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The long road to leptin

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Harrington Prize Essay

Leptin is an adipose tissue hormone that functions as an afferent signal in a negative feedback loop that maintains homeostatic control of adipose tissue mass. This endocrine system thus serves a critical evolutionary function by protecting individuals from the risks associated with being too thin (starvation) or too obese (predation and temperature dysregulation). Mutations in leptin or its receptor cause massive obesity in mice and humans, and leptin can effectively treat obesity in leptin-deficient patients. Leptin acts on neurons in the hypothalamus and elsewhere to elicit its effects, and mutations that affect the function of this neural circuit cause Mendelian forms of obesity. Leptin levels fall during starvation and elicit adaptive responses in many other physiologic systems, the net effect of which is to reduce energy expenditure. These effects include cessation of menstruation, insulin resistance, alterations of immune function, and neuroendocrine dysfunction, among others. Some or all of these effects are also seen in patients with constitutively low leptin levels, such as occur in lipodystrophy. Leptin is an approved treatment for generalized lipodystrophy, a condition associated with severe metabolic disease, and has also shown potential for the treatment of other types of diabetes. In addition, leptin restores reproductive capacity and increases bone mineral density in patients with hypothalamic amenorrhea, an infertility syndrome in females. Most obese patients have high endogenous […]

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Introduction

Long before I embarked on a career in science, I imagined that prize-worthy discoveries were the product of a moment of deep insight, a "eureka" moment, when a person of genius conjures an explanation for the inexplicable. While some of the greatest discoveries — gravitation, natural selection, and general relativity — can be fairly described in this way, this romantic view of a singular genius upending the world is, in actuality, quite uncommon. Rather, most seminal discoveries throughout history were made by multiple groups, simultaneously or near simultaneously, building on the work of others and culminating in advances that are referred to as scientific multiples. For example, a comprehensive review of all of the great discoveries prior to 1922 by Ogburn failed to identify a single advance that was not made by more than one group. Examples include the discovery of oxygen by Lavoisier, Schiel, and Priestley; the development of calculus by Newton and Leibniz; and even the theory of natural selection by Darwin and, later, Wallace, etc.(1). Indeed, it is difficult to identify discoveries that do not hew to this "rule." In modern times, multiples are less common, in part because of the rapid dissemination of information, which then has a chilling effect on competing work once a discovery is made (this is an example of what is referred to as a forestalled multiple, of which there are several other different types)(2).

Historians of science interpret the high frequency of multiples as indicating that, in general, discoveries are built on what came before and that, when a question is "in the air" and the needed technology has been developed, the answer (and opportunity) generally becomes clear to more than one individual at more or less the same time(3). This reasoning even led Ogburn to wonder whether all discoveries might be inevitable (1).

Viewed in this way, science performed at a level that ultimately attracts recognition is typically the result of an individual(s) finding themselves in a time and place that makes them aware of an opportunity, recognizing the importance of that opportunity (as compared to other competing ones) and then pursuing their goal with rigor and intensity and as much creativity as they are capable of bringing to bear on the problem Thus, the process of discovery is not necessarily about having an idea that no one else has had, but rather recognizing an important opportunity when it comes one's way, choosing to focus on that objective to the exclusion of others and executing it capably.

This discovery of leptin and my path to it illustrate these points. It was my intention to become a physician, and toward that end, I entered the combined RPI Albany Medical College six-year medical program. The program requires students to perform research during the first two sum-

Conflict of interest: Leptin is now an approved drug. While Rockefeller University owns the patent for leptin, the author is named as an inventor on the patent for leptin and, as per University policy, receives a portion of the milestone and royalty payments made to Rockefeller University by Astra Zeneca, the company that owns the license to the patents.

