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REVIEW

Leptin and Beyond: An Odyssey to the Central Control of Body Weight

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The 2010 Lasker Award for basic medical research was shared by Douglas Coleman and Jeffery Friedman for their discovery of leptin, a breakthrough that revealed insight into the genetic basis of obesity. This mini-review aims to review landmark studies on the physiologic system of body weight control. The basic research on the leptin system has broad implications for the genetic control of body weight, thus contributing to solve the global obesity crisis.

"The elation of peering into the depths of nature and being the first to see something new is impossible to describe."

— Jeffery M. Friedman

INTRODUCTION

Douglas Coleman and Jeffery Friedman shared the 2010 Albert Lasker Basic Medical Research Award for their discovery of a fat cell-produced hormone, leptin (Greek *leptos* means thin). The discovery was a landmark event in modern physiology [1]. Friedman is widely recognized for cloning the leptin gene *ob* that reverses obesity and metabolic abnormalities in a severely obese mutant mouse strain *ob/ob* [1,2]. Leptin is an adipocyte hormone that informs the brain of the status of energy stores in peripheral tissues [1-3]. The discovery of leptin not only closed the long hypothesized physiologic feedback loop that controls energy homeostasis around a set point, but also markedly accelerated our knowledge of the roles that genetics and neuroendocrinology play in obesity, a severe, costly public-health problem [1-8]. Here I briefly review the seminal work done by Friedman and his precursors and summarize the history and current research

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†Abbreviations: JAK, Janus Kinase; STAT, Signal Transducer and Activator of Transcription; ARC, arcuate nucleus; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamic; LH, lateral hypothalamic; PCR, polymerase chain reaction; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; AgRP, Agouti-related peptide; α-MSH, α-melanocortin-stimulating hormone; MCR, melanocortin receptors; AMPK, adenosine monophosphate activated kinase; ROS, reactive oxygen species; mTOR, mammalian target of rapamycin

exploring the central neural circuits that regulate metabolic physiology.

THE PATH TO DISCOVERY

Since Hippocrates' influential observation that weight can be controlled by "deciding" to eat less and exercise more, the view that body weight is under the tight control of an endocrine system was not quite widely shared [2,3]. The old dogma was challenged when Gordon Kennedy proposed the lipostat theory in 1953 [2,9,10]. It was stated that fat mass serves as the set point of energy balance, in which fat depots feedback onto the hypothalamus (the brain region responsible for feeding behavior) and signal the energy requirement [9]. Kennedy proposed that the feedback factors could be circulating metabolites. However, as would be illuminated decades later, they can also be hormones.

Experimental support for this regulatory system emerged slowly. In 1959, based on the Hetherington and Ranson study in 1942 showing lesions in hypothalamus can cause obesity in rats, G.R. Hervey presciently proposed the existence of a signal that suppresses food intake and weight in rats via the parabiotic experiments [11,12]. He surgically connected the circulatory system via subcutaneous tissues between normal rats and those with lesions in the ventral medial hypothalamus (VMH†), which were known to cause obesity [10,12]. The VMH-lesioned rats were obese as expected. Surprisingly, the normal rats that were connected to the obese decreased their food intake and lost substantial weight [12]. Without a functional VMH, the rats could not respond to the satiety signal, thus they became obese and overproduced the signal as Hervey postulated. Through circulation exchange, the excess of the satiety signal suppressed the food intake and weight in normal mice. This hypothesis is remarkably prescient considering the complexity of parabiotic models [1]. The task of identifying the circulating satiety signal was daunting, and thus left the results attractive but inconclusive.

