* involved in controlling food intake. This could explain the extremely heterogeneous and complex nature of the genetics of human obesity. While studies of identical twins reared apart versus together have argued that a large percentage of this disorder is genetically determined, mapping of obesity genes in humans indicates the involvement of a large number of loci (Bouchard, 1995).

What little we know about human obesity genes also suggests the interconnection of systems that were hitherto deemed separate. Perhaps the strongest data correlating a human genetic polymorphism with obesity is a variant in the human beta 3 adrenergic receptor, observed in several different studies. The association of this variant with genetically distinct populations, including Pima Indians (Widen et al., 1995), Norwegian (Widen et al., 1995) and French Caucasians (Clement et al., 1995a) might suggest a consistent physiological defect, but this single variant has been associated with body mass index (degree of fatness) in one study, degree of insulin resistance in another and early age of onset of diabetes in a third. If these studies are all correct, one can only conclude that this reflects the biochemical connection between these systems and the ultimate phenotype influenced by the genetic backgrounds of these different populations. Since beta 3 adrenergic receptors are predominately expressed in BAT, and possibly WAT in humans, this further suggests physiological loops involving adipose tissue and a number of different sites.

Ultimately, our increased appreciation for the complex and cyclical nature of energy balance controls suggests caution if not modesty as our reductionist approaches, so useful in generating specific molecular targets, are applied to the in vivo settings. It is possible that unexpected problems will develop as we test new drugs to control food intake, increase energy expenditure, or directly manipulate fat cell development. However, these very same interrelationships among complex physiological systems may allow for compensation and allow new "set points" to be achieved without disastrous complications. An example of this could be the development of antagonists to PPARγ as a treatment for obesity through the direct inhibition of fat cell formation. Even if we assume that such molecules will be developed, and that they effectively decrease fat cell number without adversely affecting insulin sensitivity, the continuation of energy intake at the same rate that induced obesity would require that this energy be stored as lipid in other tissues, such as liver or muscle, or remain in the blood stream. These are all very problematic outcomes. Only if reduced fat cell number causes a compensatory decrease in food intake or increase in energy expenditure could such an approach be useful. Fortunately, such a scenario is now conceivable. A reduction in adipose cell number would presumably lead to an increase in the size of those cells and hence, increased leptin synthesis. Furthermore, blockage of PPARγ itself might have a similar effect at the level of the leptin promoter. If increased leptin in this context caused reduced food intake and increased energy expenditure, a beneficial cycle and development of a new, lower set-point might be achieved. Of course, this scenario is highly speculative but illustrates the complications and opportunities that this new knowledge now affords.

Acknowledgments

Journal limitations on the number of permissable citations have prevented us from citing many additional important contributions made by workers in these fields. We regret that these references could not be more exhaustive. We gratefully acknowledge the helpful discussions and critiques provided by Drs. Regina Brun, Bradford Lowell, Barbara Kahn, Rudolf Leibel, and Steve Farmer. We also thank the members of our laboratories for their many contributions to the ideas presented here. This work was supported by grants R37DK28082 (JSF) and R37DK315405 (BMS) from the National Institutes of Health.

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