LESSONS FROM RODENT MODELS OF OBESITY

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ATSTRACT
Rodent models in which monogenic alterations cause obesity in the absence of environmental changes have confirmed earlier inferences regarding the biologic/genetic control of energy balance in mammals. Spontaneous models of obesity in rodents have identified fundamental molecular/cellular systems underlying the control of feeding and energy homeostasis, including the melanocortin system and CNS circuits that respond to leptin. Engineered mutations in rodents have revealed additional genes and pathways participating in the control of body weight. Most genes that impact body weight and adiposity affect the brain systems that control "regulatory" and/or hedonic aspects of feeding behavior. Effects on energy expenditure are frequently present, but of smaller magnitude. Differences in adipocyte physiology between obese and lean individuals appear to be largely secondary phenomena . Most of the rodent monogenic obesities have human orthologs that result in comparably severe obesity-related phenotypes. While these mutations are rare, they confirm that the molecular predicates for the control of body weight in humans are fundamentally the same as those in the rodents. Engineered mutants in rodents have also permitted the analysis of putative genetic contributors to obesity in humans, as well as providing an initial blueprint of the molecular components, pathways and physical interconnections of these systems.

INTRODUCTION

Historically, obesity has been considered a disorder of voluntary behaviors, exacerbated by the ready availability of food and reduced need for energy expenditure afforded by modern societies. Rodent models in which monogenic alterations provoke obesity in the absence of environmental changes have, however, conclusively demonstrated the biologic control of energy balance in mammals. Indeed, orthologs of many of these genes cause or contribute to obesity in humans. Spontaneous mono and polygenic models of obesity in rodents (along with obesity phenotypes of engineered mutations) have identified fundamental molecular/cellular systems underlying the control of feeding and energy homeostasis. Importantly, most genes that impact body weight and adiposity affect the brain systems that control feeding. Genetic studies in rodents have provided an initial blueprint of the molecular constituents and interconnection of these systems.

The first law of thermodynamics and body weight regulation.

The first law of thermodynamics (the conservation of energy) dictates that body energy stores reflect the difference between energy taken in and energy expended. More intake with relatively less expenditure leads to energy storage (generally, in adipose tissue); conversely, when expenditure exceeds intake, energy/fat stores decline. In this context, energy is generally taken in by eating (or drinking calorie-containing beverages). Behaviorally, two related systems govern eating (1): The circuits that control the incentive and reward values (wanting and liking) of food, and the satiety system, which promotes meal termination associated with the sensation of "fullness." Each of these systems is subject to long and

short-term regulation. They are physiologically integrated, but for simplicity frequently studied and described as distinct entities.

Energy loss/expenditure includes energy consumed but not absorbed (and which therefore passes out of the body in the stool; this is ~5% under most normal circumstances); energy metabolized in the process of breaking down and storing nutrients (diet-induced thermogenesis); energy metabolized to maintain baseline cellular functions at rest (resting metabolic rate, BMR); and energy consumed in physical activity (non-resting energy expenditure; NREE) (2). RMR accounts for about 70% of total energy expenditure (TEE) in sedentary adults, and is determined by body composition, age, wakefulness, and genetic factors. NREE is the next largest contributor, averaging approximately 20% of TEE. In sedentary individuals, low-level physical activities (fidgeting, short bouts of ambulation, etc.) make up most of NREE. Smaller amounts of energy (7%) are accounted for by diet-induced thermogenesis. Recently, there has been growing interest in the contributions of the gut microbiome to systemic energy homeostasis by possible effects on the efficiency of nutrient utilization in the gut and the consequences of such bacterial metabolism for release of metabolites that could affect energy intake or expenditure (3).

Adipose tissue represents the major repository for ingested energy that exceeds immediate needs (2). The energy density of adipose tissue is nearly 10-fold greater than muscle (protein) or liver (glycogen). The ability to store such energy protects against environmental vicissitudes that might result in starvation, fetal wastage, and inability to provide sufficient breast milk to the young. Therefore, it is likely that evolution has promoted genes/alleles that favor energy storage and conservation. The existence of environments in which excess calories are readily available with minimum or no effort is a very recent occurrence in human evolution. Human genetic "makeup" is presumably designed for the opposite circumstance. Contrary to earlier prevailing views, adipose tissue is not a passive energy depot, but participates in homeostatic processes that regulate food intake (e.g. production of leptin), and storage (e.g., insulin) and release (e.g., catecholamines) of the acylglycerides stored within them (4).

Spontaneous obesity in rodents provides the first clues to the genetic underpinnings of energy balance

In 1902, French geneticist L. Cuenot described the obese *Yellow* (A^y/a) mouse, which had been bred and maintained by European mouse fanciers since the 1800s (5). This was the first report of a spontaneously obese mouse, which prompted investigation of additional spontaneous obese mouse models, including by investigators at the Jackson Laboratories (Jax) in Bar Harbor, Maine.

The autosomal recessive *obese (ob)* mutation was discovered at Jax in 1949-50, after spontaneously arising in a non-inbred strain (6). Sixteen years later, a phenotypically similar mouse was identified (7). The diabetic state of these latter animals (studied on the diabetes-prone coisogenic KsJ background) distinguished them from *ob* (studied on the B6 background) and hence the mutation was designated *diabetes (db).* In 1990, Coleman and colleagues described additional, milder recessive obese mutations in mice: *tubby (tub),* and *fat (fat)(8,9)*. Fewer obesity-related spontaneous mutations have been detected in rats, due in part to the absence of explicit screening of large numbers of progeny for such phenotypes, and the greater cost of rat husbandry. In addition to identifying mutations in genes identical to some of the murine genes above (e.g. leptin receptor mutations in *db* mice and the Zucker and Koletsky rats(10,11)), however, the OLETF obese rat has also been described (12).

Each of these mutant animals is hyperphagic compared to controls. Furthermore, classic genetic studies revealed that each of these obese phenotypes had predictable, monogenetic heritability, demonstrating the genetic underpinnings of feeding, as well as overall energy balance. The subsequent finding that

some of these rodent obesity genes control body weight in humans confirms that biologic/genetic factors control feeding and the predisposition to obesity in humans, as well as in rodents (13).

Transgenic models

In addition to spontaneous models of obesity, genetic engineering (generally in mice) has provided many examples in which genetic alterations modulate body weight and adiposity. In the early 1980s, genetic manipulation techniques became available in rodents, enabling the analysis of systemic and organ-specific effects on physiology of one or more genes selected by the investigator (14). Several genes important for energy balance that have been examined by such approaches are discussed below. Historically, gene manipulation in mammals has been accomplished by one of two distinct means: standard transgenesis and gene targeting.

In standard transgenesis, artificial genes (often comprising promoter sequences designed to produce desired patterns of expression plus the coding sequences for the molecule to be expressed) are directly introduced into a fertilized oocyte, which is then implanted in a female surrogate to permit the development of the transgenic animal (15). In this method, the site of the transgene insertion in the genome is random; hence, the insertion may inadvertently disrupt endogenous genes, and the expression pattern may be influenced by the site of insertion as well as by the promoter sequences used. The use of very large genomic regions (such as those derived from bacterial artificial chromosomes (BACs)) to drive the gene of interest can mitigate some, but not all, of these expression issues (16). Multiple independent transgenic lines must therefore be screened to identify correctly-expressing progeny.

Gene targeting is accomplished by using homologous recombination to introduce genetic sequences designed to modify specific genes, while leaving the rest of the genome intact (17). Generally, manipulated DNA sequences are introduced into undifferentiated murine embryonic stem (ES) cells to recombine with native DNA, producing a modified ES cell line in which a specific gene is inactivated (a "knockout" or KO) or altered by editing or by the introduction of new genetic material (a "knockin" or KI) . The modified ES cells are then injected into blastocysts, which are implanted into surrogate mothers. The resultant pups generally contain cells derived from the ES cells along with cells donated by the recipient blastocyst (hence, these animals are termed "chimeras"). The chimeras are then bred in an effort to obtain germline transmission of the ES cell-derived genes. Thus, while gene targeting is quite specific in terms of the types and locations of manipulations, the use of ES cells requires a greater up-front investment of time and resources (generating the homologous targeting construct, screening ES cell clones for correct targeting, breeding for germline transmission, etc.) than does standard transgenesis.

New technologies have emerged over the past several years that promise to facilitate gene targeting. These generally involve the use of site-specific nucleases (zinc finger nucleases, TALENs, CRISPRs, etc.) to create breaks in the genomic DNA of fertilized oocytes or ES cells (18,19). These site-specific breaks can be employed not only to produce KO animals, but also to increase the efficiency of site-specific recombination within the oocyte, so that the co-injection of homologous templates can be used to generate KI animals without the use of ES cells.

In addition to the production of standard KO animals, and animals with edited coding or regulatory sequences in specific genes, gene targeting is also commonly used to produce conditional null or specific gene-expressing animals, often by employing the Cre recombinase/LoxP system (19). This bacteriophage-derived system is composed of two components- Cre (a site-specific DNA recombinase)

and LoxP (the short DNA sequences recognized by Cre). In most versions of the system, Cre removes the DNA sequences that are flanked by LoxP sites ("floxed"). By combining tissue-specific Cre expression with floxed genes of interest, the floxed genes may be disrupted in a tissue- and time-specific manner. Cre-expressing animals may be generated by standard transgenesis (with the caveats, above, regarding site of integration), or can be delivered to specific sites in the genome by homologous targeting. Animals carrying floxed alleles are produced by delivering LoxP sites to the desired locations in the genome by homologous targeting.

While many homologous targeting events are designed to be benign, they may have unintended consequences for the expression of the targeted gene (e.g., alterations in expression) or surrounding genes; these must be controlled for carefully. Another important consideration in mouse models of obesity generated by gene targeting is genetic background effects. Targeting has been most consistently successful in the 129 mouse strain because the ES cells of 129 mice are relatively easy to culture and manipulate. However, this strain exhibits increased levels of anxiety in response to environmental stressors that could potentially distort food intake and metabolic phenotypes (20). ES cells from C57BL/6 require carefully controlled *in vitro* conditions, and even then, often fail to transmit the introduced mutation to the germ line. Serial backcrossing of a progenitor 129 or other strains, e.g., C57BL/6, can be used to transfer the mutation to a more suitable "background". For example, mice overexpressing melanin-concentrating hormone (MCH) have an obese phenotype only when backcrossed for 7 generations onto the C57BL/6J background. On an FVB background, they appear to have a normal phenotype with regard to body composition (21).

