

OBESITY SYSTEMS

Seeking satiety: From signals to solutions

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Remedies for the treatment of obesity date to Hippocrates, when patients with obesity were directed to “reduce food and avoid drinking to fullness” and begin “running during the night.” Similar recommendations have been repeated ever since, despite the fact that they are largely ineffective. Recently, highly effective therapeutics were developed that may soon enable physicians to manage body weight in patients with obesity in a manner similar to the way that blood pressure is controlled in patients with hypertension. These medicines have grown out of a revolution in our understanding of the molecular and neural control of appetite and body weight, reviewed here.

INTRODUCTION

The last decade has ushered in a transformation in the medical treatment of obesity. The identification of key hormonal signals, in particular gut and adipocyte hormones acting in the brain, is transforming the management of this and other disorders. Substantial pharmacologic weight reduction of up to 25% is already achievable, and recent findings suggest that efficacy approaching that of bariatric surgery may soon be possible (1). These advances have built on a deeper understanding of the genetic and neural mechanisms that control appetite and have profound implications not only for public health but also for society as a whole, given that obesity is associated with a set of highly prevalent conditions collectively known as metabolic syndrome, which includes diabetes, heart disease, and hypertension (2). Even modest weight loss of 5 to 10% of body weight ameliorates the severity of these comorbidities, but historically, diets or other nonsurgical interventions have been largely ineffective for achieving even this limited weight loss over the long term (3). Because the metabolic syndrome is the most prevalent cause of morbidity and mortality in the developed world and its incidence is increasing in the developing world, recent advances in treating obesity are likely to have a major public health impact. In addition, the efficacy of these treatments, together with compelling genetic evidence, further establishes that obesity is, at its root, a biologic problem. Obesity is arguably the most stigmatized condition in the developed world, and, as has been the case for other stigmatized disorders, these new treatments should also (hopefully) lessen this stigma (4).

The assimilation, storage, and disposition of nutrient energy are controlled by a complex homeostatic system central to the survival of all living organisms (5). The function of this system depends on the sensing of an array of signals that reflect the nutritional status of an organism and include interacting short- and long-term systems. The role of these systems was captured in *Don Quixote*, where Cervantes wrote, “Hunger is the best sauce in the world.” Cervantes had intuited that the pleasure we derive from food and the amount of it that we consume are controlled by internal signals that reflect our nutritional state. For example, in one study, individuals who had lost 10% of their body weight reported greater satisfaction after

consuming 50 grams of sucrose compared with that they experienced when they were at their stable weight (6). In another study, administration of the adipocyte hormone leptin altered neural activity in brain reward centers (7). These studies directly show that signals reporting an individual’s nutritional state influence the subjective response to a nutrient.

In 1969, Hervey formally postulated that appetite is regulated by feedback mechanisms in which control centers in the brain are regulated by a variety of signals from the gastrointestinal tract, adipose tissue, and elsewhere (8). Signals reflecting the aggregate size of fat stores are especially important because in vertebrates, particularly among land-dwelling mammalian species, the ability to store large quantities of energy-dense fuel in the form of adipose tissue triglycerides permits survival during periods of food deprivation (9). Adequate fat stores are also necessary to maintain reproductive capacity, particularly in females. However, excess adipose tissue also carries a greater risk of predation and also decreases the dissipation of heat, which is necessary for sustained physical activity such as hunting (10). Consistent with this, the weight gain that was observed in the classic Vermont Prisoner study after prisoners were induced to increase their food consumption was soon followed by a rapid return to their starting weight (11). This observation plus the high failure rate of dieting suggest that biologic factors resist weight change in either direction, leading to the conclusion that the amount of fat stored in adipose tissue is under intense selective pressure. Thus, different populations will develop different amounts of adiposity depending on the prevailing risks (starvation versus predation) in a given environment. Overall, the selective pressures associated with excessive leanness or adiposity have led to the evolution of interacting short- and long-term physiologic systems that maintain homeostatic control of short-term energy intake and the size of fuel stores over different time scales (5, 9). In this complex and indispensable system, short-term signals from the gut and long-term signals from adipose tissue interact to regulate food intake and maintain homeostatic control of fat mass while maintaining adequate amounts of circulating nutrients.