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mers of their undergraduate years, after which a standard four-year medical school curriculum begins. I conducted two separate research projects during these summers, both of which were distinguished by their profound lack of distinction. In the first, I was assigned a project to test the effect of glucocorticoids on animals subjected to hypovolemic shock (unpublished data). I was instructed to work on dogs, but being a dog lover, refused, and instead worked on piglets (to which I also became attached). Unbeknownst to my advisor, I also rescued a German Shorthaired Pointer that had been the subject of a nonlethal experiment performed by a lab mate. The culmination of that summer's work was the monumental finding that there was not a beneficial effect of glucocorticoids for the treatment of shock and that I had a new dog (I named him Bernie).

In the second summer, I worked with Dr. Patrick Wong, a junior faculty member, who studied bradykinin. Under his tutelage, I performed a set of studies in rodents to test whether salt loading had an effect on bradykinin responsiveness and found that its hypotensive effect was equivalent in all three groups (unpublishable data, see below). For reasons I still cannot fathom, I thought this finding was of great importance and, together with Patrick, submitted a paper on our work to the *Journal of Clinical Investigation*. We received two reviews. The first was negative but was intended to be helpful. The second simply read, "this paper should not be published in the JCI or anywhere else." At this point, I was unconvinced that I had the potential to be a scientist and concluded that publishing a scientific paper represented a level of achievement that would likely elude me forever.

I was fortunate, however, because, for reasons known only to him, John Balint, Patrick's chair, thought I might have some aptitude for research, and in 1980, after I completed an internal medicine residency, he introduced me to Mary Jeanne Kreek, who studied the molecular basis of addiction at the Rockefeller University (4). My plan was to take a gap year before starting a fellowship in gastroenterology. However, in the course of this year, I became enamored with research. Instead of continuing my medical training, I became a graduate student in James Darnell's laboratory, with the aim of learning molecular genetics.

In Mary Jeanne's laboratory, I quickly became fascinated with the possibility that molecules could control behavior. In my first year, Mary Jeanne introduced me to another investigator, Bruce Schneider, who was going to help me develop a radioimmunoassay for β endorphin. While this project didn't pan out, I did learn about Bruce's work on cholecystokinin and a controversy that had erupted about whether this gut hormone played a role in the pathogenesis of the obesity in genetically obese *ob/ob* mice. I learned that the *ob* mouse had been identified by chance by George Snell and colleagues at The Jackson Laboratory in 1950 (5). This fully penetrant autosomal recessive mutation causes extreme obesity and had been mapped to mouse chromosome 6, though the nature of the defective gene was unknown (6). Indeed, hundreds of different hypotheses had been put forth about the function or identify of the *ob* gene, but none proved correct (7).

One of the putative candidate genes for the *ob* gene was cholecystokinin (CCK). CCK is an intestinal hormone secreted from gut endocrine cells after a meal that then stimulates gall bladder contractions and pancreatic secretion. CCK was later found in brain and became the first of many brain-gut peptides (8). In the years before I joined the Kreek laboratory, CCK had also been shown to act as an endogenous appetite suppressant (9). Later, Roslyn Yalow, a Nobel Prize winner who developed RIAs (published in the *JCI*), reported decreased levels of CCK in the brains of *ob* mice and suggested that the defects in CCK might be the cause of the *ob* phenotype (10). However, Bruce, who had been Yalow's trainee, had the opposite data (11), and the controversy between them about the role of CCK in the phenotype of *ob* mice festered. I became deeply interested in this topic, which served as a motivation to begin graduate school to learn the emerging tools of molecular biology.