PATHWAYS INITIALLY REVEALED BY SPONTANEOUSLY OCCURRING MUTATIONS

Obese and *Diabetes* reveal the endocrine control of energy balance.

The *obese* (*ob*; now *Lep^{ob}*) mouse was identified as an autosomal recessive mutation in a noninbred strain (Stock V) at Jackson Laboratory in 1949 (6). Mice segregating for the *obese* gene were backcrossed for many generations to generate a congenic line on the C57BL/6J background strain. *Lep^{ob}* mice on C57BL/6J, despite their early-onset obesity and transient glucose intolerance, are not diabetic; however, the *Lep^{ob}* mutation coisogenic on the diabetes-prone C57BL/KsJ line results in severe, early-onset type 2 diabetes (22).

Diabetes (db; now *Lepr^{db})* is a spontaneous recessive mutation that was first noted in a C57BL/KsJ mouse colony at the Jackson Laboratory. The KsJ *Lepr^{db}* mutant is hyperphagic and obese, but also develops severe type 2 diabetes (7). Backcrossing *Lepr^{db}* onto the C57BL/6J background attenuates the diabetic phenotype; C57BL/6J-*Lepr^{db}* is virtually identical to C57BL/6J-*Lep^{ob}*.

Douglas Coleman, at Jackson Labs, looking for the molecular predicates of the lipostatic system posited by Kennedy (23) and Hervey (24), performed parabiosis (joined circulation) studies coupling *Lep^{ob}* mice to either wild-type or *Lepr^{db}* mice (25). The *Lep^{ob}* mouse became lean when joined to a wild type, but, when joined to a *Lepr^{db}* mouse, the *Lep^{ob}* mouse died of starvation. These findings led Coleman to hypothesize that a blood-borne factor regulating body weight might be deficient in *Lep^{ob}*, but circulating at high levels in the blood of *Lepr^{db}* mice. He suggested that *obese* was the secreted factor and *diabetes* its receptor (25,26).

In 1994, the gene encoding *Lep^{ob}* was isolated by positional cloning (27) by a group at Rockefeller University, and its product, leptin, was shown to be produced primarily in adipocytes. Leptin is a type 1 cytokine, similar in structure to IL-6. *Lep^{ob}* mice lack circulating leptin by virtue of an R105X mutation

that creates a premature stop codon sequence in the leptin gene, resulting in a truncated protein that is rapidly degraded (27).

The gene (*Lepr*) that encodes the receptor for leptin (LepR) was identified by expression cloning (28); the first genetic mutation in *Lepr* was identified in the *Lepr*^{db} mouse (10,29,30), thus confirming the conceptual model proposed by Coleman: the obesity of the *Lepr*^{db} mouse was due to a mutation that precluded leptin signaling by the receptor that binds the *ob* gene product.

Alternative splicing of the *Lepr* transcript produces multiple isoforms of the receptor (which is a cytokine receptor similar to members of the IL6 receptor family): LepRa, -b, -c, -d, and so forth (**Figure 1**). The mutation in the *Lepr*^{db} mouse results from a splicing defect that causes the 3′ terminal exon (18a) of leptin receptor isoform a (*Lepr-a*) to be inserted into *Lepr-b.* A stop codon at the end of exon 18a prevents transcription of the *Lepr-b* terminal exon, so that LepRa is produced in place of LepRb (10,29,30). Because the *Lepr*^{db} mouse synthesizes all leptin receptor isoforms except LepRb, it is clear that this isoform (which contains JAK box and STAT3 domains) is critical to the control of energy homeostasis (31). Indeed, restoration of LepRb on a background null for all other LepR isoforms restores energy balance (32).

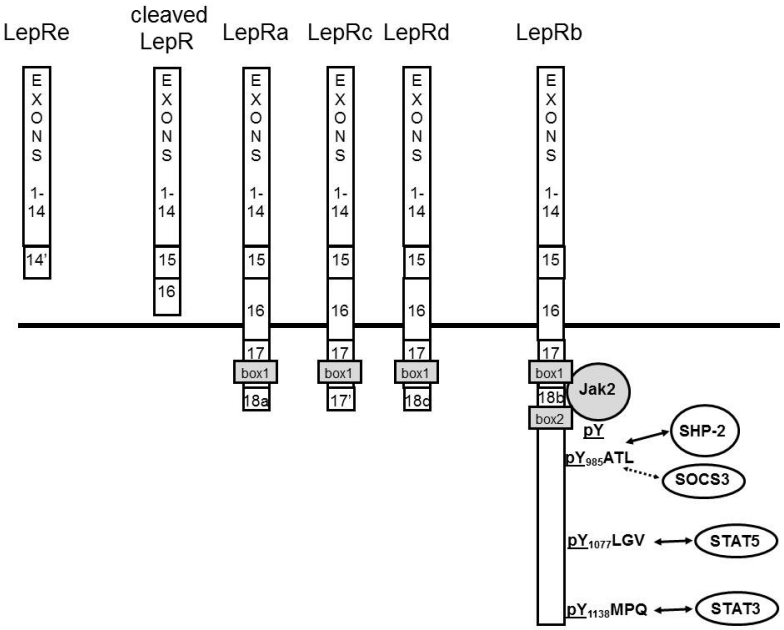

Figure 1. LepR isoforms and function. LepRa (Ra) represents the mostly highly expressed short form of LepR; LepRb (Rb) is the long form. Exon 17 contains half of a Jak docking site (BOX1) common to Ra, Rb and Rc, while exon 18b contains additional motifs required for full Jak2 binding (BOX2) and STAT3 signaling (31,33). Circulating leptin binding protein consists of extracellular domain that has been cleaved from the cell surface, along with the LepRe splice variant that lacks a transmembrane domain. Humans do not generate the splice variant, so that all LepRe is produced by cell surface cleavage, presumably by membrane associated metalloproteases (33).

LepRa, -c, -d and the other so-called “short” isoforms contain the same first 17 exons as LepRb, but diverge within the intracellular domain. LepRb is the only isoform that mediates classical Jak-STAT signaling, as this isoform alone contains the motifs required to interact with Jak2 and to bind STAT proteins for downstream signaling (Figure 1)(34). While the function of LepRb is clear, the functions of

the short isoforms are not, although they have been speculated to function in leptin transport into the brain and/or a source of cleaved, circulating extracellular LepR (which, along with LepRe comprises the major circulating leptin-binding protein) (35). The biological role(s) of soluble LepR isoforms (sLEPR) are unclear. Human obesity and fasting are associated with decreased circulating sLEPR; pregnancy with increased sLEPR. sLEPR can block LEP transport across the BBB(36-40).

The physiologic function of leptin.

Disruption of *Lep* function results in hyperphagia and obesity, and leptin administration to Lep^{ob} mice (but not $Lepr^{db}$ animals), reduces food intake and adiposity, sparing lean tissue (41-43). Thus, Lep^{ob} and $Lepr^{db}$ mice demonstrate that fat mass (along with both energy intake and expenditure) can be controlled by a single molecule. Leptin represents a powerful biologic controller of feeding and energy balance, revealing the existence of an endocrine system that controls feeding and energy balance. In humans, leptin deficiency also elicits a severe obesity phenotype: A rare, recessively inherited *LEP* mutation was discovered in two children who are members of a highly consanguineous Pakistani family (44). As with the Lep^{ob} mutation in mice, this frameshift mutation introduces a premature stop codon that truncates the leptin protein. While rare, additional leptin-deficient individuals (all of whom are severely obese) have been identified. Daily subcutaneous administration of recombinant leptin dramatically and selectively reduces body fat to normal levels in these individuals (45). A few humans homozygous for *LEPR* mutations have also been identified; these individuals present a severe obese phenotype similar to those lacking leptin, although – as anticipated - they are not responsive to exogenous leptin (46). In these patients, growth hormone deficiency and central hypothyroidism are phenotypes seen more frequently than in leptin deficiency *per se*. It is important to note that mice (47) and humans (48) heterozygous for null mutations of either LEPR or LEP are more obese than suitable controls. It is thus possible that individuals heterozygous for functionally null mutations of these and other genes encoding molecular components of the various signaling pathways regulating energy homeostasis discussed in this review constitute a significant proportion of the very obese. Additionally, heterozygosity for several of these mutations would be expected to produce even greater levels of obesity. The increasing use of exome sequencing in evaluating instances of severe obesity will lead to the detection of more instances of obesity caused by such oligogenic mechanisms.

While the role for leptin in the control of appetite and adiposity initially dominated the thinking about its biology, it rapidly became clear that leptin has other functions, and that the effects of high leptin are not as dramatic as those of low leptin. Indeed, obese rodents and humans exhibit high circulating concentrations of leptin, commensurate with their high levels of leptin-producing adipose tissue (49,50). Similarly, in contrast to the Lep^{ob} mice, increasing leptin to supraphysiologic levels in normal animals modestly and briefly blunts food intake and body weight [effect may be more striking than this]. Likewise, supraphysiological doses of leptin have only modest effects on body weight in obese and non-obese humans (51). Thus, the absence of leptin appears to convey a more powerful signal than does its excess. Also, Lep^{ob} mice (and their human counterparts) display additional phenotypes, including impaired growth and gonadal axis function, diminished immune function, infertility, and decreased energy expenditure due to low sympathetic nervous system tone and thyroid function- all of which are reversed by leptin treatment (52). The lack of leptin also promotes increased hepatic glucose production, and leptin treatment suppresses hyperglycemia in models of several diabetes, including T1D (53). This constellation of phenotypes resulting from low leptin mirrors the physiologic response to starvation; indeed, leptin treatment attenuates many of these consequences of very low adiposity (54). Thus, normal leptin concentrations signal the repletion of energy (fat) stores to mitigate hunger and enable energy expenditure, while low leptin indicates the dearth of adipose reserves and promotes food-seeking

and the conservation of remaining fat by reducing energy expenditure. The concentration of leptin constituting such a signal of adequacy of fat stores may differ among individuals, reflecting genetic, developmental and acquired differences in the CNS molecules and circuits comprising this system (55).