Prior to the era of genetic models of energy balance, the lesions study was probably the primary approach to analyze and locate regulatory sites, yet it was imprecise. In 1949, a colony of mice showing severe obesity was identified at The Jackson Laboratory, which brought the genetic approach into the field [13]. The mutation was mapped to chromosome 6 and designated *obese* (*ob*). A second mouse strain with obesity syndrome was identified by Doug Coleman and his associates in 1966 [14]. Mice homozygous for the mutation that was designated *diabetes* (*db*) showed dramatic early onset obesity, insulin resistance, hyperphagia (over-eating), and physical inactivity. The important findings came out when Coleman et al. conducted a series of parabiotic experiments with *ob/ob*, *db/db*, and wild-type mice. When *ob/ob* mice were connected to either wild-type or *db/db* ones, they decreased feeding and lost weight, and this effect was reversed after disconnection. The counterpart animals were unaffected by connection with *ob/ob* animals [15]. In sharp contrast, wild-type mice stopped eating and lost weight substantially when connected to *db/db* mice. Yet *db/db* animals were not affected. Coleman proposed that *ob/ob* mice lacked a circulating satiety factor that regulates feeding and weight. Furthermore, *db/db* mice overproduced the circulating factor but could not respond to it. Based on Hervey's observation, Coleman surmised that the hypothalamus probably contains the center that responds to the circulating satiety factor [15]. In summary, observations from multiple laboratories came to the hypothesis that body weight is under the control of an endocrine system in which fat depots gauge the energy balance and probably release a circulating hormone, encoded by the *ob* gene, to inform the hypothalamus via its receptor, the DB protein, of the satiety/hunger status.

The essential endeavor after Coleman's seminal parabiotic studies was to identify the nature of the *ob* gene. There were two alternative approaches. One was the traditional biochemical approach, which attempted to purify the hormone; the other

was the novel genetic approach taken by Jeffery Friedman: positional cloning [2]. Through a heroic eight-year odyssey, Friedman and his colleagues finally cloned the gene in 1994. They demonstrated that the *ob* gene as a 4.5 kb transcript expressed exclusively in adipose tissue [2,16]. Soon after this classic report, the Friedman laboratory and two other groups demonstrated that administration of the recombinant OB peptide ameliorated the *ob* phenotype, thus substantiating the role of OB as a hormone regulating body weight [17-19]. Moreover, brain administration of OB peptide influenced feeding behavior, suggesting OB can act directly on the neural circuits that regulate feeding and energy balance [19]. It was consistent with Coleman's prediction that the circulating factor, OB, acts on the hypothalamic satiety center. Based on these findings, Friedman named the peptide "leptin" from the Greek root *leptos* for "thin" [1].

An avalanche of discoveries followed. Lee et al. from the Friedman laboratory and Chen et al. identified the *db* locus as a gene encoding a family of leptin receptors that are alternatively spliced, with the spliced form OB-Receptor b (OB-Rb) having a long intracellular domain functional in the hypothalamus [20,21]. Among the six alternative spliced forms, only OB-Rb is expressed strongly in the hypothalamus and was predicted to mediate signaling via the Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway. Naturally, Vaisse et al. from the Friedman laboratory demonstrated that systemic administration of leptin activates STAT3 specifically and exclusively in the hypothalamus of wild-type and *ob/ob* mice but not *db/db* mice, which lack leptin receptor [22], suggesting that leptin receptor mediates the leptin's effect onto STAT3.

These findings, together with other studies, elucidated the endocrine system that regulates body weight via metabolism and feeding behavior (Figure 1). The status of fat stores in the adipose tissues dictates the synthesis and release of the hormone leptin. Weight loss causes a decrease in serum leptin levels, while feeding cues, such as insulin and glucose, increase serum leptin levels [3,23,24]. Leptin feeds back on the regulatory centers in the brain, thus controlling metabolism and feeding. Absence of either the hormone or the receptor predisposes rodents to obesity. Thus the network of the leptin system has a profound implication on obesity and human metabolic diseases.

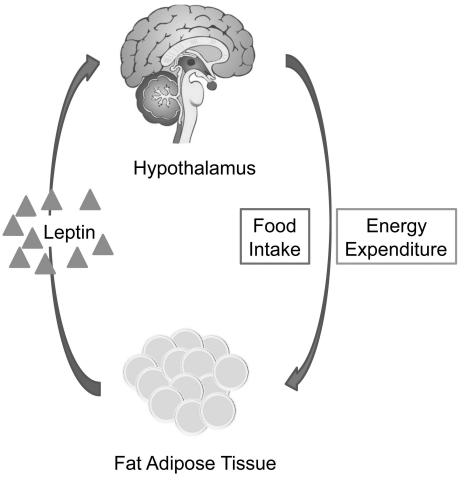

Figure 1. Leptin and Regulation of Body Weight. Leptin is synthesized and released by fat adipose tissue, which constitutes a negative feedback loop that regulates body weight. It circulates in the blood and acts on the hypothalamus via binding to leptin receptors. The plasma leptin level is proportional to the fat mass. When fat mass decreases, plasma leptin level falls, initiating the activation of orecxigenic responses and the repression of anorexigenic responses. Thus food intake is enhanced and energy expenditure is inhibited until fat mass is restored. When fat mass increases, plasma leptin level increases, thus inhibiting food intake and increasing energy expenditure via concerted actions of hypothalamic neural circuits. Overall, the body weight is maintained in an intricate balance by the leptin signaling system.