While a graduate student, I attended a series of seminars by the pioneers in the evolving field of human molecular genetics. On the basis of these seminars, I began to consider that it might be possible to isolate the defective gene in *ob* mice. The objective was similar to that which captured my attention in Mary Jeanne's laboratory, namely, how a defect in a single gene and the molecule it encoded could control behavior, with, in this case, profound effects on food intake, body weight, and many other biologic processes. As a first step, Bruce, Don Powell, and I set out to test Yalow's hypothesis by cloning the *CCK* gene (12) and, in studies performed by Peter D'Eustachio, determining its chromosomal location. (These studies were performed at the same time that I pursued my PhD research on liver-specific gene expression.) Peter found that CCK did not map to chromosome 6, thus excluding it as an *ob* candidate gene, and we later showed that CCK mapped to mouse chromosome 9 (13). This, of course, raised the question as to the identity of the *ob* gene, and it seemed possible that positional cloning could provide an answer. This approach enables the identification of genes by mapping their precise position on a genetic and physical map of the chromosome. Positional cloning of single genes, however, is now largely an atavism, as it has been replaced by whole-genome sequencing.

My excitement for this project was heightened further when I later became aware of work done in the 1960s and 1970s by Doug Coleman, also at The Jackson Laboratory. Coleman used the technique of parabiosis to study *ob* mice and *db* mice, another genetically obese strain (14), and concluded that the *ob* gene encoded a novel appetite-suppressing hormone, and *db* encoded its receptor (15). Coleman had found that when *ob* mice are surgically joined to normal or *db* mice (on the same inbred strain background), they ate less and lost weight. In contrast, normal mice paired with *db* mice starved to death. From this, Coleman concluded that *ob* mice lacked a circulating factor that could be provided by the conjoined partner and that then suppressed food intake and body weight. He further suggested that *db* mice lacked a receptor to detect the weight-suppressing factor in their blood and so overexpressed it, producing levels so high that conjoined mice (who are sensitive to the factor) stopped eating. Implicit in this hypothesis was the prediction that the *ob* gene was under feedback control and that obesity would be associated with increased levels of *ob* RNA and the protein it encoded. In time, all of these predictions proved to be correct. Learning about Coleman's hypotheses further heightened my interest in

cloning the *ob* gene and also led my group to begin efforts to also clone the *db* gene.

While no mouse genes had been cloned using positional cloning at this time, the muscular dystrophy and chronic granulomatous disease genes had been identified (16, 17), and it seemed likely that additional genes such as the one for cystic fibrosis would follow. This set of technical advances put forth the possibility that the *ob* gene could be identified. Thus, when I completed my PhD studies in 1986 and started my own laboratory, my group began efforts to clone the *ob* and *db* genes. This endeavor occupied my attention of that of a large group of talented colleagues for the next ten years. This was a painstaking, anxiety-provoking, repetitive, and in many ways stultifying process that, after a ten-year odyssey ended with a moment of discovery and a sense of exhilaration that is one of the defining moments of my life.

Early one morning, a little more than 20 years ago, I developed a Northern blot showing changes in the levels of adipose tissue RNA detected by a probe named 2G7 (18). The RNA in this experiment was prepared from both WT and *ob* (obese) adipose genes, and 2G7 was a clone that mapped to the region of chromosome 6, where the *ob* gene had been found to reside. The 2G7 probe identified a 4.5-kB RNA in adipose tissue that was absent in RNA from CMC dac *ob/ob* mice but was increased by 20-fold in adipose RNA from C57Bl/6J *ob/ob* mice that was first characterized by George Snell and colleagues in 1950 (5). CMC dac *ob/ob* mice were unpublished at the time but were kindly provided by Skippy Lane of The Jackson Laboratory, as were many other mice that were critical for this study.

The demonstration that the same RNA was absent in one mutant and induced in the second mutant provided definitive proof that 2G7 was an exon from the *ob* gene. These data confirmed that the *ob* gene had been isolated, because, had the observed RNA changes been secondary to some other genetic defect, the expression levels would have to be similar in both *ob* mutant strains. Further studies revealed that a viral insertion into CMC dac *ob/ob* mice interfered with *ob* expression, while in C57Bl/6J *ob/ob* mice, a point mutation in the second exon of the *ob* gene introduced a nonsense mutation at amino acid 105 of this 167–amino acid protein (18, 19). The marked overexpression of the *ob* gene in C57Bl/6J *ob/ob* adipose tissue further suggested that the gene was under feedback control, with an increased level of gene expression in the obese state, consistent with the data from the classic parabiosis experiments by Coleman (15). However, Coleman's experiments did not predict where the hormone that was missing in *ob* mice was expressed, though prior experiments from Hervey predicted that the receptor would be expressed in the hypothalamus (20).