Transgenic animals that lack adipose tissue exhibit a syndrome that mirrors that of lipodystrophic humans (who lack adipose tissue on a congenital or acquired basis): In spite of their leanness, lipodystrophic people and animals exhibit hyperphagia along with a predisposition to insulin resistance, diabetes and other endocrine and metabolic abnormalities that are not corrected even with caloric restriction (56,57). Due to their dearth of adipose tissue, leptin levels are low and leptin treatment improves their hunger and endocrine/metabolic abnormalities. Indeed, leptin was recently approved for the treatment of lipodystrophy syndromes in humans (58).

A leptin-regulated neural network underlies energy balance.

The similar phenotypes of *Lep*^{ob} and *Lepr*^{db} mice (along with the inability of leptin to alter physiology in *Lepr*^{db} mice) indicates that leptin action on LepRb-expressing cells must mediate its effects. Consistent with its behavioral effects (e.g., on feeding) and its effects on the neuroendocrine and autonomic systems, most LepRb-expressing cells lie in the brain. Indeed, transgenic overexpression of LepRb throughout the central nervous system (CNS) partially corrects the obesity syndrome of *Lepr*$^{db\text{-}3J}$ mice (which lack all LepR isoforms) (32). Similarly, ablation of CNS LepRb using a neuron-specific Cre in combination with a floxed (*Lepr*^{flox}) allele promotes hyperphagia, neuroendocrine failure, and obesity (59). Some tissues outside of the CNS express LepRb, but the physiologic role for leptin action on these non-CNS cells remains unclear.

Within the brain, the majority of LepRb-expressing neurons are found within the hypothalamus and brainstem, consistent with the known roles for these structures in the control of feeding and endocrine and autonomic function (60-62). While LepRb ablation in the nucleus tractus solitarius (NTS) in the brainstem reduces satiety (consistent with the known role of this brain structure), pan-hypothalamic ablation of LepRb promotes a phenotype very similar in quality and magnitude to that of whole-body null *Lepr*^{db} animals (63). Furthermore, ablation of LepRb from broadly-distributed hypothalamic *vGat*- or *Nos1*-expressing neurons promotes dramatic hyperphagia and obesity (64,65). Smaller, more circumscribed sets of hypothalamic LepRb neurons have also been implicated, as well. Within the arcuate nucleus (ARC), an important satiety center in the brain, excision of *Lepr*^{flox} by *Pomc*^{cre} and *Agrp*^{cre} modestly increases feeding and adiposity (66,67). Ablation of LepRb in the SF1-expressing ventromedial hypothalamic nucleus (VMH) blunts the increase in energy expenditure that accompanies increased adiposity, and deletion of SF1 in the lateral hypothalamic area (LHA, which is associated with motivation) diminishes motor activity and promotes obesity (68,69). LepRb neurons in the ventral premammillary nucleus (PMv) play roles in reproduction (70). Importantly, many additional groups of LepRb cells in the hypothalamus (especially the ARC and dorsomedial hypothalamic nucleus (DMH)) and brainstem have as yet undetermined functions.

Spontaneously-arising *Agouti* mice reveal the crucial role for the hypothalamic melanocortin system in energy balance.

Expression of the agouti gene (*a*) normally occurs intermittently in the hair follicle resulting in the production of alternate yellow and black pigment bands of the resulting hair; this admixture produces the agouti coat color (71). The molecule acts as primarily as an inverse agonist at the melanocortin receptor (MC1R in skin).

The *Yellow* mutation of the *agouti* locus (A^y/a) is also termed 'lethal yellow', since homozygotes for the allele are prenatal lethal. *Yellow* was bred by mouse fanciers in Europe beginning in the 1800s, and was notable for the dominant inheritance of its striking yellow coat color and obesity proportional to the intensity of the yellow coat (5). In 1960, another spontaneous *agouti* mutation was detected in the Jackson Laboratory colony; viable yellow (A^{vy}) (72). The original, lethal, *yellow* mutation is a deletion of the *Raly* gene, which causes a fusion of the constitutively active *Raly* promoter to the *agouti* gene, resulting in ectopic continuous overexpression of *agouti* in all somatic (including brain) cells. A^{vy}/a is also the result of ectopic overexpression of *agouti*; this mutation results from insertion of a retrovirus-like repetitive intracisternal A particle (IAP) into a noncoding exon of *agouti*. The resulting splice variant fuses the constitutively active *Raly* promoter to the *agouti* gene, allowing constitutive overexpression of *agouti* in all somatic (including brain) cells.

The increased body weight of A^y/a and A^{vy}/a mice results mainly from hyperphagia, and reflects both increased fat mass and lean body mass (with increased body length) (73). By contrast, Lep^{ob} *and* $Lepr^{db}$ mice have a selective expansion of fat mass due to increased food intake, decreased energy expenditure, preferential storage of excess calories as fat, decreased body length and lean body mass (74). Thus, *agouti* overexpression affects food intake similarly to leptin, but alters energy expenditure less dramatically.

The *agouti* gene encodes agouti signaling protein (ASP), a peptide with a high affinity for melanocortin receptors. The yellow coat color of the A^y/a mouse results from continuous overexpression of *agouti* in the skin which blocks (mainly by inverse agonist effects) alpha-melanocyte-stimulating hormone (a-MSH) signaling at melanocortin-1 receptors (MC1R) in the hair follicle (71,75). Since a-MSH activates melanocytes to initiate synthesis of eumelanin (black pigment) instead of phaeomelanin (yellow pigment), antagonism of a-MSH by ASP elicits a yellow coat color. ICV administration of a-MSH and a-MSH agonists decreases food intake and body weight; overexpression of *agouti* in the A^y/a brain antagonizes the anorectic action of a-MSH signaling as well as blunting the endogenous activity of the receptor, thus causing hyperphagia.

Melanocortin receptors- the role for MC4R in energy balance.

The brain contains two predominant melanocortin receptor isoforms- melanocortin receptor-3 and -4 (MC3R and MC4R, respectively) (76). Both isoforms are potently activated by a-MSH. MC4R is expressed in the PVN, DMH, VMH and LHA(75), all of which are hypothalamic sites crucial for the control of food intake. Mice homozygous for a targeted deletion of *Mc4r* display substantial hyperphagia, 3- to 5-fold increased adiposity, and 50-100% increased body weight compared to littermate controls, while maintaining the same absolute lean body mass as +/+ littermates (77). Heterozygosity for the *Mc4r* null mutation elicits an intermediate phenotype.

$Mc4r^{-/-}$ mice also have increased linear growth, as is characteristic of A^y/a mice (77). *Mc4r*-deficient mice maintain core body temperature when exposed to a cold challenge (78), suggesting that sympathetic tone is not reduced to the same extent as it is in Lep^{ob} and $Lepr^{db}$ mice. Oxygen consumption of $Mc4r^{-/-}$ mice is reduced by 20% as compared to weight-matched controls, however, indicating that MC4R mediates some control of energy expenditure, in addition to affecting feeding.

Approximately 4% of morbid human obesity (BMI > 40 kg/m^2) is due to mutations in *MC4R* (79-81). Preserved lean mass and increased stature are also evident in the human MC4R deficiency syndrome, as in rodent models (82). Most obesity associated with *MC4R* mutations has been attributed to heterozygosity for such mutations (83). Severe childhood obesity results from a null MC4R receptor, generated by missense, frame shift, deletion, and nonsense mutations (82). *MC4R* mutations are

codominantly inherited, and heterozygous family members are overweight, suggesting that these mutations impair the function of the normal gene product, unlike null mutations in *Mc4r*$^{-/-}$ mice. Genome-wide association studies (GWAS) have revealed common non-coding polymorphisms within *MC4R* that are associated with increased adiposity (84). These are likely variants affecting the transcription rate of the gene.

Agouti-related peptide (AgRP) blocks MC4R signaling.

An homology search to identify a protein with a normal physiological function comparable to ASP in the brain revealed a candidate with 25% amino acid homology to ASP (Agouti-related peptide; AgRP) (71). Eutopic *Agrp* expression is restricted to a set of neurons in the ARC that contain the orexigenic neuropeptide Y (NPY), and which are activated by fasting or leptin deficiency, consistent with a role in controlling (promoting) food intake (85). *In vitro* binding studies showed that AgRP binds the MC1, MC3 and MC4 receptors, and mice globally overexpressing AgRP (like Agouti mice) are hyperphagic and obese compared to nontransgenic littermates (86). AgRP differs from ASP in that it does not block eumelanin synthesis to elicit a yellow coat color when transgenically overexpressed in mice. MC4R exhibits significant constitutive activity *in vitro*; ASP and AgRP not only block binding of a-MSH to the MC4R, but also suppress MC4R constitutive signaling, i.e., act as inverse agonists (71). In support of a physiological role for the orexigenic action of AgRP, fasted animals show increased expression of ARC *Agrp* mRNA, as do *Lep* ^{ob} and *Lepr* ^{db} mice (86). ICV administration of AGRP to rats elicits a long-lasting hyperphagic response (71).

Interestingly, however, mice congenitally null for *Agrp* or *Npy* exhibit minimal alterations in energy balance, as do compound *Npy*$^{-/-}$*;Agrp*$^{-/-}$ mice (87). This lack of phenotype apparently reflects developmental compensation/reprogramming, however, since mice in which AgRP neurons are ablated early in development exhibit normal energy balance, while ablation of these cells in adults results in aphagia and death by starvation (88,89). Thus, AgRP likely plays an important physiologic role in the promotion of feeding, presumably by blocking melanocortin receptor action.

MC3R also contributes to the control of adiposity.

