

NEUROENDOCRINE CONTROL OF BODY ENERGY HOMEOSTASIS

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ABSTRACT

The brain integrates the response to a variety of signals of energy need and availability to match food intake with energy expenditure, thereby maintaining body weight stability. Early work with rodent models with disrupted energy balance (generally obesity) identified many hypothalamic genes and signaling pathways that impact energy homeostasis. More recent studies have identified hindbrain circuits that interact with peripheral metabolic signals and hypothalamic circuits to impact energy balance. Feeding, signals of energy utilization, and hormonal signals of energy stores (such as leptin) modulate gene expression and neurotransmission in specialized circuits within the hypothalamus and brainstem to control food intake. While many of these circuits also control energy expenditure, the effects on body weight that arise from alterations in energy expenditure are generally more modest than the effects of produced by changes in feeding. Although most of the mechanistic work that defined the systems that control energy balance utilized rodent models, these systems have human orthologs whose disruption produces phenotypes comparable to those observed in rodents, confirming their conserved function.

INTRODUCTION

Historically, obesity was thought to represent a disorder of voluntary behaviors, (albeit exacerbated by the ready availability of food and the reduced need for energy expenditure afforded by modern societies); many continue to hold this belief even today. In reality, we now understand that food intake and body weight represent biologically controlled homeostatic variables, much like blood pressure. This understanding flows from the discovery of spontaneously occurring single gene mutations that promote obesity independently of environmental alterations, along with the more recent description of human genetic variants that influence weight gain. Furthermore, research building upon these genetic observations has identified many of the biological systems that mediate the control of energy homeostasis, most of which reside in or converge on the central nervous system (CNS).

Changes in body weight reflect an alteration of energy balance, where energy intake (calories from eating or drinking) and energy expenditure (either as locomotor activity, basal metabolism, or thermogenesis) become unequal. For instance, food intake in excess of energy expenditure promotes the accretion of excess weight. Adipose tissue represents the major repository for ingested energy that exceeds immediate needs (1) and excess adipose tissue represents the hallmark of obesity.

The energy density of adipose tissue is nearly 10-fold greater than muscle (protein) or liver (glycogen) (2). The ability to store energy in adipose tissue protects against environmental vicissitudes that might result in starvation, fetal wastage, and the inability to provide sufficient breast milk to the young. Therefore, evolution has likely selected for genetic variants that favor energy storage and conservation. The existence of environments in which excess calories are readily available with minimum or no effort occurred very recently in human evolution, while the human genetic blueprint evolved under the opposite circumstance. Thus, the modern obesity epidemic may represent, at least in part, a physiologic mismatch between the evolutionary pressures that bias toward energy storage and the modern, nutrient- and calorie-rich environment.

The brain plays a central role in maintaining energy balance. CNS circuits continuously assess and integrate peripheral metabolic, endocrine and neuronal signals, and modulate both behaviors and peripheral metabolism to respond to acute and chronic needs (3). The brain modifies energy intake and expenditure to match energy demands on an ongoing homeostatic basis, establishing a metabolic “set-point”.

A BRIEF HISTORICAL PERSPECTIVE ON THE MECHANISMS THAT CONTROL ENERGY BALANCE

Role for the Hypothalamus

The description of Frölich syndrome (hyperphagic obesity and hypogonadism in patients with pituitary tumors) initially suggested that the pituitary gland might control energy balance (4). Others noted that pituitary tumors often impinge on the overlying hypothalamus, however, and suggested that the hypothalamus might represent the main modulator of feeding. Indeed, experiments by Hetherington and

Ranson in 1940 demonstrated that lesions of the ventral medial portion of the hypothalamus increased feeding and promoted weight gain in rats, while lesions in the lateral hypothalamus led to decreased feeding and weight loss (5). In addition to demonstrating the importance of the hypothalamus to energy balance, these findings also led Eliot Stellar to suggest the concept of a “satiety center” situated in the ventral medial portion of the hypothalamus and a “hunger center” located in the lateral hypothalamus (6).

This two-center model also fits with the notion that two behavioral systems govern feeding: the incentive and reward value system that modulates the wanting and liking of food, and the satiety system that promotes meal termination (associated with the sensation of “fullness”). While these systems are physiologically and anatomically integrated, simplicity often dictates their description and study as distinct entities. We now understand that the meal-terminating systems in the brainstem as well as the brain reward circuits work in conjunction with the hypothalamus to mediate the overall control of food intake and energy homeostasis. Furthermore, recent studies have demonstrated greater anatomic heterogeneity in the hypothalamic systems that control energy balance than suggested by the simple two-center model, as well as revealing finer functional complexity- with distinct subsets of neurons in the hypothalamus controlling individual aspects of food intake and energy expenditure.

Genetic Models of Obesity Prove the Lipostatic Model of Energy Balance

Animals (including humans) maintain remarkably constant adipose triglyceride stores (7), suggesting that the brain and periphery must communicate to coordinate feeding and energy expenditure so as to maintain this balance. Around the same time that lesioning studies demonstrated the importance of the hypothalamus for the control of energy balance (5, 8), Kennedy proposed the lipostatic hypothesis of hunger:

that an inhibitory factor produced by white adipose tissue in proportion to fat mass suppresses eating and body weight gain (9). He further suggested that lesions of the ventral medial hypothalamus increase food intake because of the removal of the site of action of the inhibitory signal from the fat.

A strain of mice displaying dramatic hyperphagia and obesity from the time of weaning arose spontaneously at the Jackson Laboratory in 1949-50; the autosomal recessive allele conveying this phenotype was designated *obese (ob)* (10). Sixteen years later, a phenotypically similar mouse was identified (11). The diabetic state of these latter animals (studied on the diabetes-prone coisogenic KsJ background) distinguished them from *ob/ob* mice (which had been studied on the relatively diabetes-resistant B6 background), leading to the *diabetes (db)* designation for the new mutation.

Seeking the molecular predicates of the lipostatic system posited by Kennedy (9) and Hervey (12), Douglas Coleman at Jackson Labs performed parabiosis studies coupling the circulation of *ob/ob* mice to either wild-type or *db/db* mice (13). While *ob/ob* mice became lean when joined to a wild type, they died of starvation when joined to a *db/db* mouse. These findings led Coleman to hypothesize the deficiency of a blood-borne body weight-regulating factor in *ob/ob* mice and the unresponsiveness of *db/db* mice to this factor. Specifically, he suggested that the *ob* locus produced the secreted factor while the *db* locus encoded its receptor (13,14). In 1994, the Friedman group at Rockefeller University positionally cloned the gene mutated in *ob/ob* mice and demonstrated that it encoded a secreted factor (which they termed “leptin”) produced primarily by adipocytes (15). Exogenous leptin rescued the phenotype of *ob/ob* (now, *Lep^{ob/ob}*) mice, and decreased feeding and body weight in wild-type animals. Soon thereafter, several groups cloned the leptin receptor (LepR) and demonstrated the disruption of the crucial “long” LepR isoform (LepRb) in *db/db* (*Lep^{db/db}*) mice (16–19).

The identification of leptin thus demonstrated the essential veracity of the lipostatic hypothesis. Interestingly, subsequent work has revealed a more complicated biology for leptin (whose absence sends a stronger signal than its excess (see below)), as well as suggesting the existence of additional factors that may contribute to the lipostatic control of food intake and energy balance.

THE HYPOTHALAMUS AND THE HYPOTHALAMIC MELANOCORTIN SYSTEM

The hypothalamus coordinates a host of homeostatic systems (e.g., sodium and water balance, reproduction, body temperature) in addition to energy balance. Given its need to coordinate these various functions, the hypothalamus must sense a broad array of nutrients, metabolites, hormones, and other factors (20). Of the many distinct nuclei (collections of neuronal cells) in the hypothalamus, the arcuate nucleus (ARC) plays a unique role in sensing peripheral signals. The ARC lies at the base of the hypothalamus adjacent to the median eminence (ME), a circumventricular organ that lies outside the blood brain barrier to permit direct sampling of the blood (20).

Importantly, the initial lesions of the ventral medial hypothalamus reported by Hetherington and Ranson included the ARC, as well as the ventromedial hypothalamic nucleus (VMH), the dorsomedial hypothalamic nucleus (DMH), and the periventricular hypothalamic nucleus (PVH). Lesions of the VMH nucleus alone failed to recapitulate the hyperphagic obesity caused by the larger (original) ventral medial lesions (21), suggesting important potential roles in the control of energy balance for one or more of these other hypothalamic nuclei.

The Arcuate Nucleus

Its proximity to the ME, together with its projections to deeper hypothalamic areas involved in the control of feeding (e.g., the DMH, PVH and the lateral hypothalamic area (LHA)), suggest that the ARC

serves to sense humoral signals and convey this information to downstream structures to modulate homeostatic systems (Figure 1). Indeed, the core of the CNS melanocortin system, which integrates peripheral signals of energy balance and modulates feeding and energy expenditure, lies in the ARC (22).