INTERACTING SHORT- AND LONG-TERM SYSTEMS REGULATE FOOD INTAKE AND BODY WEIGHT

Both short- and long-term body weight regulatory systems operate via feedback mechanisms in which changes in hormonal and metabolic signals elicit responses in specific brain circuits that regulate

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ingestive behavior and bioenergetics to maintain energy homeostasis. Overall, these highly integrated systems are composed of afferent signals, integratory centers in the brain, and efferent pathways that control behavior and metabolism and, in aggregate, serve a vital evolutionary function by ensuring that appropriate numbers of calories are consumed, used, and stored.

The short-term system is composed of a multitude of hormonal, metabolic, and neural signals that encode and relay information reflecting recent food intake (12). These signals ensure that there are adequate circulating nutrients over the course of a day while also preventing excessive distention of the gastrointestinal tract or ingestion of noxious agents. Sensing of the caloric content of food was first shown in animal studies that measured the amount of food that was consumed as its nutrient content was diluted. The data showed that animals regulated the number of calories consumed, not its volume, indicating that there are molecular signals that reflect the nutritional value of food (2). In addition to regulating the concentrations of insulin and other hormones, these nutrients in the gut lumen stimulate enteroendocrine cells to secrete peptide hormones, including gastric inhibitory peptide (GIP), glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), and peptide Y (PYY), that can regulate ingestion by modulating the activity of hunger- and satiety-controlling neurons. In addition, distention of the stomach activates vagal afferent nerves that stimulate specific populations of brain stem neurons to reduce feeding and prevent overconsumption. These circuits, which are primarily centered in the brain stem and other subcortical sites, are part of an alert system that is activated by excess food consumption or noxious stimuli, resulting in nausea and reduced food intake (13). These circuits also modulate reward (14). Modulation of these pathways is thus associated with sensations with which we are all intimately familiar: hunger, pleasure, satiation, fullness, nausea, or even vomiting. However, although physiologic amounts of these short-term signals transiently regulate food intake, the endogenous peptides have only limited effects on body weight. Altered function of the short-term system also contributes to the weight-reducing effect of bariatric surgery (15).

The most recent generation of obesity therapeutics are peptide derivatives of these short-term hormonal signals. The first of these obesity treatments followed the development of GLP-1 agonists as agents that stimulate insulin secretion with potent antidiabetic effects in humans. Although its effects on glucose metabolism are physiologic, further modifications to GLP-1 that extended its half-life were found to result in a substantial reduction of food intake and body weight in animals and humans (16, 17). Semaglutide, an optimized derivative of GLP-1, has shown substantial efficacy for the treatment of obesity, and newer molecules with agonist activity at multiple receptors are even more potent, as discussed below (18). Despite its efficacy in many patients, the therapeutic response to semaglutide is variable, and tolerability is sometimes an issue. However, newer agents that activate multiple gut hormone receptors, such as tirzepatide or retatrutide, may offer superior efficacy with comparable or improved tolerability. Although these agents have been shown to be safe, the long-term treatment response is not known, and based on recent evidence showing recidivism in many patients after bariatric surgery (19), prolonged treatment with these gut hormone-based treatments may ultimately result in at least partial weight regain because of activation of the long-term system. This suggests a possible future need for additional,

complementary approaches for the treatment of obesity, potentially using leptin and melanocortin agonists or other agents to modulate the long-term system to induce or maintain weight loss and prevent recidivism.