Centrally administered AgRP causes hyperphagia in *Mc4r*$^{-/-}$ mice (90), supporting a role for an additional brain melanocortin receptor in the regulation of body weight. The MC3R is expressed in the ARC, DMH and VMH (75). In comparison to the MC4R, the MC3R has reduced affinity for AGRP, and increased affinity for a-MSH. *Mc3r*$^{-/-}$ mice develop late onset obesity accompanied by a 2-fold increase in fat mass (91,92). These effects are considerably smaller than the 3- to 5-fold increased adiposity observed the *Mc4r* null mouse. The *Mc3r*$^{-/-}$ mouse displays normal food intake, but reduced locomotor activity and increased respiratory quotient (reduced fatty acid oxidation) on high-fat chow as compared to contols, suggesting alterations in energy partitioning when challenged with a high-fat diet. Thus, while MC3R does not impact feeding to the same extent as MC4R, it nonetheless plays a role in the control of energy expenditure/metabolism, and nutrient partitioning, and thus plays a role in the control of adiposity. GWAS have not identified SNPs in the region of *MC3R* as risk alleles for increased body weight, however.

Proopiomelanocortin (POMC).

POMC, the precursor peptide for melanocortin receptor agonists and endorphins with effects on ingestive behaviors, is expressed in both the anterior pituitary and the hypothalamus (76). In the anterior pituitary, POMC is processed to ACTH and b-lipotropin. In the intermediate lobe of the pituitary, and in the hypothalamus, ACTH is processed further to a-MSH and CLIP. *Pomc*$^{-/-}$ mice generated by gene targeting weigh twice as much as wild-type littermates at 12 weeks of age, and are hyperphagic when

presented with either standard or high-fat chow (93). Daily intraperitoneal injection of a-MSH to *Pomc*$^{-/-}$ mice caused a 46% weight loss over a 2-week period with a concomitant darkening of coat color. To investigate specifically the role of a-MSH in body weight regulation, *Pomc* null mice have been rescued by transgenic overexpression of POMC in the pituitary but not the brain (94). Homozygous pituitary rescue mice are 33% heavier than *Pomc* null mice, indicating that a-MSH deficiency in the brain causes the obesity phenotype and that circulating glucocorticoids restored with pituitary *Pomc* replacement have an additive effect to increase body weight.

Functionally consequential mutations in *POMC* have been identified in humans. Human subjects have been described who are 1) compound heterozygous for mutations in exon 2 of *POMC* that result in premature termination of transcription as well as a frameshift mutation that disrupts the common binding site of a-MSH and ACTH, or 2) homozygous for a nucleotide transversion mutation in exon 3 that truncates POMC protein at codon 79, resulting in trace or undetectable amounts of circulating a-MSH and ACTH (95). These individuals exhibit early onset obesity and red hair because of the a-MSH deficiency, and are adrenal insufficient due to a lack of circulating ACTH. In a number of instances the accompanying adrenal insufficiency has led to death in infancy. Hence, suspicion of this diagnosis should be considered to constitute an urgent medical issue.

Syndecans.
Cell surface heparan sulfate proteoglycans (HSPGs) modulate ligand-receptor interactions at neural synapses (96). *In vitro* studies suggest that HSPG syndecan-1 may bind to AgRP, facilitating AgRP binding to MC4R. In accord with this model, transgenic mice overexpressing syndecan-1 exhibit late-onset obesity. The endogenous hypothalamic analogue of syndecan-1 is syndecan-3, and fasted mice show a four-fold induction of syndecan-3 mRNA in hypothalamic areas involved in energy balance. When challenged by a 16-hour fast, syndecan-3$^{-/-}$ mice exhibit blunted refeeding, presumably due to the decreased binding of AgRP to MC4R.

Fat reveals roles for peptide processing systems in the control of energy balance

Fat.
The *fat* mutation was first identified at the Jackson Laboratory in a colony of inbred HRS/J mice (9). *Cpe*^{fat} mice exhibit apparent hyperinsulinemia as early as four weeks of age followed by obesity at 8 to 12 weeks. Approximately 77% of the measured insulin is proinsulin, thus bioactive insulin levels are normal. By a positional candidate gene approach, a T→C point mutation in *Cpe*^{fat} (which results in a S202P transversion) was identified in carboxypeptidase E (CPE) (97). CPE is an enzyme that cleaves COOH-terminal dibasic residues arginine and lysine in prohormone precursors of insulin, enkephalin, POMC, NPY, melanin concentrating hormone (MCH), cholecystokinin (CCK), oxytocin (OXT), and vasopressin (AVP). Transgenic overexpression of insulin in *Cpe*^{fat} mice does not correct the obesity, suggesting that aberrant processing of one of the other peptides targets of CPE contributes to their increased fat mass (98). POMC is cleaved by prohormone convertase 1 (PC1, also called PCSK1 or PC1/3) and PC2 (PCSK2) to generate a precursor peptide that is further cleaved by CPE to generate active aMSH; miscleavage of POMC may account for the obesity in *Cpe*^{fat} mice.

One missense polymorphism in human *CPE* has been identified: a C→T transversion (99). This mutation results in a non-conservative R283W amino acid substitution that greatly decreases CPE enzymatic activity and is associated with early onset type 2 diabetes.

Prohormone convertases.
Mice null for PC1 are runted, hypoadrenal, and hypogonadal, presumably due to impaired processing of

POMC, GhRH and GnRH (100). The runting and other endocrine phenotypes apparently mask effects on adiposity. Mice homozygous for the milder *Pcsk1*^{N222D} mutation develop hyperphagic obesity associated with impaired processing of POMC to α-MSH; these animals are not dwarfed. (101). Proinsulin, prothyrotropin releasing hormone, progastrin, proneurotensin and prodynorphin are also incompletely processed in these animals. Human PC1 deficiency caused by missense and splice site mutations in the *PC1* gene also results in a disorder characterized by obesity and hypocortisolemia as well as hypogonadism (102).

Animals null for PC2 are not obese, although they exhibit phenotypes consistent with other defects in peptide processing (103); this lack of obesity may result from the partial activity of the POMC PC1 product on MC3/4R even in the absence of PC2, combined with other hormonal and neural changes in these animals. No humans defective in PC2 function have been identified to this point.

Prolyl carboxypeptidase.

PRCP is a serine protease that cleaves the COOH-terminal amino acid from substrate proteins where the penultimate amino acid is a proline residue (substrate preferences are X-P-F-COOH and X-P-V-COOH) (104). In general, PRCP inactivates biologically active peptides. The COOH-terminal sequence of the 13 amino acid α-MSH molecule is PV; removal of V abrogates the ability of α-MSH to decrease food intake. *Prcp*-null mice are lean with increased sensitivity to exogenous α-MSH. Presumably, mutations in PRCP also alter the inactivation of other peptides.

OTHER SPONTANEOUSLY-OCCURRING RODENT MUTATIONS LEADING TO OBESITY

Tubby.

The *tubby* mutation arose spontaneously at Jackson Laboratory in the C57BL/6 strain (9). These mice have a mild, late-onset obesity apparent by 8 to 12 weeks of age that is associated with hyperinsulinemia without hyperglycemia. *Tubby* results from a G→T transversion that interferes with normal intron excision (105). The result is an aberrant transcript in which a 44-base pair deletion at the 3′ end of the gene is replaced with a 24-base pair intronic segment that is usually spliced out. *Tub*$^{-/-}$ mice are phenotypically indistinguishable from *tubby*, suggesting that the phenodeviant that occurred in the Jackson Laboratory colony had a loss-of-function mutation in the *tubby* gene. Lack of detectable *tubby* protein or transcript in *tubby* mice further supported this finding.

The precise physiological mechanism(s) underlying the obesity of tubby mice remain unknown (105), but the *tubby* mutation also produces retinal and cochlear degeneration, which is seen in primary ciliopathies such as the Bardet-Biedl and Alstrom syndromes. (See below). The *tubby* gene product, TUB, binds to membrane phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) and is released upon PtdIns(4,5)P2 hydrolysis. A variety of data suggest an important role for TUB in GPCR signaling and trafficking, as well as insulin and leptin signaling, via the primary cilium (106).

Mahogany and mahoganoid.

The spontaneous, autosomal recessive coat color mutations, *mahogany (mg)* and *mahoganoid (md),* were first reported over forty years ago (71). When crossed to *A*^{y}*/a* mice, both *mg* and *md* darken coat color and attenuate obesity. Positional cloning of *mg* identified a gene orthologous to the human immune-response protein attractin (*atrn*), the gene product of which accumulates on the surface of activated T cells and subsequently facilitates the interaction between T cells and antigen by “attracting” macrophages (107). *Atrn* encodes a single transmembrane protein with a glycosaminoglycan side chain that has been suggested to chaperone *agouti* to melanocortin receptors. *Atrn* is expressed widely in the brain, as well as the skin, heart, kidney, liver and lung of wild-type mice. The *Atrn*^{mg} mutation in mice is

due to a ~5-kb retroviral insertion in intron 11 that disrupts *Atrn* expression, and which permits a relative increase in eumelanin expression in the hair follicle, resulting in dark fur, presumably as a consequence of increased melanocortin signaling at the melanocortin-1 receptor (MC1R). *Atrn*^{mg} mutants also have 10-15% reduction in body weight and have 20-40% less body fat content than littermate controls. Deficiency of *Atrn* in *A*^{y}*/a* mice reduces body weight and adiposity by increasing energy expenditure rather than reducing food intake, suggesting melanocortin-independent mechanisms of action. Indeed, *Atrn* ^{mg} mice may have neurological deficits (108), and the *zitter* mutation, which causes hypomyleination in rats, results from an 8-bp deletion in *Atrn* at a splice donor site that decreases *Atrn* expression. Thus, *Atrn* function may not be entirely A^{y}/melanocortin-dependent.

The *mahoganoid* locus, *Mgrn1* (*mahogunin;* RING finger 1), is located 2 cM from the centromere of chromosome 16 (109,110). Five mutations at this locus have been identified: *md*, *md*^{3J}, *md*^{4J}, *md*^{5J}, and *md*^{6J}. The *Mgrn1*^{md} mutation is a 5-kB IAP element intronic insertion between exons 11 and 12, which attenuates expression. Similar to the *mg* phenotype, the *Mgrn1*^{md} mouse is lean, and the allele reduces body weight and darkens the yellow coat color of *A*^{y}*/a* mice. The *Mgrn* gene product contains a RING finger domain consistent with ubiquitin E3 ligase function. Although the spontaneous mutations at the *Mgrn1* locus do not cause neurological degeneration, the *Mgrn1*$^{md\text{-}nc}$ mutation generated by caffeine mutagenesis results in histopathological changes similar to those seen in *Atrn*^{mg} and the *zitter* rat (111). Both mahoganoid and mahogany convey their effects on ASP signaling by effects on endosomal trafficking of MC4R (112).