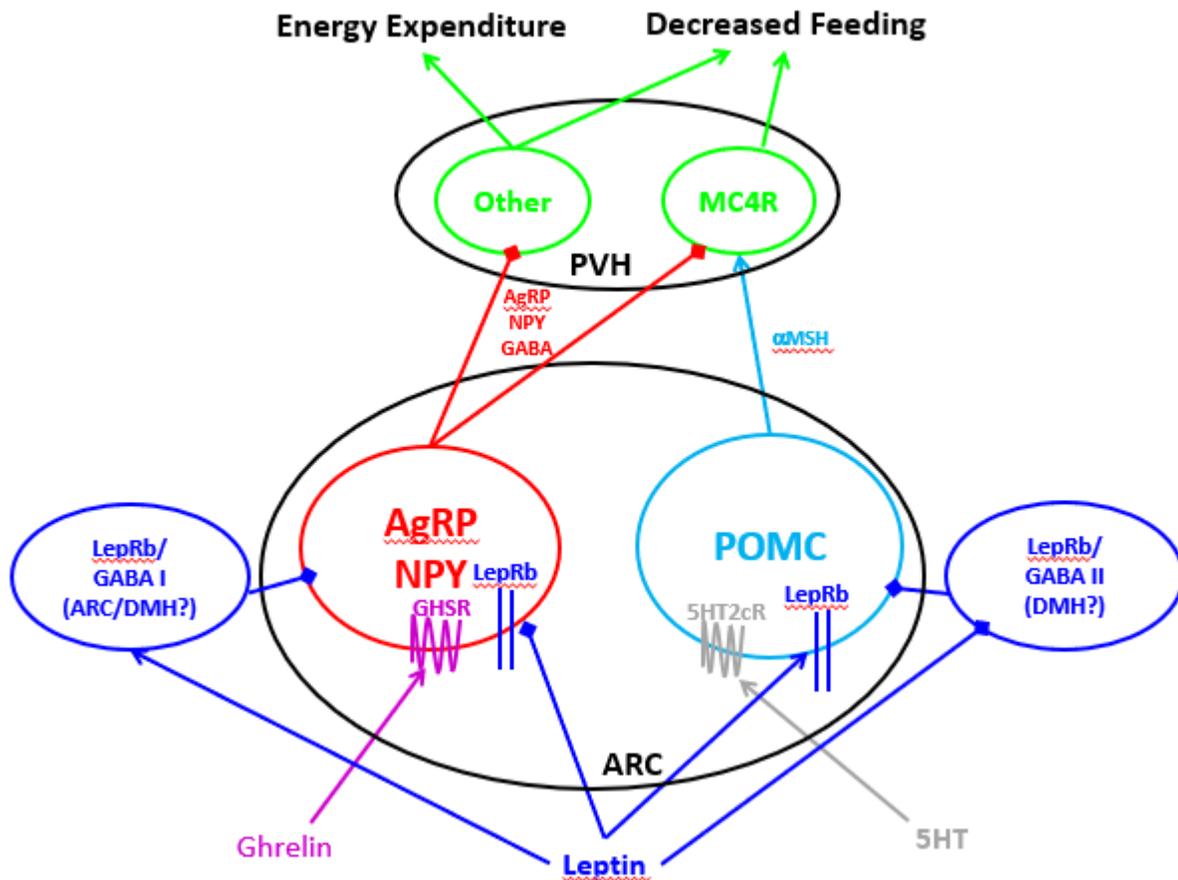


Figure 1. The hypothalamic melanocortin system. ARC POMC neurons produce αMSH and other POMC-derived peptides that act on downstream MC4R-expressing cells, such as PVH MC4R cells that play crucial roles in the suppression of food intake. ARC AgRP neurons (which also contain the inhibitory neurotransmitters NPY and GABA) release AgRP to antagonize MC4R signaling (increasing food intake) and also inhibit other PVH neurons to increase food intake and decrease energy expenditure. Signals of energy surfeit (including leptin) promote POMC neuron action; serotonin (5HT) also promotes POMC neuron action via 5HT2cR on these cells. In contrast, leptin inhibits AgRP cells, while orexigenic ghrelin also activates them. Not only does leptin act directly on these cells, but leptin action on unidentified LepRb/GABA neurons represents a major modulator of the melanocortin system.

A^y Reveals the Role for the CNS Melanocortin System in Energy Balance

In 1902, French geneticist L. Cuenot described the obese *Yellow* (A^y/a) mouse. Also termed 'lethal yellow' because homozygotes for A^y die before birth, A^y was bred by mouse fanciers in Europe beginning in the 1800s, and was notable for the dominant inheritance of a striking yellow coat, along with obesity proportional to the intensity of the yellowness of its coat (23). In 1960, another spontaneous mutation at the *agouti* locus arose in the Jackson Laboratory colony- viable yellow (A^{vy}) (24). Expression of the wild-type *agouti* gene (*a*) normally occurs intermittently in the hair follicle, generating alternate yellow and black pigment bands of the resulting hair, producing the agouti coat color (25). The original A^y mutation represents a deletion within the gene encoding the RNA-binding protein *Raly* (*Raly*), which fuses the constitutively active *Raly* promoter to the *agouti* gene, resulting in constitutive ectopic overexpression of *agouti* in all somatic (including brain) cells (26). A^{vy} also results from ectopic overexpression of *agouti*-due to the insertion of a retrovirus-like repetitive intracisternal A particle (IAP) into a noncoding exon of *agouti* (27).

The *agouti* locus encodes agouti signaling protein (ASP), a peptide with high affinity for melanocortin receptors. The yellow coat color of the A^y/a mouse results from continuous overexpression of ASP in the skin, which blocks alpha-melanocyte-stimulating hormone (α -MSH) signaling at melanocortin-1 receptors (MC1R) in the hair follicle (25,28). Since α -MSH activates melanocytes to initiate the synthesis of eumelanin (black pigment) instead of pheomelanin (yellow pigment), antagonism of α -MSH/MC1R signaling by ASP elicits a yellow coat color.

The brain also contains a melanocortin system, and this CNS melanocortin system controls energy balance (22). ICV administration of α -MSH or other

melanocortin agonists decreases food intake and body weight (29). Overexpression of ASP in the A^y/a brain antagonizes the anorectic action of α -MSH signaling and blunts the activity of brain melanocortin receptors, thus causing hyperphagia.

Melanocortin Peptides and Receptors

The post-translational modification and cleavage of the proopiomelanocortin (POMC) precursor peptide produces several melanocortin peptides, including adrenocorticotrophic hormone (ACTH), α -MSH (more prominent in rodents), β -MSH (more prominent in humans) and γ -MSH; POMC processing also produces the opioid peptide, β -endorphin (22). Within the CNS, the major population of POMC-producing cells resides in the ARC (a smaller population of brainstem POMC neurons may produce low levels of POMC and plays unclear roles in brain melanocortin signaling) (22). CNS melanocortin peptides act via the melanocortin-3 and -4 receptors (MC3R and MC4R) on target neurons. The ARC also contains neurons that produce agouti-related protein (AgRP, an antagonist/inverse agonist for MC3R and MC4R), along with the inhibitory neurotransmitters neuropeptide Y (NPY) and gamma amino butyric acid (GABA) (30)(31). Thus, the core of the CNS melanocortin system comprises anorexigenic (appetite-suppressing) ARC POMC neurons, opposing orexigenic (hunger-inducing) ARC AgRP neurons, and MC3R and MC4R-containing target neurons throughout the CNS (22) (Figure 1).

ARC POMC Neurons

Signals of positive energy balance, such as leptin, tend to activate POMC neurons and increase their *Pomc* expression (32). Artificially activating ARC POMC neurons decreases food intake (33,34). While ARC POMC neurons also contain the neuropeptide CART (and a few POMC neurons contain various

amino acidergic transmitters) (35,36), most data suggest that melanocortin peptide action mediates the majority of the POMC neurons' ability to suppress food intake and increase energy expenditure (37). The ablation of ARC *Pomc* expression promotes hyperphagic obesity similar to that of *A^y* mice (34)(38). The first evidence for a human melanocortin obesity syndrome resulted from the astute recognition of a rare agouti-mouse-like syndrome in two families, resulting from null mutations in the *POMC* gene (39–41). These patients have ACTH insufficiency, red hair, and obesity, resulting from the lack of ACTH peptide in the serum and a lack of melanocortin peptides in skin and brain, respectively. This obesity syndrome demonstrated that the CNS melanocortin circuitry subserves energy homeostasis in humans as it does in the mouse.

The predictable, monogenetic heritability of the hyperphagic and obese phenotype caused by *A^y*, *ob*, and *db* demonstrates the genetic underpinnings of feeding control and overall energy balance. The subsequent finding that the orthologs of 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 (42).

ARC AgRP Neurons

Fasting and signals indicating negative energy balance activate ARC AgRP neurons, while signals of positive energy balance (e.g., leptin) inhibit these cells. ARC AgRP neuron activation promotes feeding and decreases energy expenditure, while neuronal ablation results in lethal anorexia, consistent with the strong orexigenic nature of these cells (43,44). AgRP acts as an inverse agonist at MC3/4R, decreasing receptor activity and thus promoting positive energy balance by increasing food intake and decreasing energy expenditure (25). While the ablation of *Agrp* and/or *Npy* in ARC AgRP neurons minimally affects energy balance in wild-type animals, it attenuates the

obesity of leptin-deficient animals (45). In contrast, blockade of GABA release from these neurons, via the cre recombinase-mediated deletion of the vesicular GABA transporter (vGat), results in leanness and interferes with the response to food restriction, suggesting that these neurons (and especially GABA release therefrom) are crucial for promoting food intake, especially in response to signals of negative energy balance (46). Importantly, the ARC contains additional populations of (non-AgRP-containing) GABA neurons that may mediate orexigenic signals in a manner similar to AgRP cells (47).

Downstream Targets of the ARC Melanocortin System

Melanocortin-mediated stimulation of MC3/4R decreases food intake and increases energy expenditure to promote negative energy balance in animals and humans (48–50). Mice null for *Mc4r* display substantial hyperphagia and increased adiposity/body weight, and also display increased linear growth, as is characteristic of *A^{y/a}* mice (51). *Mc3r*-null mice display a more modest energy balance phenotype than *Mc4r*-null mice, with only modestly increased adipose mass, decreased lean mass, reduced fast-induced refeeding (52,53), elevated basal and fasting-induced corticosterone (53), and defects in circadian rhythms and meal entrainment (54). Thus, MC4R represents the major melanocortin receptor that mediates the control of food intake and body weight. Regions that contain large populations of MC3R- and MC4R-expressing neurons include the PVH, LHA, DMH, VMH, and ARC (the VMH and ARC contain MC3R only) (55).

While a syndrome resulting from *MC3R* mutations in humans has not yet been definitively identified, MC4R clearly plays an important role in the control of body weight in humans, as well. Heterozygous frameshift mutations in the human *MC4R* locus associate with physical findings virtually identical to those reported for the mouse (51), with increased adipose mass, increased linear growth and lean mass,

hyperinsulinemia greater than that seen in matched obese control subjects, and severe hyperphagia. *MC4R* haploinsufficient adults also exhibit reduced sympathetic tone and mild hypotension (56). *MC4R* haploinsufficiency in humans represents the most common monogenic cause of severe obesity, accounting for up to 5% of cases (57–59).