This long-term system maintains homeostatic control of adipose tissue mass by setting the gain on the short-term system and by regulating energy expenditure, peripheral metabolism, and other physiologic processes (20, 21). Thus, interoceptive signals that reflect the overall energy state of the organism (that is, reflecting increased or decreased fat mass) modulate feelings of hunger or satiety as well as food preference. In addition, the short- and long-term systems are highly integrated. It has been shown that brainstem neurons in the nucleus tractus solitarius (NTS) can also modulate the activity of Agouti-related protein (AGRP) and proopiomelanocortin (POMC) neurons in the arcuate nucleus, key elements of the long term system and targets for GLP-1RAs (22, 23). Mutations in the components of the long-term system, including leptin, the leptin receptor, and melanocortins, can cause obesity, and Mendelian defects in these genes account for a substantial portion of class 3 obesity in humans (24). Although short-term signals can modulate the long-term system (25), mutations in the components of the short-term system do not substantially alter body weight. The discovery of the components of this long-term system has also led to the development of treatments for additional diseases associated with nutritional alterations, including lipodystrophy, hypothalamic amenorrhea (HA), and potentially others (9).

There is often a lag between basic science discoveries and their translational applications. Advances over the last several decades have identified many of the specific molecular mechanisms that underlie these short- and long-term systems (Fig. 1). As mentioned, both systems are in large part composed of peripheral hormonal signals that act on brain pathways that regulate appetite. Whereas most of the short-term signals were identified as part of a search for peripheral agents that regulate insulin secretion or satiety, nearly all of the long-term system components were identified in genetic studies of severe obesity in mice and humans (24). These latter studies have revealed that class 3 obesity has substantial genetic loading and is largely a central nervous system (CNS) disorder. This extraordinary progress in defining obesity-related signals and brain circuits has laid the foundation for new therapeutic approaches such as dual- and triple-gut hormone receptor agonists. Below, we review these basic scientific advances and their therapeutic and broader implications.

THE SHORT-TERM SYSTEM

The absorption of food by the digestive system is an essential component of energy homeostasis. In addition to the coordinated increase in the size of the brain and gastrointestinal tract during mammalian evolution, these two organs also developed an integrated bi-directional inter-organ signaling system (26). The communication between the gastrointestinal tract and CNS circuits regulating appetite and energy homeostasis has been subject to intense research efforts for more than a century. Over this time, a framework has emerged in which chemical (or nutrient) and mechanical stimuli in the gastrointestinal tract induce neuronal and endocrine signals that are integrated and processed by neural centers along with other relevant exteroceptive (environmental) and interoceptive (internal) signals such as leptin and ghrelin. These brain centers in