THE USE OF TRANSGENIC MODELS TO STUDY SYSTEMS INVOLVED IN ENERGY BALANCE

Hypothalamic circuits important for energy balance

AgRP/NPY neurons, their mediators and modulators.

The AgRP-expressing neurons of the ARC also contain NPY, as well as the fast inhibitory neurotransmitter, GABA (113). These neurons are inhibited by leptin and activated by fasting and leptin deficiency; their activation promotes feeding and decreases energy expenditure, while their ablation results in lethal anorexia (88,89).

Mediators of AgRP/NPY neuron function.

As noted above, *Agrp* and *Npy* proteins have been ablated individually and in combination, with little effect upon energy balance in wild-type animals, although their ablation modestly attenuates the obesity of *Lep*$^{ob/ob}$ animals (87). In contrast, blockade of GABA release from these neurons, via the cre-mediated deletion of the vesicular GABA transporter (vGat) results in leanness and interferes with the response to ghrelin or food restriction, suggesting that these neurons (and especially GABA release therefrom) is crucial for promoting food intake, especially in response to signals of negative energy balance (113). Detailed studies of animals ablated for AgRP neurons have also suggested that GABA release from AgRP cells into the brainstem parabrachial nucleus is especially important for the stimulation of feeding by AgRP neurons (114).

NPY receptors.

NPY receptors are G-protein coupled receptors; six NPY receptors have been identified: Y1, Y2, Y3, Y4, Y5, and Y6 (115). Y1 and Y5 are localized to the hypothalamus and ICV administration of Y1- and Y5 receptor antagonists reduce food intake. Mice with targeted Y1 disruption show a variable and sex-dependent alterations in energy balance [139]; however, Y5-deficient mice develop mild obesity (116). Indeed, *Lep*$^{ob/ob}$*;Y5*$^{-/-}$ mice are not different than *Lep*$^{ob/ob}$ mice in terms of energy balance. In addition,

both Y1- and Y5-deficient mice are hyperphagic in response to centrally administered NPY, suggesting the existence of an additional NPY receptor or receptors that regulate food intake.

Hypothalamus-specific deletion of the Y2 receptor by viral delivery of Cre recombinase in $Y2^{Flox}$ mice results in a significant decrease in food intake and body weight(117). The endogenous peptide YY_{1-36}, a Y2 ligand co-localized with GLP-1 in the L-type endocrine cells of the GI mucosa, stimulates food intake; however, its cleavage product peptide YY_{3-36} (PYY_{3-36}), a Y2 agonist primarily secreted from endocrine cells lining the gastrointestinal tract, decreases food intake (118). Thus, the Y2 receptor may have dual functionality that is determined by the PYY moiety that binds to it.

Ghrelin, GHSR, and G*OAT.*

Ghrelin is a hormone released from cells in the epithelium of the stomach, duodenum, ileum, cecum and colon (119); its pharmacologic administration promotes dramatic feeding (120). The receptor for ghrelin is the growth hormone secretagogue receptor (GHSR), and ghrelin acylation is required for GHSR activation. Ghrelin is acylated (octanoylated) by ghrelin O-acyl transferase (GOAT) in the cells that synthesize it (121). Diurnal release of ghrelin into the circulation coincides with the initiation of meals, and decreases over the course of each meal (122); ingested fatty acids are required for ghrelin acylation, so that active ghrelin only increases prior to meals in animals that have fed over the prior 24 hours. GHSR is highly expressed on AgRP/NPY cells (as well as some other cells) in the hypothalamus, and ghrelin activates AgRP/NPY cells. Ghrelin is also expressed in the epsilon cells of the islets of Langerhans where it may act as a brake on glucose-induced insulin release by direct effects on the beta cell and/or antagonism of GLP1 secretogogues (123).

Consistent with the modest baseline phenotypes of mice null for the individual neurotransmitters employed by AgRP/NPY neurons, mice null for ghrelin, GHSR, or GOAT exhibit no detectable alterations in baseline energy balance, and only modest defects in refeeding (124). It is possible that some of the lack of effect of *Npy*, *Agrp*, *Ghrelin*, *Ghsr*, or *Goat* deletion reflects developmental reprogramming that occurs with defects in AgRP/NPY neurons during circuit formation, however, since ablation of these neurons early in development produces little effect on body weight, while their ablation in adults results in lethal anorexia (88,89).

Serotonin (5HT) receptor 2c.

The 5HT2cR is expressed in the ARC, PVN, LHA, and anterior hypothalamic nucleus (AH) of the hypothalamus (125). Agonists of the 5HT2cR promote weight loss, and several are in clinical trials or approved for the treatment of obesity. Deletion of *5ht2cr* produces hyperphagic obesity that is accentuated by high fat diet. A subset of ARC POMC neurons express *5ht2cr*, and the $Pomc^{cre}$-mediated reactivation of a null *5ht2cr* allele in these cells attenuates the food intake and obesity in the *5ht2cr* null mice (126). The effect of 5HT2cR activation may vary by nucleus, but, in aggregate, *5ht2cr* mutant mice confirm the important role for this receptor in energy balance.

Single-minded-1 (SIM1) and the PVH.

In mice, *Sim1* encodes a transcription factor required for the development of the PVH, an integrative hypothalamic nucleus in which a-MSH, NPY and 5-HT are released (127). Many PVH neurons contain MC4R, Y1R and/or 5-HT2cR. Ablation of the PVH in rodents produces a profound hyperphagia (128). The PVH contains a diverse constellation of neuronal subtypes, including those that express oxytocin (OXT), corticotropin releasing hormone (CRH), vasopressin (AVP), thyroid hormone releasing hormone (TRH), and others. Many of these molecules are thought to participate in energy balance, as well as their well-recognized neuroendocrine functions.

Human Single-minded-1 (SIM1) deficiency was discovered by karyotyping in three case studies of young obese patients with small deletions or translocations at the human *SIM1* locus on chromosome 6 (127). Homozygous deletion of *Sim1* is embryonic lethal in mice. *Sim1*$^{+/-}$ mice are normal until 4 weeks of age, when they develop hyperphagic obesity (129). These mice display reduced numbers of neuronal nuclei in the PVH with a proportional decrease in overall size of the PVH. Presumably, the decreased number of PVH neurons in these mice diminishes anorexic "tone" from the PVH, leading to hyperphagia and obesity in the *Sim1*$^{+/-}$ mice, as well as in rare human patients with SIM1 mutations. Additionally, deletion of *Mc4r* with *Sim1*^{cre} recapitulates the hyperphagia and obesity of *Mc4r*$^{-/-}$ mice, as does the ablation of *Mc4r* in the PVH by virus-mediated cre delivery (130,131). Thus, PVH SIM1-expressing neurons represent crucial direct targets for MC4R action and for energy balance. Understanding the roles for the various subsets of PVH SIM1 neurons in the control of energy balance has been more difficult, however. Ablation of *Mc4r* from OXT, AVP, and CRH neurons does not alter energy balance (131).

Oxytocin.

A variety of pharmacologic data suggest important roles for PVH-derived OXT in the control of feeding; the injection of OXT into the region of the NTS promotes satiation (132). However, genetic data argue against an important role of OXT or OXT neurons in energy balance. Not only do *Oxt*$^{-/-}$ animals display no alteration in feeding or energy balance, but neither the activation nor the ablation of PVH OXT neurons in adult animals alters food intake (133,134).

Corticotropin releasing hormone.

CRH increases glucocorticoid secretion via the hypothalamic-pituitary-adrenal axis, but also acts on a number of CNS circuits. Centrally administered CRH produces decreased food intake and weight loss (135); conversely, elevated CRH promotes activation of the HPA axis and promotes Cushing's syndrome with increased central adiposity due to peripheral glucocorticoid excess. While inactivation of *Crh* causes glucocorticoid deficiency, it has no impact on energy homeostasis (136). Similarly, while antagonism of the receptors for CHR (CRH1 and CRH2) leads to increased food intake, decreased energy expenditure and increased body weight (137), CRH receptor-deficient mice display normal regulation of body weight (138). Thus, while PVH CRH neurons and CRH signaling are crucial for the control of the HPA axis and for stress responses, CRH and its receptors do not appear to play an important role in the control of energy balance by the PVH.

Vasopressin (AVP).

In addition to magnocellular AVP neurons (mainly located in the SON) that project to the posterior pituitary to control fluid balance, PVH AVP cells project widely throughout the brain. While the deletion of *Mc4r* from these cells does not alter energy balance, the pharmacogenetic activation of these cells modestly suppresses food intake, suggesting that these cells may play some role in the control of energy balance, even though they do not represent direct targets of melanocortin action (131,139).

Steroidogenic factor-1 (SF1) and the VMH.

Steroidogenic factor 1 (*Sf1; Nr5a1*) is a transcriptional modulator expressed in the dorsomedial portion of the VMH- a hypothalamic nucleus implicated in the regulation of body weight (140). The VMH contains neurons that express LepRb, MC3R and other receptors involved in body weight regulation. Although *Sf1*-deficient mice were first described in 1994, early death due to adrenal insufficiency prevented characterization of this mouse in adulthood. By performing adrenal transplantation, it was possible to observe late-onset obesity in *Sf1* -deficient mice, which resembles the mild obesity phenotype of MC3R deficiency. *Sf1*-deficient, adrenal-transplanted mice appear normal until 8 weeks of age, when their body weights diverge from their wild-type littermates. By 6 months, *Sf1*$^{-/-}$ mice are 72% heavier than controls

due primarily to increased body fat, with no differences observed in linear growth. The obesity of these animals appears to result largely from decreased energy expenditure. *Sf1*^{cre} has been used to delete LepRb from the VMH; this manipulation decreases energy expenditure and accentuates obesity in high-fat diet-fed animals (68). Many SF1-containing VMH neurons also contain the neuropeptide PACAP (the product of the *Adcyap* gene), which may contribute to the control of energy expenditure (141). Thus, *Sf1*-mediated manipulation of the dorsomedial VMH has revealed a crucial role for this region in the control of energy expenditure and thus overall energy balance.