Site-specific deletion studies have demonstrated a crucial role for MC4R in the PVH for the control of food intake and energy balance (60,61). While AgRP neurons project to and inhibit ARC POMC neurons via direct GABA action (62), this projection appears to play little role in the promotion of feeding by AgRP neurons (63). Rather, AgRP neurons most strongly increase feeding via their projections to the PVH (LHA projections also participate)(64). Thus, the PVH plays crucial roles in the control of feeding by POMC and AgRP neurons.

Interestingly, while AgRP neuron activation promotes feeding most strongly via the PVH, AgRP neuron inhibition decreases food intake at a distinct site: detailed studies of animals ablated for AgRP neurons demonstrate that the withdrawal of GABAergic inhibition from cells in the brainstem parabrachial nucleus (PBN) mediate this affect (65) (See below for additional details).

Paraventricular Nucleus of the Hypothalamus (PVH)

The PVH represents a major output nucleus for the hypothalamus, from which integrated information is transmitted to effector systems, such as the pituitary gland, the autonomic system, and behavioral control circuits (66,67). The identification of small deletions or translocations at the human Single-minded-1 (*SIM1*) locus on chromosome 6 in three young obese patients suggested a crucial role for the PVH in energy balance in humans (68). *SIM1* encodes a transcription factor that is expressed throughout the PVH and is required for the development of the PVH (68). While

homozygous deletion of *Sim1* is embryonic lethal in mice, animals heterozygous for *Sim1* are normal until 4 weeks of age, when they develop hyperphagic obesity (69). 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 *Sim1* haploinsufficiency diminishes anorexic “tone” from the PVH, leading to hyperphagia and obesity in mice as well as in rare human patients with *SIM1* mutations.

As with other hypothalamic nuclei, the PVH contains a constellation of diverse neuronal subtypes. Identifying the PVH subpopulations that mediate effects on food intake and energy expenditure represent a crucial research direction. Unsurprisingly, PVH MC4R neurons potently suppress food intake (60,61,70). Interestingly, however, PVH-projecting ARC AgRP neurons regulate cells that lack MC4R (in addition to regulating MC4R neurons), suggesting the existence of additional PVH populations that play roles in the control of energy balance (71). *Nos1*-expressing PVH cells represent one important subset of appetite-regulating non-MC4R PVH cells (72). Other important non-MC4R PVH neurons include prodynorphin (*Pdyn*)-expressing cells (71).

Prominent populations of PVH neurons include those that contain hormones/neuropeptides, including oxytocin (OXT), corticotropin releasing hormone (CRH), and thyrotropin releasing hormone (TRH), arginine vasopressin (AVP), and oxytocin (OXT) (61,64,70,73). These peptides also control other endocrine and CNS functions: TRH and CRH stimulate the thyroid and adrenal axes, respectively; AVP contributes to fluid balance; and OXT regulates uterine function and social interactions (74–78). While these peptidergic PVH neurons do not contain MC4R, the injection of OXT into the hindbrain promotes satiation (64). Genetic data from mice argue against an important role of OXT or OXT neurons in energy balance, however. Not only do *Oxt*-null 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 (72,79). Furthermore, all of these peptide-containing PVH populations are only weakly anorexigenic in mice, and OXT, AVP, and CRH neurons do not mediate melanocortin responses (61). Thus, peptidergic PVH neurons play little role in the control of feeding, at least in mice, while distinct *Mc4r*-, *Nos1*-, and *Pdyn*-containing PVH neurons (along with potentially other PVH neuron types that will be important to identify) play crucial roles in the control of feeding and energy balance. Interestingly, a recent GWAS analysis identified a polymorphism near the human anaplastic lymphoma kinase (*ALK*) locus that correlates with thinness. Decreased expression of this gene reduces adiposity in a variety of animal models and *Alk* expression in the PVH appears to mediate its effects on body weight (80). Identifying the cell type(s) that mediate the effects of *Alk* on body weight will be very informative.

Dorsomedial Nucleus of the Hypothalamus (DMH)

The DMH has long been implicated in energy balance regulation, as well as in the modulation of body temperature, arousal and circadian rhythms of locomotor activity (81). This nucleus receives direct input from the ARC and also contains LepRb-expressing neurons (82,84). While the exact molecular phenotype(s) of energy balance-regulating DMH cells remain poorly defined, recent studies have suggested that the LepRb-containing cells in this region play crucial roles for maintaining energy balance (85). Indeed, the viral-mediated disruption of DMH LepRb in adult mice augments food intake and promotes obesity (86). Furthermore, subpopulations of GABAergic DMH neurons play important roles in the leptin-mediated control of ARC POMC and AgRP cells (and thus, food intake) (85,87,88). TrkB-containing DMH neurons also contribute to the control of homeostatic feeding behavior (89). Thus, while details continue to emerge, the DMH plays crucial roles in leptin action, the control of the hypothalamic

melanocortin system, food intake, and overall energy balance.

Ventromedial Nucleus of the Hypothalamus (VMH)

The VMH contains neurons that express LepRb, MC3R and other receptors involved in body weight regulation. Neurons in the dorsomedial portion of the VMH (dmVMH) express the transcription factor, steroidogenic factor 1 (*Sf1*; *Nr5a1*) (90). Although *Sf1*-deficient mice were first described in 1994, their early death due to adrenal insufficiency initially prevented the study of these mice in adulthood. Later, adrenal transplantation enabled the long-term survival of these mice, permitting the detection of late-onset obesity in *Sf1*-deficient mice (91), consistent with a role for the VMH in the control of energy balance. The obesity of *Sf1*-null mice results largely from decreased energy expenditure, however (91). Furthermore, *Sf1*-cre-mediated ablation of LepRb doesn't alter food intake, but rather decreases energy expenditure (thereby accentuating obesity in high-fat diet-fed animals) (92). Many *Sf1*-containing VMH neurons contain the neuropeptide PACAP (the product of the *Adcyap* gene), which contributes to the control of energy expenditure (93). Thus, *Sf1*-mediated manipulation of the dorsomedial VMH has revealed a crucial role for this region in overall energy balance, albeit by the modulation of energy expenditure, rather than food intake. Indeed, the dmVMH is generally thought to serve as an autonomic control center that modulates a variety of parameters driven by the sympathetic nervous system (SNS). In addition to controlling energy expenditure, the dmVMH also plays important roles in nutrient mobilization (as during the response to hypoglycemia) (94–97).

Lateral Hypothalamic Area (LHA)

While a network of systems that suppress food intake (albeit in a manner antagonized by AgRP neurons) reside in the ARC, DMH, and PVH, the LHA is often

thought of as a region that promotes feeding. Well-known LHA neuronal subtypes include two distinct sets of excitatory neurons that receive input from leptin and melanocortins and contribute to the control of feeding and energy balance. One population contains the neuropeptide melanin concentrating hormone (MCH; not related to POMC or any of its derivative peptides) (98). First studied in mammals because of the increased expression of *Mch* mRNA in *Lep^{ob/ob}* and fasted mice, administration of MCH increases food intake and body weight gain and decreases energy expenditure (98). Furthermore, animals null for *Mch* (or its receptor) are lean (99). The MCH receptor localizes to 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 contain the neuropeptide, hypocretin (HCRT; also known as orexin) (100,101). Based upon early acute pharmacologic studies, HCRT was originally conceived of as an orexigen, since HCRT stimulates food intake when injected centrally during the light cycle. Consistently, fasting increases *Hcrt* mRNA expression and activates HCRT neurons (101). Subsequent work has revealed that animals null for HCRT or its receptors become mildly obese without observable alterations in food intake, however (102). Furthermore, mice (and dogs and humans) null for *Hcrt* or lacking HCRT neurons exhibit narcolepsy and increased body weight and adiposity (103). Thus, rather than having a primary role in the control of feeding, HCRT neurons promote alertness and activity, and most of the effect of *Hcrt* mutation on energy balance results from decreased physical activity and energy expenditure, while HCRT administration promotes activity (and food intake) during the resting phase of the diurnal cycle.

The LHA also contains LepRb neurons that control HCRT neurons; these contain neurotensin and lie intermingled with the HCRT cells (104-107). Ablation of LepRb from these LHA cells prevents the normal

regulation of HCRT neurons and results in decreased locomotor activity and energy expenditure. Both LHA LepRb neurons and HCRT cells project to the ventral tegmental area (VTA), which contains a large number of dopaminergic neurons that represent the core of the mesolimbic reward system (see below for further discussion of reward pathways). Thus, while lesioning studies suggest that the integrity of the LHA is required for motivation and normal feeding behavior, most data suggest that it plays little role in the normal modulation of food intake.

PERIPHERAL SIGNALS THAT MODULATE ENERGY BALANCE VIA THE HYPOTHALAMUS

Homeostatic regulation of energy balance requires the brain to maintain appropriate energy levels by monitoring peripheral signals of energy status and metabolism to modulate food intake and a variety of autonomic and neuroendocrine determinants of energy utilization. This requires the ability to sense circulating signals of metabolic status.

Leptin

The discovery of leptin revealed the existence of an endocrine system that senses and modulates adipose stores. Disruption of leptin signaling results in hyperphagia and obesity, and leptin administration to leptin-deficient *Lep^{ob/ob}* mice (but not LepRb-null *Lep^{db/db}* animals), reduces food intake and adiposity, sparing lean tissue (108–110). While the role for leptin in the control of appetite and adiposity initially dominated the thinking about its biology, it has become clear that the effects of elevated leptin are not as dramatic as those of low leptin. Indeed, diet-induced obese rodents and humans remain obese despite exhibiting high circulating concentrations of leptin, commensurate with their high levels of leptin-producing adipose tissue (111,112). In contrast to the *Lep^{ob/ob}* mice, where leptin administration results in remarkable reversal of the obesity phenotype,

increasing leptin to supraphysiologic levels in normal animals only modestly and briefly blunts food intake and body weight. Likewise, supraphysiological doses of leptin promote only modest effects on body weight in obese and non-obese humans(113). Thus, the absence of leptin conveys a more powerful signal than does its excess.