Considering both leptin deficiency and unresponsiveness account for obesity in mouse models, it was expected that leptin deficiency would be a major factor in obese humans. Indeed, low leptin levels were found in a small but significant fraction of obese humans (5 percent to 10 percent) [3]. However, leptin treatment of obese humans had variable effects [25]. Surprisingly, high

serum leptin is associated with obesity (known as leptin resistance), indicating either the adaptation of the leptin system to a new set point or the dysfunction of leptin signaling [3,26]. Therefore, leptin is not the whole story for central regulation of body weight but rather a landmark for a new initiative exploring the mechanisms.

LEPTIN AND INTERPLAYS OF CENTRAL NEURAL CIRCUITS IN THE HYPOTHALAMUS

In the energy balance equation, energy store is determined by energy intake and energy expenditure, both of which are regulated by the hypothalamus [27]. Leptin acts primarily on the hypothalamus, influencing behavior and metabolism [16-19]. The discovery of leptin prompted researchers to explore the central neural circuits and their interactions with the leptin signaling.

Since leptin actions on the hypothalamus depend on the presence of leptin receptor, studies on the localization of leptin receptors ushered in soon. Fei et al. from the Friedman lab found OB-Rb, the one responsible for the *db* phenotype, is expressed in the arcuate nucleus (ARC), ventromedial (VMH), dorsomedial (DMH), and lateral (LH) hypothalamic nuclei, but is not detectable in other brain regions via polymerase chain reaction (PCR) [28]. These regions are known to regulate food intake and energy expenditure. Lesion studies in rodents (in some cases, humans) demonstrated that disruption of the ARC, VMH, and DMH resulted in hyperphagia and obesity [27,29-34], whereas lesions in the LH caused hypophagia [35]. The dual-center model identifies the VMH as the "satiety center" and the LH as the "hunger center" [36]. Thus the studies by Fei et al. and others highlight the dominant role of leptin signaling in the central regulation of energy balance [28,37-39].

Another equally important direction was the hypothalamic target molecules of leptin. Right after the discovery of the *ob* gene, Stephens et al. proposed leptin regulates food intake and metabolism in part via inhibition of the synthesis and release of neuropeptide Y (NPY), which stimulates food intake and decreases thermogenesis [40]. However, even before the discovery of leptin, arcuate nucleus NPY neurons via their connection to pro-opiomelanocortin (POMC) cells were proposed to regulate feeding [41]. Indeed, later studies showed that leptin inhibits neural pathways that stimulate food intake (orexigenic) and decrease energy expenditure, as well as activates neural pathways that inhibit feeding (anorexigenic) [3,10,42]. Orexigenic neuropeptides include NPY and Agouti-related peptide (AgRP), whereas anorexigenic signals contain α-melanocortin-stimulating hormone (α-MSH, a cleaved product of POMC).

The neurons that are marked by AgRP or POMC and the neurons expressing melanocortin receptors (MCR) comprise the melanocortin system, which regulates long-term energy balance [10,42]. ARC nucleus is composed of two specific neurons, POMC and NPY/AgRP [42]. An exceptionally attractive and simple model depicting the interplays between leptin signaling and the central regulation of feeding is described thusly: Leptin activates POMC neurons via firing rates and gene expression; activated POMC neurons release α-MSH into the synapses, which activates the projected neurons via binding to MCRs and leads to anorexigenic responses and increased energy expenditure. Simultaneously, leptin inhibits NPY/AgRP neurons, offsetting the antagonistic effect of AgRP on MCRs [43,44]. NPY/AgRP neurons stimulate orexigenic responses and inhibit POMC neurons via direct synaptic connection [41,45]. Notably, there is no feedback from POMC neurons to NPY/AgRP neurons, indicating the default function of the neural circuit is to promote food intake [10,42]. The importance of the melanocortin system is exemplified not only by these findings that leptin directly acts on this circuit in the hypothalamus but also by the fact that loss of function of the MC-4R, the major MCR, is the most common genetic cause of human obesity, accounting for 3 precent to 5 percent of severe obesity in humans [46,47].