Reward circuitry:

The lateral hypothalamic area (LHA) and mesolimbic dopamine (DA) system.

While the ARC, PVH, and (to a lesser extent) VMH mediate net anorexic tone, the LHA (together with the mesolimbic DA system) modulates behavioral incentive- including the drive to eat (1). While lesions of the ARC or LHA promote hyperphagia and obesity, destruction of the LHA abolishes the motivation to feed, resulting in starvation. While many details of these reward circuits remain to be discovered, LHA neurons modulate the mesolimbic DA system by projections to the ventral tegmental area (VTA; where the DA cell bodies lie) and the striatum (a crucial target of VTA DA neurons).

The VTA and DA.

Mice that lack tyrosine hydroxylase (TH) cannot make the precursor for catecholamines and are deficient in DA, noradrenaline and adrenaline; these animals die between embryonic day 11.5 and 15.5; restoring TH in noradrenergic neurons generates viable mice that synthesize noradrenaline and adrenaline normally, but do not synthesize DA in neurons of the mesolimbic DA system (142). DA-deficient pups nurse normally until 2 weeks of age, but thereafter fail to thrive due an inability to wean themselves onto solid food unless supplemented with the DA precursor, L-DOPA, suggesting that DA is required for normal ingestive behavior (as well as activity). However, ingestive behavior data that implicate dopamine as a stimulator of food intake may be confounded by the roles of dopamine in the initiation of motor activity and reward mechanisms.

The LHA.

Both leptin and the melanocortins have been implicated in the control of two important sets of neurons that lie within the LHA. One population contains the neuropeptide melanin concentrating hormone (MCH; not related to POMC or any of its derivative peptides) (143). MCH promotes feeding, and animals null for MCH (or its receptor) are lean (144). The MCH receptor is located on the primary cilium, and some of the effects of ciliopathies on adiposity may be conveyed by effects on this receptor (see discussion of ciliopathies below). A distinct set of LHA neurons express the neuropeptide hypocretin (HCRT; also known as orexin) (145,146). Based upon early acute pharmacologic studies, HCRT was originally conceived of as an orexigen; subsequent work has revealed animals null for HCRT or its receptors to be mildly obese, however (147). Indeed, narcolepsy, which results from the loss of HCRT action in mice and humans, is associated with increased adiposity (148). Most of the effect of HCRT administration or *Hcrt* mutation on energy balance results from decreased physical activity and energy expenditure. Similarly, the LepRb-containing neurons that control HCRT neurons have been identified- these contain neurotensin (NT) and lie in the LHA, intermingled with the HCRT cells (149-151). Ablation of LepRb from these LHA cells prevents the normal regulation of HCRT neurons and results in decreased motor activity and energy expenditure. Both LHA LepRb neurons and HCRT cells project to the VTA, and parameters of DA neuron function are altered in mice lacking LepRb in NT neurons, as well as in *Lepr*$^{ob/ob}$ and *Lepr*$^{db/db}$ mice.

Genes involved in insulin and leptin signaling.

Transcription factors involved in leptin signaling.

LepRb, like other Type 1 cytokine receptors, activates signal transducers and activators of transcription (STATs) as a major component of its signaling pathway (31). During leptin signaling, tyrosine phosphorylated residues on LepRb recruit STAT3 and STAT5, which are then phosphorylated by Jak2 to promote their trafficking to the nucleus. In the nucleus, STATs bind DNA and modulate gene expression. STAT3 mediates the majority of leptin action, since disruption of the binding site for STAT3 on LepRb causes a severe obesity phenotype in mice that is similar to the obesity syndrome of *Lepr*$^{db/db}$ mice (152). Similarly, disruption of *Stat3* in the forebrain or in LepRb-expressing POMC, or AgRP neurons results in obesity in mice (153,154). While the brain-wide disruption of the genes encoding both isoforms of STAT5 (*STAT5a* and *STAT5b*) causes mild late-onset obesity, the deletion of *Stat5a/b* specifically in LepRb neurons produces no detectable phenotype, suggesting that STAT5 signaling is not required for leptin action *in vivo* (155-157). STAT5 represents a major mediator of GM-CSF signaling, however, and mice null for GM-CSFR in the brain animals are obese, suggesting that the role for STAT5 in energy balance may be linked to the action of GM-CSF or other cytokines different than leptin (156).

Insulin receptor.

Like leptin, insulin circulates in proportion to fat mass, and alters neuropeptide expression in the hypothalamus via receptors located in the ARC, PVN, and DMH (158). ICV insulin has been reported to decrease food intake in rats and mice. Furthermore, mice deleted for insulin receptor (*Insr*) throughout the CNS display a modest late-onset obesity (more prominent in females), and are more susceptible to diet-induced obesity than wild-type mice (159). Thus, CNS INSR signaling plays a role in energy balance. Deletion of *Insr* in skeletal muscle causes modest increases in adiposity, presumably by decreasing insulin-stimulated glycogen storage in muscle and concomitantly increasing glucose uptake in adipose tissue (hence, due to changes in nutrient partitioning) (160). Conversely, deletion of *Insr* from adipose tissue produces lipodystrophy (161).

The IRS-protein/PI 3-kinase pathway.

The tyrosine phosphorylation of insulin receptor substrate proteins (IRS-proteins; IRS-1, -2, -3, and -4) represents the first downstream step in insulin signaling (162). Tyrosine phosphorylated IRS-proteins engage downstream molecules, such as those in the phosphatidylinositol 3-kinase (PI3-kinase) pathway, to mediate insulin action. While deletion of *Irs1* interferes primarily with peripheral insulin action and the growth axis, deletion of *Irs2* affects pancreatic beta cells and the brain to cause insulin deficient diabetes (due to islet failure) and obesity. Restoration of *Irs2* in the islets of *Irs2*$^{-/-}$ mice or brain-specific ablation of *Irs2* results in normoglycemic obesity, consistent with a role for brain IRS2 signaling in energy balance (163). Indeed, deletion of *Irs2* from LepRb-expressing neurons promotes obesity, albeit a milder obesity than observed in animals deleted for *Irs2* throughout the brain. While leptin modulates the IRS-protein→PI3-kinase pathway, deletion of *Irs2* itself does not interfere with leptin action, suggesting that IRS2 may primarily play a role in brain insulin action (164). Deletion of *Irs4*, which is expressed in neurons of the hypothalamus, modestly alters energy balance. A variety of subunits and downstream effectors of the PI3-kinase signaling pathway have also been deleted in several neuronal populations in mice (35). These produce phenotypes generally consistent with the notion that PI3-kinase is important for the proper function of the POMC and AgRP neurons that modulate energy balance- at least in part by controlling the firing of these important neurons. Similarly, ablation of the gene encoding the PI3-kinase inhibited transcription factor, FOXO1, tends to augment insulin and leptin action *in vivo (165)*.

mTOR and autophagy.

The mechanistic target of rapamycin complex 1 (mTORC1) is activated by PI3-kinase signaling and

nutrient (especially amino acid) availability to promote cellular anabolic processes while blunting autophagy (166). ICV amino acids activate hypothalamic mTOR and promote satiation, while blockade of hypothalamic mTORC1 using the inhibitor, rapamycin, promotes hyperphagia- suggesting a role for mTORC1 in producing satiety (167). The role for mTORC1 in the hypothalamic control of energy balance may be complicated, however, as neuronal firing also activates mTORC1, and mTORC1 is increased in AgRP/NPY neurons during fasting (168). Furthermore, lifelong activation of mTORC1 or inactivation of autophagy (via deletion of *Atg7*) in POMC neurons promotes hyperphagic obesity in mice (169,170).

Tyrosine phosphatases and other inhibitors of insulin and leptin signaling.

Protein tyrosine phosphatase-1B (PTP1B) dephosphorylates cognate tyrosine kinases (including those associated with INSR and LepRb) to terminate signaling (171,172). In addition to exhibiting increased insulin sensitivity, *Ptp1b*$^{-/-}$ mice are lean compared to controls and are resistant to weight gain on a high-fat diet, suggesting increased leptin action in these animals. Indeed, animals in which *Ptp1b* is disrupted throughout the brain, or specifically in LepRb or POMC neurons demonstrate increased leanness and enhanced leptin action (173,174). Other phosphatases may also limit insulin and/or leptin signaling: Mice null for *Rptpe* or *Tcptp* also demonstrate leanness and increased leptin sensitivity (175).

Suppressors of Cytokine Signaling (SOCS proteins), including SOCS1 and SOCS3, bind to activated cytokine receptor/Jak2 kinase complexes (including the LepRb/Jak2 complex) to mediate their inhibition and degradation (176). SOCS proteins may also inhibit INSR and other related tyrosine kinases. Leptin signaling via STAT3 promotes *Socs3* expression in hypothalamic LepRb neurons; SOCS3 protein binds to phosphorylated Tyr_{985} of LepRb to attenuate LepRb signaling (177). The physiologic importance of this pathway is demonstrated by the leanness of mice containing a substitution mutation of LepRb Tyr_{985} (binding site for SOCS3 on LEPR; see figure above) and the similar phenotype of mice lacking *Socs3* in the brain or in LepRb neurons (178,179). While LepRb Tyr_{985} also mediates the recruitment of the tyrosine phosphatase SHP2 (aka, PTPN1), data from cultured cells suggest that SHP2 mediates ERK pathway signaling by LepRb, and disruption of *Ptpn1* in the brain, in LepRb neurons, or in POMC neurons, promotes obesity (31).

SH2B1.

SH2B1 binds to activated Jak2, as well as to the INSR, TrkB, and a few other receptor tyrosine kinase complexes to increase their activity and mediate aspects of downstream signaling (180). *Sh2b1*$^{-/-}$ mice display a complex phenotype that includes obesity; brain-specific absence of *Sh2b1* also promotes obesity in mice (181,182). Thus, SH2B1 signaling in the brain is required for energy balance, perhaps due to its requirement for correct signaling by multiple receptors involved in energy homeostasis. Furthermore, the phenotype of several human patients with morbid obesity, developmental delay, and behavioral disorders are associated with chromosomal deletions (16p11.2) or coding variants involving *SH2B1 (183)*. Indeed, GWAS studies have suggested a role for common variants in *SH2B1* in human obesity (84).