Lep^{ob/ob} mice (and their leptin-deficient human counterparts) display additional phenotypes, including impaired growth and gonadal axis function, diminished immune function, infertility, and decreased activity and energy expenditure - all of which are reversed by leptin treatment (114,115). The lack of leptin also promotes increased hepatic glucose production, and leptin treatment suppresses hyperglycemia in several models of insulinopenic diabetes (116,117). Lipodystrophic people and transgenic animals that similarly lack adipose tissue exhibit leanness and low leptin levels, as well as hyperphagia, insulin resistance, diabetes and other endocrine and metabolic abnormalities that are not corrected by caloric restriction (109,110,118). Leptin replacement therapy to correct low leptin concentrations represents an important treatment for lipodystrophy syndromes in humans, decreasing their hunger and improving their endocrine and metabolic abnormalities (119).

This constellation of phenotypes resulting from low leptin mirrors the physiologic response to starvation and leptin treatment attenuates many of these consequences of very low adiposity (115). 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 NEUROBIOLOGY OF LEPTIN

The similar phenotypes of *Lep^{ob/ob}* and *Lep^{db/db}* mice (along with the inability of leptin to alter physiology in *Lep^{db/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 (83,84). Similarly, ablation of LepRb in the CNS promotes hyperphagia, neuroendocrine failure, and obesity (120). Some cells outside of the CNS might 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 reside within the hypothalamus and brainstem, consistent with the known roles for these structures in the control of feeding, endocrine and autonomic function (83,84,121). Pan-hypothalamic ablation of LepRb promotes a phenotype very similar in quality and magnitude to that of *Lep^{db/db}* animals (122). Furthermore, ablation of LepRb from broadly-distributed hypothalamic *vGat*- or *Nos1*-expressing neurons promotes dramatic hyperphagia and obesity (123,124). Smaller, more circumscribed sets of hypothalamic LepRb neurons have also been implicated in body weight control as well. Within the ARC, early developmental removal of LepRb specifically in POMC and AgRP neurons modestly increases feeding and adiposity (125,126). Interestingly, removal of LepRb from AgRP neurons in adult animals results in robust hyperphagia, obesity and diabetes, suggesting that developmental processes can largely compensate for the early lack of direct leptin action on AgRP neurons (127). Ablation of LepRb in the *Sf1*-expressing VMH blunts the increase in energy expenditure that accompanies increased adiposity, and deletion of LepRb in the LHA diminishes motor activity and promotes obesity (92,106,128). LepRb neurons in the ventral premammillary nucleus (PMv) play roles in reproduction (129). Importantly, functions for many additional groups of LepRb cells in the hypothalamus (especially in the DMH) have yet to be determined. Currently, LepRb neurons in the ARC and DMH are thought to play the most important roles in the control of feeding and energy balance by leptin.

THE MOLECULAR BIOLOGY OF LEPTIN

Alternative splicing of the *LepR* transcript produces multiple isoforms of the receptor: LepRa, -b, -c, -d, etc (Figure 2). The *LepR^{db}* mutation mouse results from a splicing defect that causes the LepRa-specific exon to be inserted into the mRNA that encodes LepRb,

preventing translation of the LepRb-specific coding sequences and producing LepRa in place of LepRb (16–18). Because the *LepR^{db/db}* mouse synthesizes all leptin receptor isoforms except LepRb, LepRb must be crucial for the control of energy homeostasis (130). Indeed, restoration of LepRb on a background null for all other LepR isoforms restores energy balance (19).

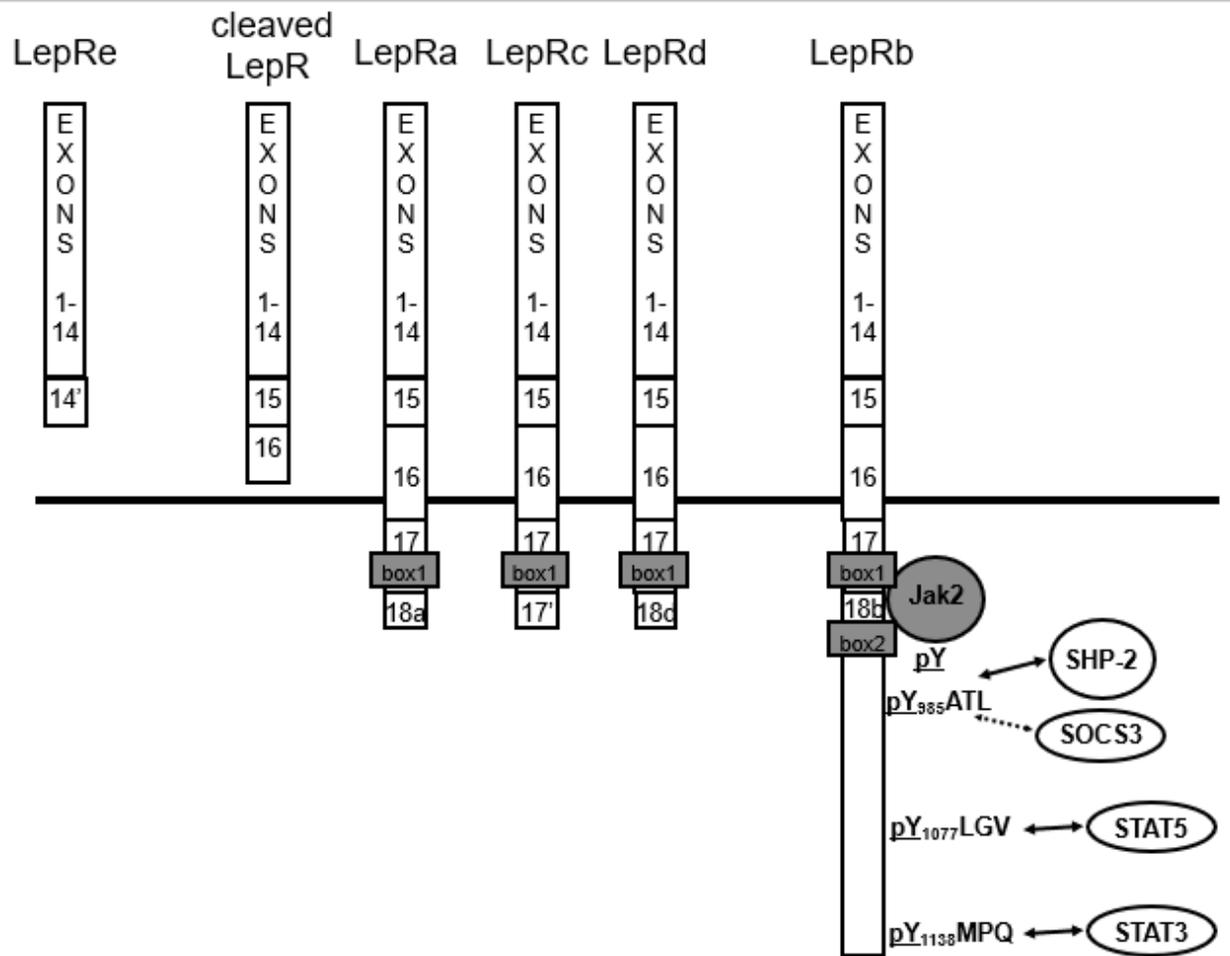


Figure 2. LepR isoforms and signaling. LepRa (Ra) represents the mostly highly expressed short form of LepR; LepRb (Rb) is the long form. Exon 17 contains half of a Jak docking site (BOX1) common to Ra, Rb and Rc, while exon 18b contains additional motifs required for full Jak2 binding (BOX2) and STAT3 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).

LepRb, like other type 1 cytokine receptors, activates a JAK family tyrosine kinase (JAK2) to initiate signaling (130). Subsequently, tyrosine phosphorylated residues on LepRb recruit STAT proteins, 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 *Lep^{db/db}* mice (131). Similarly, disruption of *Stat3* in the forebrain or in LepRb-expressing neurons results in obesity in mice (132,133). While the brain-wide disruption of the genes encoding both isoforms of STAT5 (STAT5a and STAT5b) causes mild late-onset obesity, the disruption of *Stat5a/b* specifically in LepRb neurons produces no detectable phenotype, suggesting that STAT5 signaling is not required for leptin action *in vivo* (134–136). STAT5 represents a major mediator of GM-CSF signaling, however, and mice null for GM-CSFR in the brain 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 (135).

Insulin

Like leptin, insulin circulates in proportion to fat mass, and alters neuropeptide expression in the hypothalamus via receptors located in the ARC, PVH, and DMH (137). 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 (138). In addition, insulin acts centrally to decrease hepatic

glucose output, in part via the inhibition of AgRP neurons (139,140).

The insulin receptor (INSR), a tyrosine kinase, recruits and tyrosine phosphorylates insulin receptor substrates (IRS proteins; IRS-1, -2, -3, -4) which engage downstream signals, including the phosphatidylinositol 3-kinase (PI3-kinase) pathway. Deletion of *Irs1* interferes primarily with peripheral insulin action and the growth axis, *Irs3* is rodent-specific and adipocyte-restricted, and the deletion of *Irs4* minimally alters energy balance (141). In contrast, deletion of *Irs2* causes insulin-deficient diabetes (due to islet failure) and obesity. Restoration of *Irs2* in the islets of *Irs2*-null mice or brain-specific ablation of *Irs2* results in normoglycemic obesity, consistent with a role for brain IRS2 signaling in energy balance (142). While leptin modulates the IRS-protein/PI3-kinase pathway and the deletion of *Irs2* from LepRb-expressing neurons promotes obesity (albeit a milder form of obesity than observed in animals deleted for *Irs2* throughout the brain), deletion of *Irs2* does not interfere with leptin action, suggesting that IRS2 may primarily play a role in brain insulin action (143).

A variety of subunits and downstream effectors of the PI3-kinase signaling pathway have also been deleted in several neuronal populations in mice (144). 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.