Putting this all together in that moment in a darkroom, it became evident in an instant that, not only had the *ob* gene been cloned, but the data were consistent with Coleman's predictions. The data thus suggested the hypothesis that the *ob* gene encoded a novel adipocyte hormone that functioned as the afferent signal in a negative feedback loop that maintains homeostatic control of adipose tissue mass. This hypothesis thus explained how a stable weight could be maintained over long periods of time, precisely balancing caloric output to energy expenditure. We named the new hormone leptin, derived from the Greek root "leptos" for "thin," the notion being that leptin kept a mouse (and humans) from becoming obese. While some of the features of this new hormonal system were predicted at the time, others were not. Science seldom proceeds in a straight line, and the field spawned by the identification of leptin and other genes that cause obesity is no exception.

There is no question that if we had not cloned the *ob* gene, someone else would have. This work can thus be considered a forestalled multiple; once we found the gene, others working on it stopped. Having had the opportunity to be the first to see how nature solved an age-old problem — how to maintain a stable weight while consuming millions of calories a year — was greater than anything I had ever dreamed. To a casual observer, the Northern blot showing that the *ob* gene had been identified would have appeared as a set of nondescript black blobs on a transparent background. But to me, it was beautiful, because it captured the essence of art: a somewhat abstract representation of a deeper truth. In the now 35 years since I learned, by chance, about the ob mouse, there have been surprises and disappointments. Some of the key lessons from 20 years of research on leptin are summarized below.

Wiring diagram of a complex behavior

One can describe the phenotype of *ob* and *db* mice in several different ways. Historically, these animals have been described as obesity mutations, but one could also think of them as showing a behavioral phenotype with numerous abormalities (7). They are massively hyperphagic, show a dramatic decrease in locomotor activity, are quite gentle and nonaggressive, and are not sexually active. Thus, the identification of leptin and, later, the leptin receptor (LepR) (21, 22), encoded by the *db* gene, provided an entry point for delving into the neural mechanisms that control complex behaviors. Moreover, the elucidation of the pathogenesis of the obesity resulting from the Ay (yellow agouti) mutation identified hypothalamic neurons expressing POMC, the precursor of αMSH, as key neural targets of leptin and, more generally, integrators of numerous metabolic signals (23). αMSH acts on the MC4, a GPCR, and MC4 receptor (MC4R) mutations replicate the obese phenotype of Ay mice (24). The subsequent identification of AGRP as an endogenous inhibitor of αMSH in a second group of hypothalamic neurons, also expressing NPY, added another population of leptin-responsive neurons (25, 26). We now know that leptin acts in part by activating POMC neurons and inhibiting NPY/AGRP neurons, though many other neural cell populations also play a role either as direct neural targets, or act downstream of these neurons (26–28). Indeed, enormous progress has been made in defining a set of overlapping neural circuits that control food intake and body weight. With time, these findings are likely to lead to a deeper understanding of how feeding behavior is controlled as well as advance our understanding of the control of other behaviors.

Indeed, leptin's neurobiologic effects are not limited to feeding circuits. Extreme weight loss in humans has been shown to induce a set of emotional sequelae including depression. A possible role for a reduction in leptin levels in mediating some portion of this was suggested by the finding that leptin injection into the hippocampus can improve the performance of animals

in a forced swim test (29). This assay provides a quantitative indication of the level of depression in animals and robustly predicts the efficacy of antidepressant drugs in humans. Other studies have shown that leptin has significant effects on reward processing by dopaminergic centers in the midbrain and that it can reduce the value of a sucrose reward (30). This is important, because it shows that the pleasure we derive from eating is not fixed but rather reflects the status of metabolic signals such as leptin, among others. Leptin also has potent effects on many other neural circuits including those controlling hormones that regulate reproduction and reproductive behaviors, activity, aggression, thermoregulation, and stress.