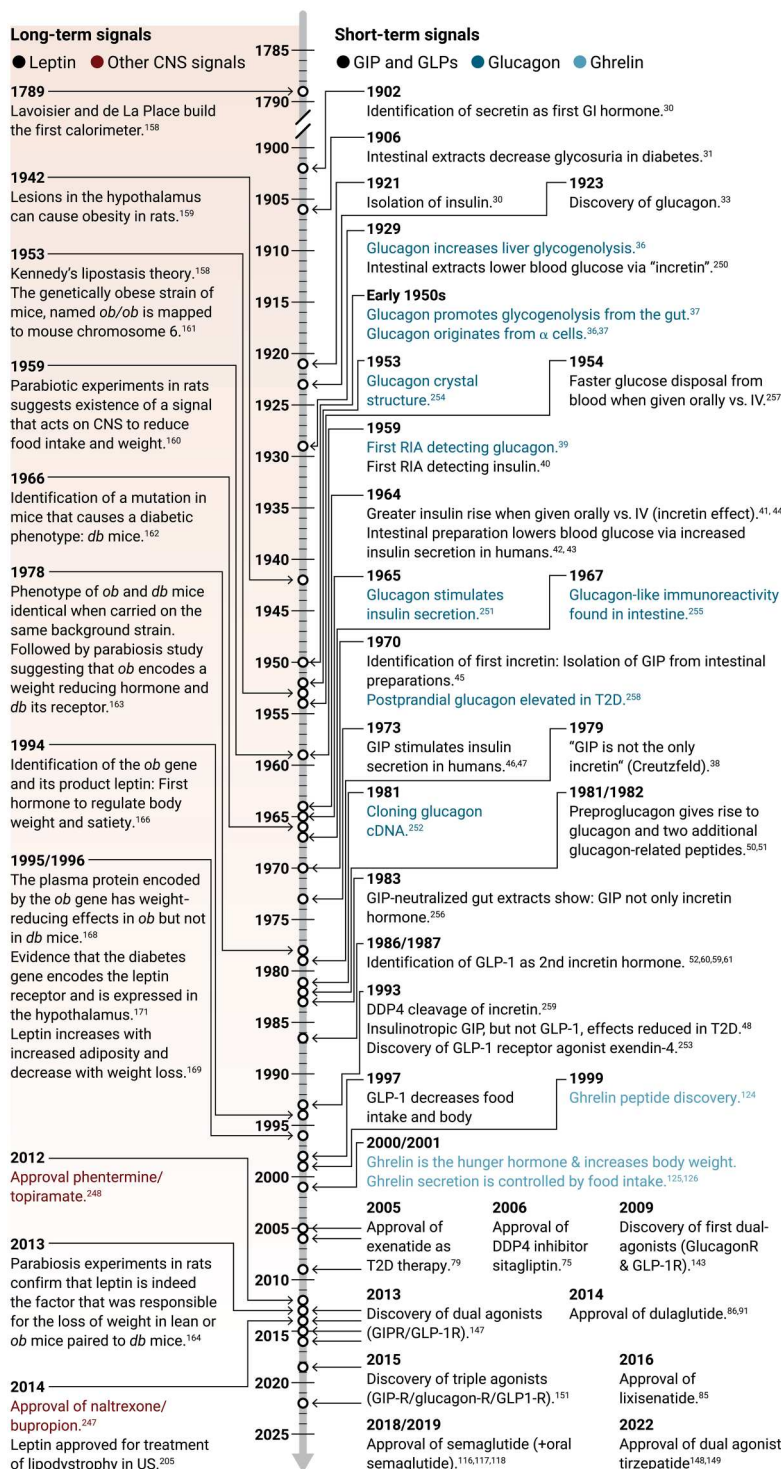


Fig. 1. Timeline of advances leading to the identification of short- and long-term satiety signals and their therapeutic applications. GI, gastrointestinal; IV, intravenous; RIA, radioimmunoassay; ob, obese; db, diabetic; R, receptor.

turn generate “outgoing” signals that adjust food-seeking and consummatory behaviors to maintain homeostasis (27). The elucidation of this framework has enabled and accelerated the identification of numerous drug targets for the treatment of diabetes and obesity, leading to the development of a growing number of therapeutics (28). The pharmacology that has emerged from the elucidation of the molecular mechanisms underlying gut-brain communication suggests that the efficacy of these medical treatments may in time match or even exceed the metabolic benefit achieved by bariatric surgery (29). The origin of this burgeoning field dates to the identification of the first peptide hormone.

Origins and the incretin concept

More than 120 years ago, Bayliss and Starling discovered a factor produced by the duodenum that was released in response to acid and that increased pancreatic secretion of bicarbonate and suppressed acid production by the stomach. Because the factor stimulated secretion from the pancreas, they named it secretin and referred to this class of signaling molecules as a “hormone,” derived from the Greek root *ormao* for “to arouse or excite” (30). This was followed by other studies using parabiosis (cross-circulation studies) connecting the pancreatic and the jugular veins in normal and pancreatectomized dogs to show that a circulating intestinal signal could lower blood glucose (31–33). This (at the time) unknown factor was referred to as an incretin. At the time, the importance of this discovery was overshadowed by the development of insulin, then thought to provide a cure for diabetes (34). One year after the discovery of insulin, glucagon, another pancreatic hormone, was isolated by Kimball and Murlin (35). Glucagon was found as a contaminant in some insulin preparations that caused transient hyperglycemia, and it was later shown to increase hepatic glycogenolysis (36, 37). In 1950, glucagon was shown to be produced by pancreatic α cells (38), which, alongside the discovery of insulin, focused further attention on the pancreas. Thus, the potential role of incretin(s) to also regulate blood glucose remained dormant until decades later (39).