Modulators of Insulin and Leptin Signaling

Many of the molecular signaling pathways that inhibit insulin and leptin action overlap. Protein tyrosine phosphatase-1B (PTP1B, a.k.a., PTPN1) dephosphorylates cognate tyrosine kinases (including those associated with INSR and LepRb) to terminate signaling (145,146). In addition to exhibiting increased insulin sensitivity, mice lacking *Ptpn1* are lean compared to controls and exhibit resistance to weight gain on a high-fat diet, suggesting increased leptin action in these animals. Indeed, animals null for *Ptpn1* throughout the brain (or specifically in LepRb or POMC neurons) demonstrate increased leanness and enhanced leptin action (147,148). In addition to PTP1B, the tyrosine phosphatase, TCPTP, which directly dephosphorylates STAT3, contributes to the attenuation of LepRb signaling. Furthermore, obesity and elevated leptin increase the expression of *Ptpn2* (which encodes TCPTP), and the deletion of neuronal *Ptpn2* decreases body weight, increases leptin sensitivity, and blunts weight gain in DIO animals (149). Moreover, the combined deletion of *Ptpn1* and

Ptpn2 in the brain augments leanness and further attenuates weight gain in DIO mice (149).

Suppressors of Cytokine Signaling (SOCS proteins, e.g., SOCS1 and SOCS3) bind to activated cytokine receptor/Jak2 kinase complexes (including the LepRb/Jak2 complex) to mediate their inhibition and degradation (150). 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 Tyr₉₈₅ of LepRb to attenuate LepRb signaling (151). The leanness of mice containing a substitution mutation of LepRb Tyr₉₈₅ and the similar phenotype of mice lacking *Socs3* in the brain or in LepRb neurons highlight the importance of these mechanisms of feedback inhibition for the control of energy balance (152,153). While LepRb Tyr₉₈₅ also mediates the recruitment of the tyrosine phosphatase SHP2 (aka, PTPN11), data from cultured cells suggest that SHP2 mediates ERK pathway signaling by LepRb, and disruption of *Ptpn11* in the brain, in LepRb neurons, or in POMC neurons, promotes obesity (130) (Figure 3).

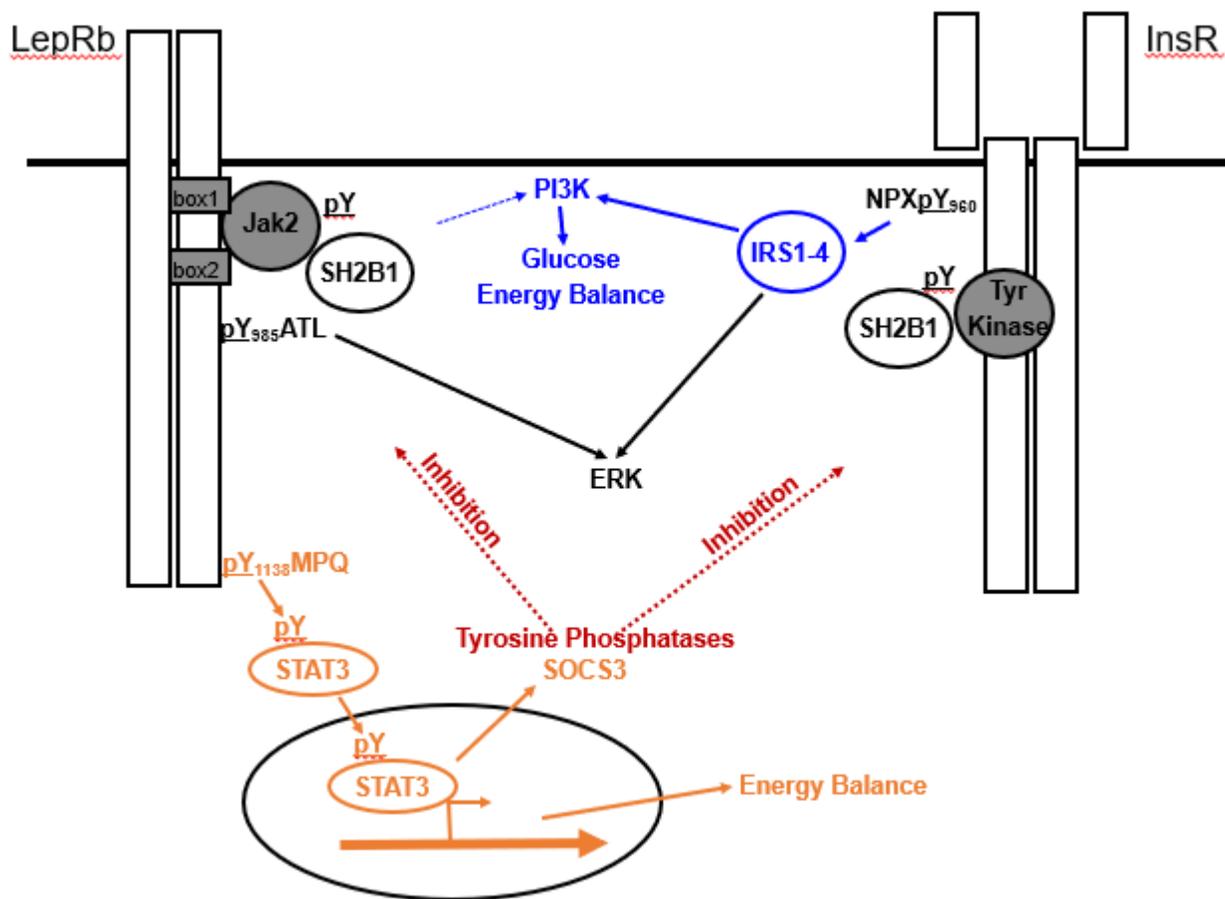


Figure 3. Signaling by and inhibition of LepRb and InsR. LepRb, which exists as a preformed homodimer in complex with the Jak2 tyrosine kinase, recruits and phosphorylates (pY) STAT3 via phosphorylated pY₁₁₃₈ to control many aspects of energy balance. InsR, which also exists as a preformed dimer, but has intrinsic tyrosine kinase activity, autophosphorylates the juxtamembrane Tyr₉₆₀ to recruit the insulin receptor substrate (IRS) proteins IRS1-IRS4. IRS-proteins strongly activate the phosphatidylinositol 3-kinase (PI3K), which play roles in the brain control of energy balance and glucose homeostasis. Leptin also activates PI3K, albeit much more weakly than InsR, and by undefined mechanisms. Both LepRb and InsR activate the ERK pathway. The adapter protein, SH2B1 also enhances signaling by both receptors. In addition to decreasing food intake and increasing energy expenditure, LepRb-mediated STAT3 signaling promotes the expression of SOCS3, which acts as a feedback inhibitor of LepRb and InsR signaling. A variety of tyrosine phosphatases also inhibit the activity of both receptors.

SH2B1 binds to activated Jak2, as well as to INSR, TrkB, and a few other receptor tyrosine kinase complexes to increase their activity and mediate aspects of downstream signaling (154). *Sh2b1*-null mice display a complex phenotype that includes obesity; brain-specific absence of *Sh2b1* also promotes obesity in mice (155,156). 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* (157). Indeed, GWAS studies have suggested a role for common variants in *SH2B1* in human obesity (59). While the deletion of *Sh2b1* from LepRb neurons in mice promotes obesity, this effect may be independent of leptin action (158), suggesting that SH2B1 impacts energy balance via its actions on other growth factor receptors.

Potential Roles for Other Adipokines and Anorexigenic Signals

Several lines of evidence suggest the existence of peripherally-derived anorexigenic signals in addition to leptin and insulin. First, because continuous administration of high levels of exogenous leptin in wild-type animals only slightly and transiently decreases feeding, while wild-type animals starve themselves to death during parabiosis to *Lepr^{db/db}* animals (13,108,113,159), there likely exists an additional hormonal signal that suppresses food intake (albeit one that requires leptin for its action). Additionally, the forced overfeeding of animals results in multi-day anorexia even in the absence of increased leptin concentrations (160). Although it is not clear that this second anorectic signal derives from adipose tissue, fat produces many signaling molecules in addition to leptin, some of which, like leptin, are cytokines (adipose-derived cytokines, or “adipokines”). While the adipokines adiponectin and resistin can alter feeding when injected into the brain (161,162), neither can suppress food intake to the extent observed in parabiosed or overfed animals. Thus, additional anorexigenic signals remain to be discovered.

The Orexigenic Ghrelin System

The diurnal release of ghrelin, which derives from the stomach, coincides with the initiation of meals and decreases over the course of each meal (163). Acutely administered ghrelin causes animals and humans to consume larger meals than normal, while

chronic ghrelin administration results in obesity in rodents (164–167). As would be expected, most obese humans have low levels of circulating ghrelin, whereas levels are elevated in patients with anorexia nervosa (168).

The growth hormone secretagogue receptor (GHSR) serves as the receptor for the acylated (active) form of ghrelin (which is acylated (octanoylated) by ghrelin O-acyl transferase (GOAT) in the cells that synthesize it) (169). 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.

ARC AgRP neurons express high levels of GHSR, and ghrelin activates these cells. Indeed, ghrelin action on AgRP neurons mediates the majority of the anorectic response to ghrelin (170,171). 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 beginning early in embryogenesis exhibit no detectable alterations in baseline energy balance, and only modest defects in refeeding (172), presumably due to compensatory processes that alter the function of AgRP neurons during development. Apart from its actions on neurons in the ARC, ghrelin administration into other areas of the brain (i.e. PVN, LHA, ventral tegmental area (VTA), dorsal vagal complex) can also stimulate positive energy balance (173–176).

THE HINDBRAIN CONTROL OF FEEDING

Most consider the hypothalamus to play a dominant role in the long-term control of food intake. Indeed, leptin, the hormonal signal of long-term energy stores, mediates its largest effects on food intake and energy balance via the hypothalamus (122,177). In contrast, hindbrain circuits respond robustly to signals of gut status (including stretch, nutrients, and toxins/irritants) to control meal termination and thus meal size.

Humoral signals from the gut act on the hindbrain area postrema (AP), which lies outside the blood-brain barrier at the base of the fourth ventricle in the caudal medulla. Other gut signals are conveyed to the hindbrain via afferent vagal fibers (whose soma lie in the nodose ganglion) (Figure 4). These signals converge on the *nucleus tractus solitarius* (NTS) and promote meal termination (178,179). Interference with components of this system (e.g., vagotomy) increases meal size, although compensatory changes in meal

frequency (presumably directed by the hypothalamus) often dictate that food intake and energy balance remain constant over the long-term (180). Outputs from the AP and NTS include the dorsal motor nucleus of the vagus (DMV), which sends parasympathetic signals to the gut to alter motility. Projections to more rostral regions, including the PBN and hypothalamic sites (including the PVH and DMH) also play roles in the suppression of food intake.