Obesity has a substantial genetic component

The identification of mutant genes that cause obesity in mice provided a molecular framework for identifying mutant genes that cause obesity in humans. Thus, mutations in leptin, the LepR (31), the MC4R, as well as PCSK1, and enzymes required for the processing of POMC cause human obesity, as do other components of the neural circuit that regulates food intake including BDNF and Sim1. Indeed, it now appears that more than 10% of morbid human obesity is a result of Mendelian defects in these (and other) genes, which, in the majority of cases, are in the MC4R and the LepR (31). This is a level of Mendelian inheritance that exceeds that for nearly every other complex trait that has been studied. The realization that obesity is often the result of genetic mutations in humans provides strong evidence that this condition is a result of alterations in a neural circuit that controls the basic drive to eat as well as metabolism (and perhaps other behaviors) and provides an alternative to the widely held view that obesity develops from a failure of willpower or consequent to the modern environment.

Furthermore, it has been noted that all of the obesity genes identified thus far are expressed in the brain. This is despite the fact that there is a large body of evidence indicating that differences in metabolic rates can predict changes in weight (32) and that an increase in peripheral metabolism, such as that which occurs after treatment with thyroid hormone or uncouplers of respiration such as dinitrophenol, leads to weight loss (33, 34). Moreover, while a defect in leptin signaling is associated with hyperphagia and a marked decrease in energy expenditure in mice, the principal effect in humans is on appetite, with little or no discernible effect on metabolism (Sadaf Farooqi, personal communication).

The heritability of obesity has been reported to be between 0.7 and 0.8, which is higher than that for most other traits (35). That there is a substantial genetic contribution to obesity is also supported by adoption and familial aggregation studies (36, 37). However, while some fraction of obesity can be attributed to the aforementioned Mendelian defects as well as to variation in genes identified in GWAS, such as *FTO*, there is reason to expect that many new genes remain to be discovered (38). The use of high-throughput genomic sequencing to look for variation in patients with extreme phenotypes is likely to lead to the identification of new genes (39). It will be of particular interest to learn whether these new genes also function in the neural circuit that is modulated by leptin. It is thus quite likely that future reviews on leptin will need to take into account additional, as-yet unidentified components of the neural circuit that regulates food intake.

Defects in leptin signaling

Leptin-deficient *ob* mice show abnormalities in most, perhaps all, physiologic systems (7). Thus, these animals show defects in the entire neuroendocrine axis and are infertile or subfertile and euthyroid sick, with markedly increased corticosterone levels. In addition to these global effects on the neuroendocrine axis, *ob* mice are hypothermic and diabetic and have shown immune and hematologic dysfunction. Indeed, after they were first identified, the complex phenotype of these animals led some to question whether the identification of the *ob* gene would advance our understanding of how food intake and body weight are normally regulated. In retrospect, the complex phenotype of these mice can be most easily understood by noting that the abnormalities they manifest are generally associated not with obesity, but rather with starvation. This prediction was supported by the observation that leptin administration suppresses the neuroendocrine response to fasting in mice (40). These findings and others suggest that a key function of leptin is to communicate information to the brain and other organs that there are adequate fat stores and that the organism is not starving. In the absence of leptin, or with the reduced levels seen after fasting, a set of physiologic responses are elicited, the aggregate effect of which is to reduce energy expenditure, at the same time that appetite is stimulated.