The discovery of incretin molecules was set in motion when bioassays for factors inducing glycogenolysis revealed that glucagon was produced not only by the pancreas but also by intestinal cells (37). The presence of glucagon immunoreactivity in the intestine was later confirmed using a specific radioimmunoassay (40). Another key finding, made possible by the first radioimmunoassays for insulin (41), was the observation that orally delivered glucose increased insulin concentrations to a greater extent than did intravenously administered glucose (42). These results invigorated the search for incretin(s) because they suggested that a circulating substance was released from the gastrointestinal tract to stimulate pancreatic insulin release. The definition of an incretin set forth by Creutzfeld was a factor that is both released from gut in response to nutrients, especially carbohydrates, and that stimulates insulin secretion in the presence of glucose, referred to as glucose-stimulated insulin secretion (39, 43–45). The first incretin was initially identified by serendipity in 1970, when Brown *et al.* (46) identified a gut hormone, originally named enterogastrone, later renamed GIP, that inhibited gastric acid production. However, interest waned when the doses of GIP required for the inhibition of acid secretion were found to be supraphysiologic. The importance of this hormone was only later appreciated when, in empiric screens for intestinal factors regulating glucose, GIP was shown to potently stimulate insulin

secretion in human and animals (47, 48). This generated intense interest in its potential as a therapeutic that unfortunately never materialized, in part because a GIP insulin stimulatory effect is not evident in patients with diabetes (49). However, recent evidence has suggested that GIP effects may contribute to the greatly enhanced efficacy of the latest anti-obesity therapeutics (50).

A decade later, Habener and colleagues (51) cloned a glucagon cDNA from anglerfish, a species in which the islets of Langerhans are anatomically distinct from the exocrine pancreas. Its sequence predicted two homologous proteins derived from the same precursor protein that they suggested was cleaved to generate glucagon and a 39-amino acid peptide initially named GLP (52). It was renamed GLP-1 because subsequent cloning of the mammalian glucagon precursor identified GLP-1 and a second peptide, GLP-2, that was later shown to regulate intestinal growth (53–55).

Chemistry matters: Discovery of bioactive GLP-1

Prior work from Creutzfeld had suggested that GIP could not account for the complete effect of an oral glucose load to stimulate insulin secretion, and shortly after the identification of GLP-1, he tested the effect of the entire 37-amino acid GLP-1 peptide on rat pancreatic islets (56). He observed only a small effect that proved difficult to reproduce, but his pioneering experiments suggested that a GLP-1-derived peptide might be the missing incretin. The basis for the limited effect Creutzfeld had observed became evident in 1986 when, in collaboration with Habener, peptide chemist Mojsov synthesized and tested several different peptides predicted by the pre-proglucagon cDNA sequence and defined the molecular forms present in intestine *in vivo*. Mojsov and Merrifield (57) had worked previously with Merrifield to synthesize the glucagon peptide. These peptides were first used to generate a set of antisera that enabled the development of a set of radioimmunoassays suitable for characterizing the molecular forms of GLP-1 after a series of chromatographic separations. Immunoreactive fractions of GLP-1 were isolated after gel filtration followed by reverse phase and then ion exchange chromatography. In a 1986 publication, these studies revealed that the predominant molecular form in the intestine is a truncated 30-amino acid peptide, GLP-1 (7-37), rather than GLP-1 (1-37) (53, 58). These same molecular forms were also seen by Drucker *et al.* (59) after transfection of a glucagon cDNA into pituitary and pancreas cell lines after a characterization of the immunoreactive material using the same analytical approach used to characterize the peptide in intestinal extracts. In 1987, Holst and colleagues confirmed that GLP-1 7-37 was present in pig intestine but initially referred to it as GLP-1 78-107 on the basis of the amino acid sequence of the precursor protein (60). This 1987 report also showed that GLP-1 (7-37) potently stimulated insulin secretion from the pig pancreas, and another report published at the same time by Mojsov *et al.* (61) showed potent insulinotropic effects of the peptide in perfused rat pancreas. The amount of GLP-1 7-37 required for stimulation of insulin secretion was in the same range as the circulating plasma concentration, making GLP-1 7-37 approximately 100 times more potent than GIP. In another publication, Drucker *et al.* (62) showed that GLP-1 (7-37) stimulated cyclic adenosine 3',5'-monophosphate and insulin release and increased insulin RNA abundance in an insulinoma cell line. The paper also stated that the effect was diminished in the presence of 5.5 mM versus 25 mM glucose. Subsequently, Weir confirmed that the insulinotropic effect was dependent on the glucose