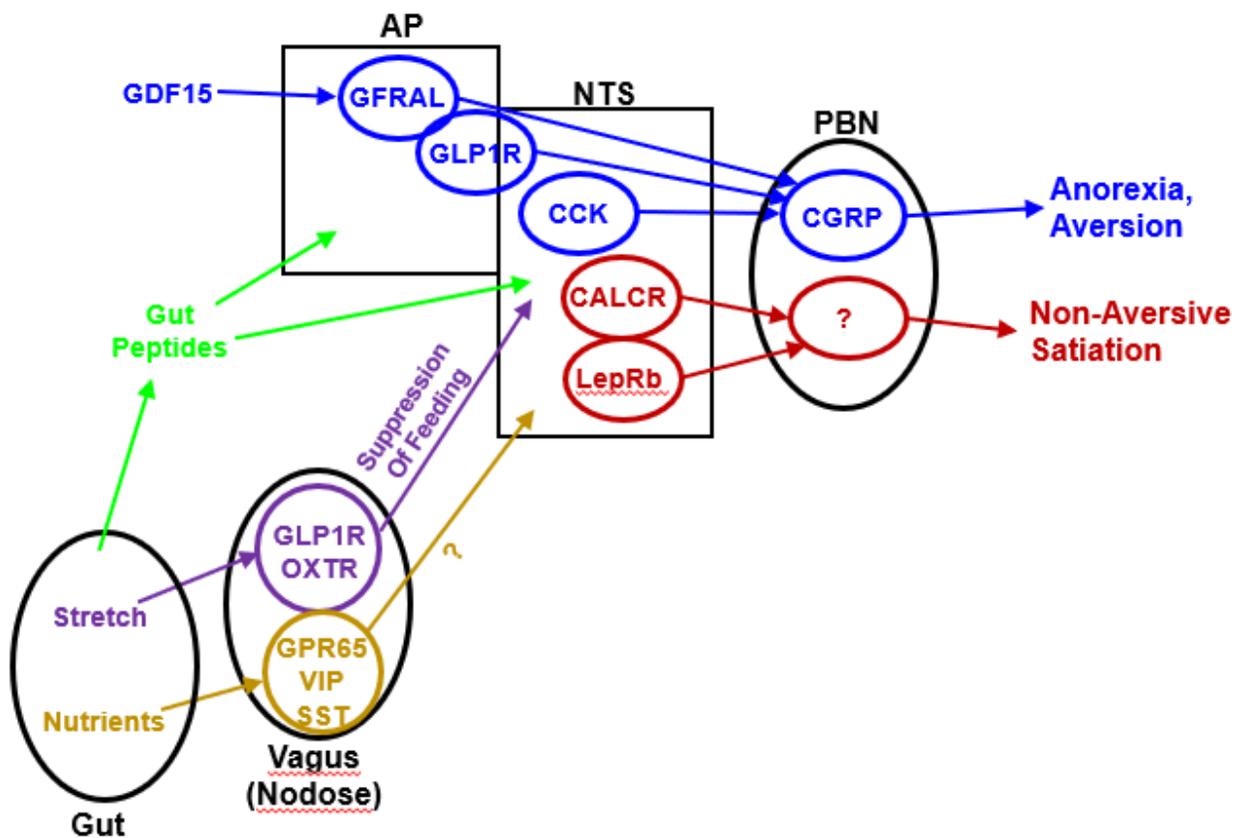


Figure 4. Emerging circuitry of gut-brain pathways that control food intake. A variety of signals converge on the hindbrain to suppress food intake. This includes a variety of gut peptides and the stress/inflammation signal, GDF15, as well as vagal sensory neurons whose soma reside in the nodose ganglion. Stretch-sensing vagal afferents that express GLP1R and/or OXTR suppress feeding via the NTS (although their particular cell targets in the NTS remain to be defined). In contrast, nutrient-sensing vagal neurons (including those that express GPR65, VIP, and/or SST) do not appear to control feeding; their precise function remains undefined. Many populations of AP/NTS neurons promote the aversive suppression of food intake by projecting onto CGRP-expressing cells of the PBN. Other neurons of the NTS (including those that express CALCR and LepRb) suppress food intake without promoting aversive effects, at least in part by activating a poorly-defined set of non-CGRP neurons in the PBN.

A number of observations suggest potential roles for hindbrain centers in the control of long-term energy balance, however, including the expression of LepRb and GHSR in the AP and NTS (83,84,181–184). Indeed, leptin modulates the physiology of hindbrain neurons and knockdown of NTS LepRb expression modestly increases food intake and body weight, especially in high fat diet (HFD)-fed rats (181,185–189). Furthermore, ablation of prolactin releasing hormone (PRLH, a.k.a., PRRP) increases feeding and body weight, and the NTS-specific re-expression of PRLH on a *Prlh*-null background restores normal feeding and energy balance (190). More recently, the silencing of several NTS cell types has been shown to increase food intake and cause obesity. Thus, the normal function of NTS systems contributes to the long-term control of energy balance. Furthermore, many appetite-suppressing medications (including agonists for gut peptide receptors) mediate their effects by activating hindbrain systems (191–194).

The Nodose Ganglion and Vagal Sensory Neurons

Gut-innervating vagal sensory neurons in the nodose ganglion consist of mechanosensory cells that increase activity in relation to increasing gastric volume and distinct chemosensory neurons that respond to the chemical characteristics of nutrients in the gut. Both mechanosensing and chemosensing vagal neurons innervate the entire gastrointestinal tract (195,196). Recent studies have interrogated the vagal sensory neurons of the nodose ganglion, revealing markers for gut-innervating mechanosensory cells (which sense stretch and pressure; these cells express the receptors for GLP1 (GLP1R) and OXT (OXTR)) and for chemosensory neurons (which sense nutrients in the gut; these cells express GPR65, vasoactive intestinal peptide (VIP), and somatostatin (SST)) (197,199). Interestingly, the activation of mechanosensory cells suppresses feeding, while chemosensory cell activation does not. Thus, the mechanosensory and chemosensory vagal

cells must innervate distinct downstream CNS targets, at least in part. The appetite-suppressing functions of several hormones and neuropeptides (including gut-derived cholecystinin (CCK)) may result from their actions on vagal neurons (200,201). While CNS OXT neurons (in the PVH) do not appear to participate in the control of feeding, the response of vagal mechanosensory neurons to exogenous OXTR agonists might mediate the appetite-suppressing effects of these agents (202).

Role for the Area Postrema in Nausea and Aversive Responses

Because AP capillaries lack tight junctions, the AP lies outside the blood-brain barrier and directly senses circulating nutrients and hormones. While the molecular characterization of AP neurons remains in its infancy, the AP contains a variety of receptors (GLP1R, GFRAL, and CALCR) that respond to appetite-suppressing hormones (203–206). Notably, ligands for each of these receptors promote aversive responses (e.g., nausea), for which the AP is well-known (207–209). Indeed, the action of autoantibodies directed to aquaporin-4 (AQP4, which is expressed around the AP) during neuromyelitis optica spectrum disorders results in AP syndrome—characterized by unremitting nausea and vomiting (and sometimes hiccups) (210–212). Neurons from the AP project into the brain, including to the NTS, DMV, and PBN.

The Nucleus Tractus Solitarius and Parabrachial Nucleus

The NTS, which lies adjacent to the AP, receives gastrointestinal input from vagal sensory neurons and from the AP. The NTS also receives taste information via the geniculate ganglion (213), although the NTS systems that integrate taste signals with information from the gut have yet to be defined. NTS neurons also express a variety of receptors that contribute to the

control of food intake (e.g., LepRb and CALCR), and thus presumably sense a variety of circulating appetite-regulating signals. Furthermore, NTS LepRb and CALCR neurons contribute to the physiologic control of food intake (185,214,215). Interestingly, while at least some AP and NTS neurons mediate the aversive suppression of food intake (i.e., cause nausea and/or vomiting, as well as decreasing appetite), the NTS LepRb and CALCR neurons suppress food intake without promoting such aversive responses (214,215).

Thus, distinct NTS systems mediate the aversive and non-aversive suppression of food intake. Indeed, it makes teleological sense that the consumption of nutrients should promote reward (to encourage the subsequent ingestion of a particular food type), rather than terminating ingestion in an aversive manner and discouraging the future consumption of the food. Consistently, the activation of certain vagal pathways can promote a rewarding response, even while suppressing feeding (198,199).

Many AP/NTS neurons that mediate the aversive suppression of food intake directly innervate calcitonin gene-related protein (CGRP)-expressing PBN neurons. Indeed, PBN CGRP neurons mediate the aversive responses to a variety of agents associated with gut irritation, including some chemotherapy drugs (216). PBN CGRP cells also appear to participate in the emotional response to a variety of fear-inducing stimuli (217). The activation of PBN CGRP cells suppresses food intake under a variety of conditions; indeed, the withdrawal of inhibitory tone from these cells mediates the lethal anorexia associated with the ablation of ARC AgRP neurons (65).

Interestingly, however, the inactivation of PBN CGRP cells minimally impacts food intake and does not alter energy balance (218); thus other neural systems must mediate the long-term control of feeding and energy balance by brainstem systems. Hence, the systems that mediate the aversive suppression of food intake may suppress long-term feeding less effectively than

non-aversive systems, at least under normal physiologic conditions. The PBN must also contain non-aversive systems for the suppression of food intake, since neither NTS CALCR cells nor PVH MC4R neurons innervate PBN CGRP cells (but rather innervate a distinct region of the PBN) and both promote the non-aversive suppression of food intake via the PBN (214).