In addition to its intrinsic importance, this aspect of leptin function provides a framework for understanding the efficacy of treating a series of leptin-deficient states in humans. In each case, leptin treatment improves one or more abnormalities generally associated with starvation. Lipodystrophy, the complete or partial absence of fat, is a heterogeneous disorder associated with leptin deficiency, a severe, sometimes intractable insulin resistance, and diabetes, as well as hyperlipidemia and nonalcoholic steatohepatitis (NASH) (41–43). Leptin is currently an approved therapy for generalized lipodystrophy and has also shown potential for the treatment of patients with partial lipodystrophy and severe metabolic abnormalities (though, at present, it is not approved for this indication) (44). Recent evidence has also suggested that lipodystrophy is a spectrum disorder and that there may be extensive overlap between a polygenic form(s) of lipodystrophy and patients previously described as being normal weight metabolically obese (NWMO) (45–47). This syndrome appears to contribute to the abnormalities in a nonobese subgroup of as many as 15% of patients with insulin resistance.

Similarly, the leanness of young women who exercise often with great avidity is also associated with relative leptin deficiency and hypothalamic amenorrhea (HA). This condition is characterized by a failure to menstruate, infertility, and premature osteoporosis. Leptin replacement therapy improves the reproductive abnormalities associated with HA and even causes a significant improvement of bone mineral density in patients with HA (48, 49). Leptin confers these beneficial effects, despite causing weight loss in treated patients (48). Similarly, patients with leptin mutations also show extreme weight loss after leptin therapy, while also showing improvements in the same abnor-

malities that are typically associated with starvation. Thus leptin-deficient patients show marked improvements in their metabolic profile, a restoration of fertility, and improvements in immune function with leptin treatment, despite losing weight. In aggregate, these data strongly suggest that organismal sensing of the overall nutritional state (i.e., adipose tissue mass) is conveyed by leptin and not by the actual amount of fat stored in adipose tissue.

In general, the more extreme the abnormalities of patients with low leptin levels, the more significant their clinical response to leptin therapy. This raises the possibility that leptin might have potential as a treatment for other pathologies that develop in settings of leptin deficiency. For example, one female patient with leptin deficiency failed to enter puberty in adolescence, even though her bone age indicated that she should have, and leptin treatment led to the onset of menses (Sadaf Farooqi, personal communication). This suggests that leptin might be used to induce puberty in very lean young women with a delayed onset of puberty. Both leptin-deficient and starved individuals show immune abnormalities, with a shift from Th1 to Th2 immunity and an increased susceptibility to infectious disease (50). Here again, leptin treatment of leptin-deficient humans and starved animals reverses these changes. Thus, it is possible that leptin could be used as an immune adjuvant in settings of extreme cachexia such as starvation, cancer, or chronic inflammatory disease. It has even been proposed that leptin might be useful in patients with end-stage anorexia nervosa, with the hope that low-dose leptin treatment could ameliorate some of the pathology associated with leptin deficiency, without significantly reducing food intake (further), and/or as an adjunct to parenteral nutrition (C. Montzoros, personal communication).

Leptin might also be of benefit for patients who do not manifest signs or symptoms of pathologic deficiency of leptin (i.e., starvation) but who nonetheless are leptin sensitive (this would be in contrast to most obese patients, who are leptin resistant; see below). Prior studies in animals have shown that leptin stimulates glucose metabolism in WT mice independently of weight loss and that it can improve the diabetes of lipodystrophic mice independently of insulin (51–53). This raised the possibility that leptin might show efficacy for the treatment of type 1 diabetes. This possibility has now been tested in streptozotocin-treated mice and rats that are either partially or completely insulin deficient. In both cases, leptin markedly lowered blood glucose levels. Indeed, in one study, untreated animals all died within one month, while treated animals survived as long as leptin continued to be expressed from an adenoviral vector (54). Further evidence has suggested that leptin elicits its antidiabetic effects by inhibiting glucagon. This has raised the possibility that leptin might also be of benefit for patients with type 1 diabetes, who often present with weight loss and hyperphagia as a consequence of complete or partial insulin deficiency. In this setting, leptin could either alleviate the demands on β cells at the onset of the disease to extend the "honeymoon" period, and/or be used to supplement insulin at later stages of the disease as a means for smoothing glucose control with less hyperglycemia. Leptin therapy might also minimize the weight gain that is associated with increased doses of insulin. Further studies will be necessary to evaluate these possibilities.