Other transcription factors.

The transcription factor BSX is found in AgRP neurons, where its expression is regulated by leptin and feeding status (184). Deletion of *Bsx* in mice reduces the increase in *Npy* and *Agrp* expression, and associated hyperphagia, in food-deprived mice, suggesting a role for BSX in the function of AgRP neurons and in feeding control. While its role in leptin action is not known, the disruption of *Atf3* (which encodes a STAT3-responsive member of the AP1 family of transcription factors) in a poorly-characterized set of hypothalamic neurons also causes obesity in mice (185).

The peroxisome-proliferator activated receptor (PPAR) family of nuclear transcription factors modulates genes encoding proteins involved in lipid homeostasis (186). PPARa induces hepatic genes that promote mitochondrial uptake and beta-oxidation of free fatty acids, and PPARa agonists (fibrates) are used clinically to lower circulating triglycerides and free fatty acids. *Ppara*-/- mice exhibit mild late-onset obesity that may partially result from decreased energy expenditure, but food intake is increased in these animals, and no increase in feed efficiency is observed, suggesting that increased food intake may ultimately drive this phenotype (187). PPARg is expressed primarily in adipose tissue, and promotes adipocyte differentiation and storage of triacylglycerols in adipose depots (186). PPARg agonists (thiazolidinediones, TZDs) increase insulin sensitivity and have been used in the treatment of type 2 diabetes. PPARg agonists promote weight gain and, although *Pparg-/-* is embryonic lethal), *Pparg+/-* mice weigh 14% less than wild-type C57BL/6 mice, and have a 70% reduction in WAT mass. These mice display an elevated metabolic rate, and also a decrease in food intake. Indeed, while the primary effect of PPARg manipulation of body weight and adiposity was previously assumed to results from direct adipose tissue action, genetic and pharmacologic manipulation of PPARg in the brain has revealed that brain PPARg action promotes increased feeding, which accounts for the energy balance effects of PPARg (188-190). A common polymorphism of PPARG has been associated with BMI in GWAS studies (84).

Mouse Models of human obesity syndromes.

Brain-derived neurotrophic factor (BDNF)/TrkB signaling.

BDNF, a member of the neurotropin family, is widely expressed in the nervous system during development, as well as being expressed within several brain regions important for energy homeostasis in adults (191). It acts via its receptor, TrkB, to control a variety of basic neural processes, including proliferation, survival, and plasticity. Given its many important roles in the CNS, alteration in BDNF expression (or that of its receptor, TrkB) would be predicted to interfere with multiple processes. Indeed, humans haploinsufficient for *BDNF* display impaired cognitive function and hyperactivity, in addition to hyperphagic obesity (192,193). Mutations in TrkB produce similar hyperphagia and obesity in rare human patients, along with impaired cognitive function and nociception (194). Interestingly, a coding polymorphism in BDNF (Val66Met) is associated both with obesity and with binge eating disorders in humans (195), consistent with the role for BDNF/TrkB signaling in energy balance, and suggesting a broader role for this system in the genetic determination of adiposity in humans. Indeed, alteration of TrkB and/or BDNF function in the hypothalamus of mice promotes obesity (196,197). Furthermore, polymorphisms in *BDNF* are associated with risk for obesity in human GWAS studies (84).

Ciliopathies.

A subset of mutations causing defects in primary cilia promote obesity syndromes (198,199). The primary cilium is found on most cells; while structurally related to motile cilia (such as flagella), the primary cilium is immotile and does not participate in propulsion. The primary cilium plays a crucial sensory role in cells, including cell-specific sensing, such as olfaction in sensory epithelium, photoreception in retinal cells, mechanical transduction in kidney cells, and signaling via a variety of cell surface receptors, including many GPCRs. A broad group of disease-causing human mutations have now been recognized to result from mutations in genes affecting ciliary functions (the “ciliopathies”). The clinical presentation of these diseases variably includes anosmia, retinal degeneration, kidney malformations, and a variety of developmental and neural defects, many of which are idiosyncratic to the particular gene that is mutated. A number of these mutations produce obesity in addition to the other phenotypes noted above, both in mice and humans. Included in these obesity-causing ciliopathies are

Bardet-Biedel Syndrome (BBS), McKusic-Kaufman Syndrome, Alström Syndrome, and, possibly, Joubert Syndrome.

Structural defects are apparent in the primary cilia of humans with BBS and the mice segregating for mutations in these genes (200,201). The structural changes may not themselves account for the functional derangements associated with these mutations. The BBS proteins, which constitute a "BBS-some" complex associated with the base of the primary cilium/basal body, participate in the trafficking of proteins to and within the cilium. Indeed, mutations affecting IFT88, a protein specific for trafficking within the cilium, in mice results in an obesity phenotype similar to that produced by mutations in BBS genes (202). While the particular protein(s) whose impaired trafficking may underlie this obesity is not yet clear, the primary cilium is crucial for signaling via a variety of receptor signaling pathways, including the WNT and SHH pathways, tyrosine kinases (such as the receptor for PDGF), MCHR, and numerous GPCRs. There is also evidence for impaired leptin receptor signaling in mice segregating for *Bbs* (203)– though attributed by some to the consequences of weight gain *per se* (204)ref] - and *Rpgrip1l* mutations (205). Such alterations could impair the development or function of a variety of neural circuits important for the regulation of energy balance. The deletion of *Ift88* from POMC neurons produces a portion of the obesity phenotype observed in the complete null, suggesting roles for multiple cell types (and perhaps multiple signaling pathways) in the complete ciliopathy phenotype (202).

FTO.

The human locus with the strongest GWAS linkage to adiposity (a polymorphism located in the human *FTO* locus) also contributes the largest amount to the genetic component of polygenic human obesity (206). Multiple mechanisms for the regulation of energy balance have been proposed for this alteration. In mice, *Fto* is expressed in the brain, including in hypothalamic feeding centers, where its expression is modulated by leptin and feeding status (207). Furthermore, its role in controlling food intake and body weight is suggested by the lean phenotype of $Fto^{-/-}$ mice, although these animals present a complex phenotype that includes runting (208). In contrast, mice ubiquitously overexpressing *Fto* or overexpressing *Fto* in the brain demonstrate increased food intake and adiposity (209). Alternatively, the *Fto* locus is adjacent to the *Rpgrip1l* gene, which encodes a protein involved in primary cilium function; the non-coding sequence variant in intron 1 of *Fto* are physically associated (in linkage disequilibrium) with alleles of a transcription factor (Cux1) binding site that, by binding in the intron affects expression of a ciliary gene, *Rpgrip1l* (205). Hence, the effects of the *FTO* alleles may be conveyed via effects on RPGRIP1L. Recently, it has been suggested that these *Fto*-associated polymorphisms lie within a long-range enhancer element that modulates the expression of a downstream gene, *Irx3 (210)*. The *Fto* polymorphism predicts the expression of hypothalamic *Irx3*, not *Fto*, in mice. Mice null for *Irx3* or that overexpress a dominant negative *Irx3* mutant in the hypothalamus demonstrate increased leanness. It is possible, of course, that the *Fto* intronic variants are affecting the expression of multiple genes other than *Fto* itself. In fact, the strength of the association is consistent with that possibility.

Prader-Willi syndrome (PWS)

PWS presents in infancy with low birth weight, hypotonia and poor feeding, with a progressive transition to hyperphagia and obesity starting after age 2 or 3 years. Additional features include short stature (correctible with growth hormone therapy), central hypogonadism, characteristic behaviors (especially around feeding), and often cognitive impairment (211,212). Most instances result from a 5-7 Mb deletion of an imprinted region (PWS region) on the paternal chromosome 15 (15q11-q13) and are non-recurrent. Within this deletion lie a number of genetic elements, including the genes encoding *MAGEL2* and *NECDIN*, which are thought to be involved in neural development and function, and a complex non-coding locus. Non protein -coding genes in this interval include a transcribed non-coding gene (*SNURF-*

SNRPN) that encodes a multitude of C/D box small nucleolar (sno-) RNA genes, including *SNORD116*. The RNA products of these SNORD genes are thought to be involved in RNA editing, perhaps of specific mRNA species. A small number of individuals with PWS phenotypes associated with microdeletions of the implicated region on chromosome 15 have reduced the number of candidate genes for this syndrome (211). These patients have demonstrated obesity, developmental delay, hypogonadism, and all major features of PWS. The minimum critical deletion region contains only non-coding genes, including, the *SNORD116* gene cluster, *IPW*, and *SNORD109A*. The *Snord116* locus has been deleted from mouse models, which display a growth defect and behavioral abnormalities, including a relative hyperphagia that develops after weaning, but which is balanced by increased energy expenditure (213). Thus, the effects of *SNORD116* likely contribute to PWS, but may not account for all of the phenotypes. The functions of *Necdin* and *Magel2* have also been examined in genetically targeted mouse models. *Magel2*$^{-/-}$ mice display early growth retardation with a mild increase in adiposity, and *Necdin*$^{-/-}$ mice display early postnatal respiratory failure along with a subset of PWS-associated behaviors (214-216). Thus, the full PWS likely results from the combined effects of multiple genes; several genes within the PWS region also likely contribute to the maximal obesity phenotype. It is not yet clear how each of the loci within the PWS alter neurophysiology and/or which neurons they might specifically affect energy balance. As with BBS, some of these genes are likely to be affecting brain structural development/connectivity as well as more conventional signaling pathways. Understanding the molecular physiology of PWS (and BBS) is likely to identify novel genes in the control of energy homeostasis in non-syndromic obesities.

MODELS THAT PROBE ROLES FOR SATIETY SYSTEMS IN ENERGY BALANCE.