Gastrointestinal Hormones that Modulate Feeding

CHOLECYSTOKININ

Secreted from neuroendocrine secretory cells (L-cells) lining the intestinal lumen in response to nutrients, cholecystokinin (CCK) represents the canonical gut-derived satiety signal. It is an acutely acting signal with a very short half-life (219). Early studies showed that exogenous CCK administered just prior to a meal reduces food intake in rats. In the last thirty years these results have been repeated and extended in numerous labs, demonstrating that the anorectic effects of CCK can be translated to virtually all species, including humans (220–222). 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 (Bray 2000) (223)). 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 (224).

THE INCRETINS

Glucagon like peptide-1 (GLP-1) functions as an incretin (enhancer of insulin secretion) (225). GLP-1 can also modulate satiety: ICV GLP-1 (or GLP1R agonists) potently suppresses food intake in rats and

mice, while the GLP1R antagonist, exendin (9-37), increases short-term food intake. 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 (226). Despite this lack of a physiological role for GLP-1 or GLP-1R in the long-term control of food intake, chronic treatment with GLP-1R agonists serves to suppress food intake and promote weight loss (227).

The suppression of food intake by GLP-1R agonist pharmacotherapy requires GLP-1R expression on glutamatergic neurons of the CNS (194). Furthermore, caudal brainstem processing suffices to suppress food intake and gastric emptying by peripherally applied GLP-1R agonists (228). Thus, the crucial GLP-1R-expressing neurons that mediate the anorectic effects of GLP-1R agonist pharmacotherapy may reside in the AP and/or NTS.

Given that brain GLP-1R mediates the appetite-suppressing effects of exogenous GLP-1R agonists and that the NTS GLP-1 neurons represent the sole source of GLP-1 in the CNS (229), these NTS GLP-1 cells have been the subject of a great deal of interest. Interestingly, however, while NTS GLP-1 cells represent a subset of the NTS LepRb cells that contribute to the control of feeding, the ablation of NTS GLP-1 fails to alter energy balance or the ability of NTS LepRb neurons to suppress feeding (215). Consistently, extending the half-life of endogenous GLP-1 by inhibiting dipeptidylpeptidase-4 (DPP4) fails to alter food intake, although it amplifies the incretin effect of endogenous GLP-1. Thus, neither endogenous NTS GLP-1 nor its CNS targets contribute meaningfully to the suppression of food intake, despite the prominent pharmacologic effects of GLP-1R agonists on these parameters.

Intestinal polypeptide (GIP, formerly gastric inhibitory

polypeptide) is secreted from K-cells in the duodenum and proximal jejunum in response to food intake (230,231) and acts as an incretin, increasing glucose-dependent insulin release from pancreatic β -cells and contributing to postprandial plasma glucose normalization. The incretin function of GIP may be mediated either directly via pancreatic GIP receptor (GIPR) activation (232) or via the activation of non-ganglionic cholinergic neurons that innervate the islets, presumably as part of an enteric-neuronal-pancreatic pathway (233). The impact of GIP on central appetite regulation is controversial, however (234,235). Indeed, while the combination of GIPR and GLP1R agonism in a single peptide appears to enhance weight loss over a GLP1R agonist alone, GIPR ligands poorly modulate food intake on their own. Furthermore, there remains some debate about whether GIPR antagonism (rather than agonism) accentuates the effects of GLP1R agonists on food intake (236).

GROWTH DIFFERENTIATION FACTOR-15

While not a gut-derived peptide, growth differentiation factor 15 (GDF15) acts via the brainstem to modulate nutrient intake. GDF15 is secreted by a large number of tissues in response to cellular stressors. Circulating concentrations of GDF15 express increase in disease states, such as prostate cancer, infection, and cardiovascular disease, and this has been associated with anorexia and cancer cachexia (237). Furthermore, a variety of clinical and genetic data suggest roles for high circulating levels of GDF15 in the nausea and vomiting associated with *hyperemesis gravidarum* during the second trimester of pregnancy (238,239). Mice with transgenic over-expression of GDF15 are leaner and are protected from diet induced obesity, and the injection of GDF15 causes hypophagia and weight loss in rodents (240,241).

Unlike GDF15, which has broad tissue expression, expression of the receptor for GDF15 (GFRAL) is restricted to the AP and NTS in adults. Intact signaling

through the hindbrain is required for GDF15-mediated weight loss, as ablation of the AP and NTS or deletion of GFRAL abolishes hypophagia and weight loss in GDF15-treated mice (205,242,243). While GDF15 produces a strong conditioned taste aversion, the downstream neural circuits by which GDF15/GFRAL activation modulates feeding behavior have yet to be elucidated. While GFRAL-null mice are protected from weight loss in response to infections, tumors, and chemotherapy, they display little (if any) alteration in body weight under normal physiologic conditions (204). Thus, GDF15 appears to link strong physiologic stressors (e.g., infection, pregnancy, cancer, and cardiovascular dysfunction) to the aversive suppression of food intake, rather than contributing to the normal control of food intake and energy balance.

PEPTIDE YY

Peptide YY (PYY), which is released from the L cells of the distal digestive tract, belongs to the pancreatic polypeptide family (including pancreatic polypeptide (PP) and NPY) and has been proposed to serve as a satiety signal (244–246). The circulation contains two forms of the peptide: PYY1-36 and PYY3-36; the latter represents the main circulating form of PYY in postprandial human plasma and is able to cross the blood-brain-barrier by non-saturable mechanisms (247,248). Both forms of PYY bind to the Y2 isoform of the NPY receptor (NPY2R) (249). While the reported effects of PYY3-36 on food intake in rodents and humans initially generated some controversy (250), recent studies support the notion that NPY2R agonists can promote a strongly aversive suppression of food intake in many species (251,252). The role for endogenous PYY in food intake remains unclear, however, and although the AP/NTS represent presumptive sites that mediate the suppression of food intake by NPY2R agonists, this has yet to be definitively established.

[Please refer to ENDOTEXT chapter *Endocrinology of the Gut and the Regulation of Body Weight and*

Metabolism by Andrea Pucci and Rachel L Batterham, for additional information]

AMYLIN

Pancreatic β -cells co-secrete the peptide, amylin, with insulin during meals. Amylin inhibits gastric emptying and systemic and central administration causes a dose-dependent reduction of meal size (253–256). Amylin binds to the amylin receptor- CALCR in complex with a receptor activity modifying protein (RAMP) (257). The amylin-responsive neurons of the AP/NTS have yet to be definitively identified, but may lie in the AP and/or NTS. Interestingly, combination treatment with amylin plus leptin elicits a greater inhibition of food intake and body weight loss in obese rats than predicted by the sum of monotherapy conditions. Peripheral administration of amylin restores leptin sensitivity in rats, crucial in the treatment of leptin resistance in obesity (258), suggesting the potential therapeutic utility of combining hindbrain- and hypothalamus-acting compounds.

Interactions Between Forebrain and Brainstem Systems that Control Food Intake

Communication between the systems that sense the gut and those that sense energy stores is crucial to control satiety appropriately for feeding state and physiologic requirements. Thus, the forebrain and hindbrain must communicate to appropriately control feeding. Indeed, hypothalamic systems impact brainstem feeding circuits: AgRP neurons tonically inhibit PBN CGRP cells, while PVH projections to distinct (non-CGRP) PBN cells suppress feeding (61,65,70,71). Similarly, the ingestion of nutrients activates a gut-vagus-NTS pathway that inhibits the activity of AgRP neurons (199), and projections from the NTS to the PVH can blunt food intake (259). A great deal more research in this area will be required

to fully understand the integration of these circuits, however.

OTHER SIGNALS THAT MAY MODULATE FOOD INTAKE

Nutrient Signaling

While their effects are not as strong as those of many hormones or neural circuits, all three groups of nutrients (carbohydrates, lipids, and proteins) have been implicated in the control of feeding. Mayer proposed the “glucostatic hypothesis” in the 1950s, suggesting that decreases in glucose utilization stimulated eating and increases in glucose utilization halted eating (260,261). Indeed, intrahypothalamic glucose administration decreases food intake and inhibits hepatic glucose production (262). The response to decreased glucose or the blockade of glycolysis, which increases food intake and hepatic glucose production, is much stronger than the response to increased glucose, however. Furthermore, most glucose-sensing neurons are modulated within the normal to low range of glucose concentrations, rather than by elevated glucose. Also, the sensor of cellular energy deficits, AMPK, has also been proposed to play a role in CNS glucose sensing (263,264), but this cellular pathway is likely to be engaged mainly by severe energy deficits in the CNS. Hence, the brain glucose- and energy-sensing systems may be mainly involved in defending against large swings in blood glucose (e.g., defending against hypoglycemia) rather than serving as a primary controller of food intake and energy balance.

While the hypothalamic sensing of long-chain fatty acids has also been suggested to suppress food intake in response to increased availability of fatty acids in states of nutrient surfeit (265,266), the physiologic relevance of such a system remains unclear. The uptake of esterified lipids into the CNS is modest and circulating fatty acids actually increase during fasting. The systems that import fatty acyl-

CoAs into mitochondria and the control of overall mitochondrial function in hypothalamic cells that control food intake and metabolism represent important determinants of energy balance, however.

Low protein diets dramatically increase food intake, and the peripheral or intra-CNS infusion of amino acids (especially the branched-chain amino acid leucine) robustly decreases food intake (267,268). While the neural pathways underlying these effects have yet to be completely elucidated, brainstem systems likely contribute, at least in part. Additionally, the mechanistic target of rapamycin (mTOR)-mediated cellular amino acid sensing system is required for the operation of the CNS systems that mediate protein appetite (269). In addition to its role in neurotransmission, glutamate acts on its receptor in the GI tract both mediate taste-sensation and to serve as a gut-derived signal to also the vagal input to the CNS (270). In one study, intra-luminal glutamate infusion resulted in reduced body weight without altering food intake (271).

Inflammation

Inflammatory signals are proposed to mediate several distinct metabolic responses. Strong acute inflammatory stimuli (including those associated with systemic infection, cancer, etc.) decrease appetite and increase energy expenditure, promoting cachexia (GDF15 may mediate a portion of this effect). Conversely, obesity is associated with increased low-grade inflammation that appears limited to particular tissues, such as adipose tissue (272). 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 INFLAMMATION

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 (273). 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-1 β converting enzyme (ICE, which is essential for IL-1 β activity), prevents LPS-induced anorexia in mice (274). GDF15, acting via AP GFRAL neurons, may also contribute to the LPS-mediated suppression of food intake.