Physiologic increases in plasma leptin levels in WT mice lead to a dose-dependent reduction of food intake and loss of weight (55). While leptin has potent effects on reducing food intake and body weight in *ob* and WT animals, its efficacy in obese animals is variable and reduced (55). Animals with mutations in the LepR fail to respond at all to leptin treatment, as do Ay mice that have a defect in melanocortin signaling (55). Diet-induced animals show only a small response, while New Zealand obese (NZO) mice, a strain that develops a polygenic form of obesity, fail to respond to leptin delivered peripherally but lose significant amounts of weight when leptin is delivered i.c.v. Each of these strains has high plasma levels of leptin, suggesting that they are leptin resistant. The most extreme case of leptin resistance is seen in the *db* mouse, which has a mutation in the LepR (21, 22). In the absence of leptin action, these animals become obese and, secondarily, overproduce the hormone (56). Thus, obesity satisfies the hallmarks of a hormone resistance syndrome, with an attenuated response to exogenously administered hormone and elevated endogenous levels. In addition, mutations in the genes *PTP1B* and *SOCS3* in the leptin signal transduction pathway increase leptin signaling and lead to a resistance to obesity, identifying biochemical mechanisms that potentially contribute to leptin resistance (57, 58). However, leptin resistance is complex and can develop at many points in the neural circuit that regulates feeding. Thus, leptin resistance can also develop downstream of leptin target neurons, as in Ay and MC4R-knockout mice, in which melanocortin signaling from POMC neurons is abrogated (23). As with other hormones, leptin resistance can also develop in response to chronically elevated hormone levels via tachyphylaxis (59). Finally, as mentioned above, in NZO mice, leptin resistance can develop because of impaired leptin transport, although little is known about the transcytotic mechanism (60).

In humans, leptin is highly potent in patients with low endogenous levels, though its effects in otherwise normal lean patients have never been comprehensively studied (41–43, 48). In contrast, leptin has variable effects as a monotherapy for obesity in the general population (61). Initial studies showed encouraging effects at very high doses (0.3 mg/kg b.i.d.), but this dose was too high for general usage, and a lower dose (0.1 mg/kg b.i.d.) did not show efficacy. However, a more recent study treating obese patients with an even lower dose (0.05 mg/kg) led to a weight loss of approximately 5%, with efficacy equivalent to that of other pharmacotherapies for obesity (62). It is thus possible that higher doses of leptin led to tachyphylaxis and that a larger study of patients treated with leptin at 0.05 mg/kg or lower could replicate the weight loss observed in the earlier study. There is also evidence that some obese patients show a greater response to leptin than do others. In light of the potency of leptin in patients with low endogenous leptin levels, it is possible that the one could enrich for a responder subset by selecting obese patients with low leptin levels. Indeed, while leptin levels are highly correlated with adipose tissue mass ($r = 0.9$), plasma leptin can still vary by tenfold or more among patients of the same BMI (56). Furthermore, mice with low lev-

els of leptin are obese and remain leptin sensitive, and patients with heterozygous leptin mutations are obese, with low leptin levels (63, 64).