A variety of gut-derived signals including peptides (such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP1), and amylin (a.k.a., islet amyloid polypeptide)) and vagal signals converge on hindbrain circuits in the NTS to promote satiation and meal termination (217). These systems are crucial for the short-term control of feeding, and their pharmacologic manipulation may be therapeutically important. Injection of CCK, GLP-1, or amylin, including into the hindbrain, promotes meal termination. Furthermore, supraphysiologic/pharmacologic agonism of GLP1 and amylin receptors not only induces satiation, but promotes modest weight loss. It is not clear that these systems modulate long-term feeding under physiologic conditions, however.

CCK.

CCK is a gastrointestinal hormone secreted in response to ingestion of a meal by enteroendocrine “I” cells located primarily in the duodenum. Many of these cells co-express other peptides affecting ingestive behavior (ghrelin, GIP, PYY). CCK induces a transitory sensation of satiety, secretion of pancreatic enzymes and gallbladder contraction. CCK-A receptors are located on vagal afferents of the stomach and the liver and transduce signals via the vagal nerve to satiety centers in the brainstem, eliciting a brief reduction in food intake (for a review, see (218)). CCK-B receptors are located diffusely throughout the brain, but their role in the satiety effect of CCK has not been demonstrated. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is an outbred strain of Long-Evans rats used experimentally as a model of type 2 diabetes. This animal has a 34% increase in food intake resulting from larger meal size, accompanied by a 23% increase in body weight at 15 weeks as compared to lean Long-Evans rats (219). In 1994, a mutation in the CCK-A receptor (CCKAR) of OLETF rats was identified; this 6847-base-pair deletion disrupts the *Cckar* promoter, reducing receptor expression. While CCK decreases meal size and duration, compensatory increases in meal frequency prevent CCK from producing long term effects on total food intake or body weight. Indeed, deletion of *Cckar* in mice does not cause obesity,

suggesting that the OLETF phenotype results from a number of genetic variants that act in concert with the *Cckar* mutation.

GLP1.

GLP-1 functions as an incretin (stimulator of insulin secretion) following its release from L-cells of the duodenum after nutrients enter the intestine (220). GLP1 can also modulate satiety: ICV GLP-1 (or GLP1R agonists) potently suppress food intake in rats and mice, while the GLP-1 receptor antagonist, exendin (9-37), increases short-term food intake. Some of the effects of GLP1 on food intake may be due to delayed gastric emptying. *Glp1r-/-* mice exhibit decreased circulating insulin concentrations during a glucose tolerance test, suggesting that GLP1R is important for glucose-stimulated insulin secretion (GLP-1 acting as an "incretin"). Body weight and food intake are unaffected by ablation of GLP-1R, however, suggesting that (like CCK and CCKAR) this system primarily modulates short-term satiation, rather than long-term energy balance, under normal physiologic circumstances.

INTERACTIONS OF THE IMMUNE SYSTEM AND ENERGY BALANCE

Inflammatory signals are proposed to mediate several distinct metabolic responses. Clearly, strong acute inflammatory stimuli (including those associated with systemic infection, cancer, etc.) decrease appetite and increase energy expenditure, promoting cachexia. Conversely, obesity is associated with increased low-grade inflammation that appears limited to particular tissues, such as adipose tissue and the hypothalamus. This low-grade "metabolic inflammation" is associated with insulin resistance and obesity. A variety of animal models have been employed to explore the interaction of inflammatory signals and energy balance/metabolism.

Systemic immune signaling promotes negative energy balance.

Lipopolysaccharide (LPS) administration, which produces some of the metabolic consequences of bacterial infection, blunts appetite; the mechanism of this hypophagia overlaps with the systems that control energy balance, as the LPS-induced anorexia requires the melanocortin system (221). Consistent with the induction of negative energy balance by systemic inflammation, alterations that blunt inflammation generally blunt inflammatory anorexia. While not altering baseline energy balance in chow-fed animals, deletion of IL-1b converting enzyme (ICE; which is essential for IL-1b activity), prevents LPS-induced anorexia in mice (222). The inflammatory system may also contribute to the control of energy balance under normal physiology, as well: adiposity is increased in *Il6*$^{-/-}$ and *Gmcsf*$^{-/-}$ mice, and in mice with impaired macrophage function due to the targeted deletion of Mac-1 or LFA-1 (or their receptor, ICAM-1) (223). Conversely, mice with constitutively increased IL-1 receptor signaling induced by targeted deletion of the endogenous IL-1 receptor antagonist, *Il1ra*, display reduced body mass compared to wild-type littermates (224).

Metabolic inflammation.

Obesity is associated with increased production of a number of cytokines (including TNFa) in adipose tissue, resulting primarily from the activation of adipose tissue macrophages and other immune cells (225,226). Indeed, a number of manipulations that decrease adipose tissue inflammation ameliorate the metabolic dysfunction associated with obesity. While interference with generalized macrophage function may increase adiposity, as noted above, other manipulations that alter their pro-inflammatory (versus anti-inflammatory) nature increase leanness and improve metabolic function (227,228). Similarly, interfering with the Nf-kb pathway (which is crucial for the response to a variety of inflammatory stimuli) in the liver improves metabolic function in obese mice. Some data also suggest a contributory role of hypothalamic inflammation, including gliosis, in promoting obesity. However, debate continues regarding

whether this inflammation provokes or attenuates obesity, virus-mediated interference with Nf-kb signaling in the hypothalamus ameliorates obesity and metabolic dysfunction (229). The ER stress in adipose tissue and the hypothalamus, potentially a consequence of metabolic inflammation, is also associated with obesity (230). Genetic or pharmacologic interference with ER stress ameliorates obesity and insulin resistance in rodent models.

ENERGY EXPENDITURE AS A DETERMINANT OF ADIPOSITY

With few exceptions, most of the systems that dramatically alter energy balance act primarily via the control of feeding; isolated alterations in energy expenditure promote more modest changes in energy balance that may be synergistic with effects on ingestive behavior, and may be detected under specific environmental and experimental conditions. Increases in energy expenditure and negative energy balance promote a compensatory increase in feeding. Similarly, decreased energy expenditure will cause the accretion of adipose mass, which tends to restrain feeding. For instance, interference with normal VMH function (discussed above) decreases diet-induced energy expenditure, and promotes increased adiposity only when animals are provided high caloric density diets. The adipokine leptin, which is responsive to acute and chronic changes in adipose tissue energy stores plays an important role in promoting these reciprocal responses.

However, the physiological responses to reductions in energy stores – increased drive to eat, reduced energy expenditure – are much stronger than the response to increased energy stores (55).

Animal models with altered energy expenditure.

Uncoupling protein 1 (UCP1, which is found primarily in brown and beige adipose tissue (BAT)) allows dissipation of the electrochemical gradient across the inner mitochondrial membrane, releasing energy as heat (231). Ablation of BAT in mice expressing diphtheria toxin A driven from the UCP1 promoter or congenital deletion of Ucp1 fails to alter adiposity at thermoneutrality, although adiposity increases slightly relative to controls in animals raised at temperatures colder than thermoneutrality, since these animals fail to substantially increase energy expenditure in response to the cold challenge (232). Similarly, the phenotype of mice null for the b_3-AR was not as severe as predicted: fat mass in male mice is only slightly increased, even in animals consuming a high-energy diet under non-thermoneutral conditions (233). Also, “b-less” mice, with a global targeted deletion of all three b-adrenergic receptor isoforms, have only slightly increased body fat (22.2 % ± 0.9 as compared to 16.2% ± 1.9 for wild type controls) on high fat diet under non-thermoneutral (233).

Increased sympathoadrenal activity in adipose tissue activates a signaling cascade that induces phosphorylation of regulatory subunits of protein kinase A (PKA), which in turn inhibits lipogenesis and increases lipolysis. Deletion of the regulatory subunit II b of PKA (RII b) , found mainly in WAT, BAT and brain, causes a compensatory increase in RI a , a subunit isoform that constitutively upregulates PKA activity (234). *RII b -/-* mice therefore have constitutively increased cAMP in response to sympathetic activation in adipose depots, with secondary elevation of metabolic rate and body temperature, and a 50% reduction in WAT pad weight despite a compensatory hyperphagia.

ALTERATIONS IN ADIPOSE TISSUE THAT AFFECT ENERGY BALANCE

Glucocorticoids and adipocyte 11-β–hydroxysteroid dehydrogenase type 1 (11βHSD-1).

11*β*HSD-1 is the enzyme that catalyzes the conversion of cortisone to biologically active cortisol. Visceral obesity is associated with elevated cortisol secretion due to increased local activity of 11*β*bHSD-*β*1 in adipose tissue, but normal levels of circulating glucocorticoids (235). Transgenic overexpression of

11βHSD-1 driven by the aP2 promoter (to confer adipose tissue specificity) produced mice that consumed 17.1% more calories than lean controls and thus gained 16% more weight by 9 weeks of age, and weighed 21% more calories than controls when administered a high-fat diet. A 3.7-fold increase in the mesenteric (visceral) fat pad weight was detected (236). These findings suggest that increased production of glucocorticoids in adipose tissue drives the visceral obesity syndrome, although the increased food intake in these animals implicates potential non-local mechanisms.

CONCLUSIONS

The mice and rats described in this essay provide proof that body weight and composition are regulated by specific genes that participate in complex neural and metabolic pathways that determine energy intake and expenditure. The identification of molecules responsible for the single gene obesities in these animals has expedited the discovery of many other molecules, pathways and developmental processes that constitute the still only incompletely understood mechanisms for energy homeostasis that interact with developmental and environmental processes to determine body mass and composition. Their existence provides definitive refutation of vitalist/psychological notions that have permeated the field of energy intake and metabolism, and provides the heuristic, reductionist framework in which ongoing research on these questions should be conducted. It is likely that major genes and their modifiers, as well as allelic variants of a larger number of genes with lesser individual impact, will eventually account for both qualitative and quantitative aspects of the critical phenotypes in rodents and humans. As this chapter demonstrates, mice and rats provide a powerful resource for the discovery and study of the constituent molecules, and for hypothesis generation regarding the same processes in humans. The ability to refine the characterization of the behavioral and metabolic phenotypes that are controlled by these genes –in rodents and humans– will add greatly to the power of genetics to reduce the complex continuous phenotypes that are the physiologic “stuff” of energy homeostasis to their constituent molecular events.

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