In short, leptin regulates energy balance by modulating the activity and gene expression of NPY/AgRP neurons and POMC neurons in the ARC. To add a new layer of regulation, Pinto et al. from the Horvath group identified rapid rewiring of the ARC neural circuit by leptin [48]. The synapses projected to NPY/AgRP neurons and POMC neurons were different between *ob/ob* mice and wild type mice. Leptin treatment normalized the synaptic density on these neurons within six hours, several hours before leptin's effects on food intake. These findings suggest leptin also acts on the hypothalamus via neural plasticity.

CONCLUSIONS AND OUTLOOK

The identification of *ob/ob* mice was the first evidence from animal studies to illuminate the genetic basis of obesity. Friedman's discovery of leptin has provided the conclusive evidence for an endocrine system that regulates feeding and metabolism via communication from fat tissues to brain, which is the primary statement of the lipostat theory. From the perspective of physiology, fat mass gauges the energy stores; leptin signaling communicates from adipocytes to hypothalamus, the center that regulates numerous physiologic and behavioral processes, including metabolism and feeding; leptin influences feeding via the ARC melanocortin system, modulating neural activity, gene transcription, and circuit plasticity; and the ARC integrates information from the VMH (the satiety center) [49] and other brain regions and instructs its downstream neural circuit to affect feeding and metabolism through hormonal and neural pathways, thus bringing the energy status to normal (Figure 1).

Over the last 16 years, researchers have learned much more about the biology and physiology of leptin. Yet much remains unknown. Whether leptin is the only signal for energy balance is an open question. The brain is in a constant demand for fuel molecules. For example, 70 percent of glucose is utilized by the brain, which represents only 2 percent of body weight. Fuel molecules and their downstream products have been hypothesized to sense the energy status *in situ*. In other words, neurons in the ARC and VMH can modulate their activity in direct response to fluctuations in glucose, free fatty acids, and amino acids [10]. Thus it is attempting to study the nutrient-sensing mechanisms within the brain, especially the POMC neurons, namely adenosine monophosphate activated kinase (AMPK) pathways, reactive oxygen species (ROS) pathways, mammalian target of rapamycin (mTOR) pathways [10].

How the ARC neurons integrate signaling and control feeding behavior is also under avid investigation. In 2005, Sternson et al. from the Friedman laboratory reported direct projections from the satiety center, VMH, to the ARC neurons. Of interest, in a population level, medial VMH neurons project excitatory synapses onto POMC neurons, but not NPY neurons [49]. NPY neurons only receive weak inhibitory synaptic projections within the ARC [49]. These findings support the idea that food intake (orexigenic) is the default function of mammalian, including human, neural circuits. Besides the crosstalk between different brain regions, there are also neural pathways connecting the hypothalamus and peripheral tissues. Using virus to trace neuronal projections, Stanley et al. from the Friedman laboratory identified subpopulations of neurons from the hypothalamus projecting to two metabolic active tissues, liver and white adipose [50]. The delineations of these neural pathways will facilitate functional analysis of how the hypothalamus regulates feeding behavior and metabolism.

How cellular leptin resistance is linked to obesity is a debated topic [26]. Analogous to insulin resistance in metabolic syndromes, leptin resistance coexists with obesity in most cases. Whether the blunted response to elevated leptin levels predates or adapts to obesity is not known [26]. Moreover, induction of cellular attenuation of leptin signaling complicates the causal relationship between leptin resistance and obesity. Of note, Knight et al. recently found diet-induced obese mice whose leptin levels

were clamped to lean levels remained sensitive to exogenous leptin, suggesting hyperlepinemia contributes to leptin resistance [51]. However, additional research is required to verify this issue.

Central regulation of body weight is closely relevant to human health. The dysfunction of leptin and its downstream components contributes to monogenic obesity. The United States is in a severe obesity epidemic. Over the past decade, obesity has become recognized as a national health threat and a major public health challenge. In 2009, only Colorado and the District of Columbia had a prevalence of obesity less than 20 percent. Thus the elucidation of leptin and its actions in the central neural circuit will help solve the national crisis. More than 50 years of research has established the lipostat model of body weight control. Yet researchers are still in the odyssey to a full understanding of molecular mechanisms.

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