The inflammatory system may also contribute to the control of energy balance under normal physiology, as well: adiposity is increased in *Il6* null and *Gmcsf* null mice, and in mice with impaired macrophage function due to the targeted deletion of *Mac-1* or LFA-1 (or their receptor, ICAM-1)(275). 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 (276).

METABOLIC INFLAMMATION

Obesity is associated with increased production of a number of cytokines (including TNF alpha) in adipose tissue, resulting primarily from the activation of adipose tissue macrophages and other immune cells (275,279). Manipulations that decrease adipose tissue inflammation ameliorate the metabolic dysfunction associated with obesity. While interference with generalized macrophage function may increase adiposity, interventions that alter their pro-inflammatory (versus anti-inflammatory) nature

increase leanness and improve metabolic function (280,281).

Some data also suggest that inflammation-associated hypothalamic processes may contribute to obesity. High fat feeding results in the activation of hypothalamic microglia (the resident immune cells of the brain) and astrocytes (282,283). Some have postulated that these activated microglia secrete proinflammatory cytokines to disrupt the control of food intake, promoting obesity. Debate continues regarding whether this gliosis provokes or attenuates obesity, however. The ER stress in adipose tissue and the hypothalamus, potentially a consequence of metabolic inflammation, has also been reported in obesity (284). Genetic or pharmacologic interference with ER stress ameliorates obesity and insulin resistance in rodent models.

ENERGY BALANCE AND MOTIVATION

The homeostatic regulation of energy balance powerfully defends against body weight excursions below the lower limits of adiposity (9), and but often fails to prevent weight gain in our world of abundance of highly palatable, high energy foods. Non-metabolic factors that contribute to overeating and obesity include food palatability, availability, sensory-specific satiety, energy density of food, consumption rate, stress, social environment and energy output/exercise (285,286). Palatability and pleasantness of food represent powerful determinants in regulating motivation to eat.

Reward Circuitry and Neurotransmitters

DOPAMINE AND THE BRAIN REWARD SYSTEM

The neural circuits that comprise the reward pathways encompass wide-ranging brain regions, including the hypothalamus, the nucleus accumbens in the basal

forebrain, the midbrain ventral tegmental area (VTA), the amygdala and the thalamus (274). The LHA connects the hypothalamus to the broader reward system through projections to the VTA, where dopaminergic cell bodies lie. From there, the mesolimbic pathways (dopaminergic projections between the VTA and the nucleus accumbens) mediate reward-based feeding (287–289).

Dopamine (DA) potently augments the drive to obtain a rewarding stimulus and is required to drive feeding behavior. DA-deficient mice 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) (290). While the specific mechanisms through which dopaminergic signaling regulates motivated feeding behavior are not yet clear, connections between the LHA and the mesolimbic system as well as integration with the leptin and melanocortin systems appear to contribute.

SEROTONIN RECEPTOR 2c

Serotonin (5-hydroxytryptamine, 5-HT), which derives from stress-modulated neurons in the midbrain raphe nuclei, acts via 5-HT receptor 2c (HTR2c) to decrease food intake and body weight, and deletion of *Htr2c* produces hyperphagic obesity that is accentuated by high fat diet. Within the hypothalamus, ARC, PVN, LHA, and anterior hypothalamic nucleus (AH) neurons contain *Htr2c* (291). A subset of ARC POMC neurons express *Htr2c*, and the *Pomc*^{cre}-mediated reactivation of a null *Htr2c* allele in these cells attenuates the food intake and obesity in the *Htr2c* null mice (292,293). *Htr2c* cells in the midbrain VTA and in the hindbrain NTS may also contribute to the control of feeding by HTR2c. The effect of HTR2c activation may vary by brain region, but, in aggregate, *Htr2c* mutant mice confirm the important role for this receptor in energy balance. HTR2c agonists promote weight loss, and several have been approved for the treatment of obesity.

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 because increases in energy expenditure and negative energy balance promote a compensatory increase in feeding. Similarly, decreased energy expenditure will cause the accumulation 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 (91,92).

The tendency for energy intake to match changes in energy expenditure is exemplified by several animal models in which alterations in energy expenditure do not lead to large changes in adiposity. 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 (294). 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 (295). Similarly, the phenotype of mice null for the beta-adrenergic receptor beta 3-AR is 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 (296). Also, “beta-less” mice, with a global targeted deletion of all three beta-adrenergic receptor isoforms, have only slightly increased body fat on high fat diet under non-thermoneutral conditions (296).

[Please refer to ENDOTEXT chapter titled *The Role of Non-exercise Activity Thermogenesis in Human Obesity* by Christian von Loeffelholz and Andreas Birkenfeld and *Control of Energy Expenditure in Humans* by Klaas R Westerterp for additional complementary information on energy expenditure]

LESSONS FROM HUMAN OBESITY SYNDROMES

While much of our understanding of the genetics and signaling pathways involved in the central control of energy balance and development of obesity has been derived from rodent models, there exist rare cases of human obesity syndromes due to genetic mutations that shed light on the pathogenesis of obesity development. Many of these mutations corroborate the evidence from animal studies. In addition, with the advent of next generation sequencing and the ability to delve deeply into the human genome, genome wide association studies (GWAS) have also begun to reveal gene variants that may contribute or predispose to obesity.

Monogenic Obesity Syndromes

MC4R

Approximately 4% of morbid human obesity (BMI > 40 kg/m²) results from mutations in *MC4R* (297–299). Preserved lean mass and increased stature are also evident in humans with *MC4R* deficiency syndrome, as in rodent models (57). Most obesity associated with *MC4R* mutations has been attributed to heterozygosity at the *MC4R* locus (58). Patients who are homozygous for a null *MC4R* mutation develop severe childhood obesity (57), while heterozygous family members are overweight. This suggests a codominant inheritance pattern in which the gene product of these mutations impair the function of the normal gene product. Genome-wide association studies (GWAS) have revealed common non-coding polymorphisms within the *MC4R* locus that are associated with increased

adiposity (59). Treatment options for patients with *MC4R* mutations remain limited, although recent studies have suggested that the newly developed *MC4R* agonist setmelanotide can produce modest weight loss in patients with *MC4R* variants that encode receptor with decreased (rather than absent) function, as well as those with *POMC* mutations (300,301).

LEPTIN DEFICIENCY INCLUDING LIPODYSTROPHY

Genetic leptin deficiency in humans is very rare, but (as in rodents) 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 (302). 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 (303). A few humans homozygous for leptin receptor 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 (304). It is important to note that mice (305) and humans (306) heterozygous for null mutations of either *LEPR* or *LEP* are more obese than 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.

Lipodystrophy represents another clinical syndrome associated with leptin deficiency. Lipodystrophy encompasses a heterogeneous group of disorders that range from inherited monogenic gene disruptions to acquired disorders due to treatment with medications such as highly active retroviral therapy for HIV. In generalized lipodystrophy, patients develop loss of subcutaneous fat tissue which results in leptin deficiency, hyperphagia, severe insulin resistance and diabetes, and visceral obesity. When leptin deficiency can be demonstrated, treatment with recombinant leptin significantly improves hyperphagia, body weight and diabetes severity (307).

CILIOPATHIES

A subset of mutations causing defects in primary cilia promote obesity syndromes (308,309). 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 are now known 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), McKusick-Kaufman Syndrome, Alström Syndrome, and Joubert Syndrome. Altered trafficking of MC4R and/or MCH receptor may play roles in the obesity of those with ciliopathies.

POMC AND PROHORMONE CONVERTASE 1 DEFICIENCIES

Mutations resulting in complete absence of the *POMC* gene product cause secondary adrenal insufficiency due to lack of ACTH; however, once glucocorticoid replacement therapy is started, children with these mutations invariably develop obesity due to hyperphagia. In patients of Caucasian ancestry, there is also a characteristic phenotype of red hair and pale skin, although this is not found in patients from other genetic backgrounds. Some *POMC* mutations affect specific melanocortin peptide products, and those that specifically alter α -MSH result in severe early-onset obesity (39,40).

The prohormone POMC is cleaved by prohormone convertase 1 (PC1). 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 (310). Other monogenetic obesity syndromes in mice and humans likely result from alterations in melanocortin signaling, including those due to alterations in other components of the peptide processing system, including carboxypeptidase E (CPE) (311).

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF/TrkB) SIGNALING

BDNF, a member of the neurotrophin 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 (312). BDNF 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, alterations in BDNF (or 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 (313,314). Mutations in *NTRK2* (which encodes TrkB)

produce similar hyperphagia and obesity, along with impaired cognitive function and nociception, in rare human patients (315). Interestingly, a coding polymorphism in *BDNF* (Val66Met) is associated both with obesity and with binge eating disorders in humans (316), 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 (317,318). Furthermore, polymorphisms in *BDNF* are associated with risk for obesity in human GWAS studies (59).

PRADER-WILLI SYNDROME (PWS)

PWS presents in infancy with low birth weight, hypotonia and poor feeding, followed by 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 (319,320). 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 (319). 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 (321). 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 (322–324). 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 to alter energy balance. Understanding the molecular physiology of PWS is likely to identify novel genes in the control of energy homeostasis in non-syndromic obesities.

[PLEASE REFER TO THE CHAPTER TITLED *THE GENETICS OF OBESITY IN HUMANS* BY SADAF FAROOQI AND STEPHEN O'RAHILLY, FOR ADDITIONAL INFORMATION ON GENETIC FORMS OF OBESITY.]

CONCLUSION

This chapter provides an overview of the complex neural and metabolic pathways that determine energy intake and expenditure. Distinct areas of the brain receive and interpret hormonal and neuronal messages from the periphery and other brain regions to integrate regulatory changes that maintain energy balance. These changes require a finely tuned balance of synaptic neurotransmitters, hormonal

feedback loops and neuropeptide expression. The identification of the molecules encoding these messages using human studies and animal models has expedited the discovery of the crucial signaling and homeostatic pathways that govern these mechanisms. 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.

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