The efficacy of leptin for the treatment of obesity has been augmented by combining it with other agents that cause weight loss, in particular, short-term signals including intestinal peptides that modulate meal pattern. For example, both leptin (0.05 mg/kg) and amylin (pramlintide), a pancreatic peptide that is approved for the treatment of diabetes, each caused weight loss of approximately 5% in a selected group of patients. However, when the two agents were combined, a synergistic effect was observed, with an average weight loss of 13%, which is better than the stipulated efficacy for an anti-obesity agent (62). Studies in animals also showed that pretreatment of diet-induced obese animals with amylin restored the ability of leptin to phosphorylate Stat3 in the hypothalamus, suggesting that this gut peptide might reduce the activity of neural circuits that promote leptin resistance (62). The efficacy of leptin in animals has also been augmented in combination with other peptides or hormones, raising the possibility that, in time, it could emerge as part of a combination therapy for obesity (65). Bariatric surgery is an alternative means of inducing weight loss, and, while invasive, this procedure can be extremely effective. Leptin levels fall after this procedure, in proportion to the amount of the weight loss, and it is also possible that leptin treatment in this setting could reduce recidivism and/or mitigate some of the sequelae of the procedure that are secondary to the relative leptin deficiency that develops (66).

Questions for the future

While much has been learned, the road ahead is likely to lead to new advances and some surprises. Still, some key questions remain unanswered including the aforementioned.

How is leptin transported into the CNS?

What regulates leptin gene expression? Leptin gene expression is correlated with the intracellular lipid content of adipocytes, suggesting that its regulation might be coupled to a lipid-sensing mechanism (56, 67). The nature of this putative lipid-sensing mechanism is unknown.

How does leptin control metabolism? What is the role of leptin signaling at peripheral tissues, or are most or all of its effects mediated by the CNS? What are the physiologic and cellular mechanisms by which leptin reduces adipose deposits in fat and other tissues? What are the physiologic and cellular mechanisms by which leptin improves glucose metabolism? Recently, our group has provided direct evidence that leptin reduces fat mass by activating sympathetic efferents to adipose tissue, though the elements of the CNS circuits regulating this have not been fully elucidated (68). However, the cellular mechanisms responsible for leptin metabolic effects on fat, liver, and other tissue have not yet been fully elucidated.

What is the physiologic mechanism(s) that causes leptin resistance? Are there blocks to leptin signaling in the neural circuit that regulates feeding that can be treated by modulating the activity of neurons downstream of leptin? Is there defective transit across the blood-brain barrier? Is it a result of sustained hyperleptinemia (59)?

Finally, how does leptin modulate a complex motivational behavior? Leptin acts directly on a number of CNS sites to reduce food intake and body weight in animals and humans and provides an entry point to study the control of feeding (26). Feeding is a complex motivational behavior controlled by many inputs including smell, taste, hormonal state, and cognition. Recently, it has been shown that leptin acts in part by regulating hedonic circuitry, but the anatomic site(s) responsible for initiating feeding behavior are unknown (30). Thus, it is not known how or even where the multiple relevant inputs are processed to formulate a "binary" decision. So, perhaps the biggest question is, how do we decide to eat or not eat? The answer to this timeless question may very well be part of the focus of future studies of leptin biology.

Summary: what have we learned?

(a) The cloning of the *ob* gene and identification of leptin have uncovered a new endocrine system that regulates food intake and body weight. (b) This system provides a means by which changes in nutritional state regulate (all) other physiologic systems. (c) Leptin mutations cause a severe obesity that can be successfully treated with the recombinant protein. (d) Leptin reduces food intake by decreasing the reward value of nutrients. (e) There are several other leptin deficiency syndromes that are treatable with leptin replacement. This includes lipodystrophy, an underdiagnosed cause of diabetes. In HA, leptin can restore fertility and improve osteoporosis. Leptin can also improve the immune abnormalities of starvation. (f) A substantial fraction of morbid obesity is the result of Mendelian defects in the neural circuit that is modulated by leptin. (g) A subset of obese individuals lose weight with leptin monotherapy. The majority are leptin resistant, but some patients respond to a leptin-amylin combination. (h) Finally, the identification of leptin and the neural circuit that controls feeding provide a framework for studying obesity and an alternative explanation to the notion that obesity is simply a lack of willpower that can be treated by advising patients to eat less and exercise more.

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