concentration, although, in this case, a large response was seen in the presence of 16.7 mM glucose, but an insulinotropic response was still seen with 6.6 mM glucose, with no effect observed at a 2.8 mM. Overall, these data satisfied all of the criteria for an incretin initially set forth by Creutzfeld (39, 63).

Endogenous GLP-1 7-37 is produced and secreted by enteroendocrine L cells in the gut, predominantly in the distal ileum and colon, and by K cells in the stomach (64). Secretion is stimulated by ingested nutrients such as glucose and other carbohydrates and, to some extent, dietary lipids (65). GLP-1 acts on GLP-1 receptors (GLP-1Rs), G protein-coupled receptors that are a member of the B class, which are predominantly expressed in the pancreas, peripheral nerves, and brain, as well as in the cardiovascular system, stomach, kidney, and adipose tissue (66, 67). Tissue-specific GLP-1R knockouts suggest that its insulinotropic effect is mediated both via direct effects on pancreatic β cells and by stimulation of local vagal and spinal afferents in intestine (68). Animal studies also showed transiently reduced food intake after GLP-1 7-37 administration in rats on a normal diet. However, whereas mice with a genetic deletion of GLP-1R show abnormal glucose tolerance and decreased insulin secretion, their food intake and body weight are normal (69–71). This short-term feeding effect of endogenous GLP-1 is mediated, at least in part, by local stimulation of vagal afferents in distal intestine after it is released from enteroendocrine cells (72). In subsequent clinical studies, acute injections stimulated insulin production and transiently reduced food intake (73).

These studies thus established that GLP-1 is an incretin and further suggested that it might have potential as an antidiabetic agent in humans. Consistent with this, Nathan working with Mojsov and Habener conducted clinical studies using GLP-1 synthesized by Mojsov and Merrifield and showed insulinotropic effects of an infusion of GLP-1 7-37 in human (74). Importantly, Nauck *et al.* (49) also showed that in contrast to GIP, the incretin effect of GLP-1 is maintained in patients with diabetes. However, its therapeutic effect was limited by its extremely rapid turnover, with a normal half-life between 1.5 and 5 min, thus requiring an infusion to elicit a meaningful response. Although the peptide is normally amidated, it is nonetheless rapidly degraded by dipeptidyl peptidase 4 (DPP4), a broadly expressed integral membrane protein that also circulates in plasma after shedding from the cell surface. DPP4 is an amidation-insensitive dipeptidase that cleaves active GLP 7-37 to inactive GLP 9-37 (75, 76). Thus, although the intestinally secreted peptide is present in sufficient amounts to act directly on the pancreas via the splanchnic circulation and also stimulates local vagal afferents, it does not appear to circulate in sufficient amounts to act on CNS receptors to suppress food intake for sustained periods (16, 77).

Step by step: From physiology to pharmacology

The short half-life of endogenous GLP-1 limited the therapeutic potential of the native peptide as an antidiabetic agent until Eng studied the effects of a GLP-1 analog isolated from the Gila monster salivary gland. Eng was intrigued by prior observations that Gila monster venom can enlarge the size of the pancreas (78) and purified the active agent as a molecule he named exendin-4. Exendin-4 had extensive sequence homology with GLP-1 and had similar insulinotropic effects on pancreatic β cells. However, this Gila monster peptide is resistant to DPP-4 and has a half-life of hours rather than minutes. The stability of this molecule enabled

clinical studies to evaluate its antidiabetic effect, and in a series of clinical trials (79–81), exendin-4 showed substantial efficacy in patients with type 2 diabetes, with lowering of HbA1c even among those with poor glucose control. Exenatide has a half-life of 2.4 hours and is administered twice daily, but its half-life was further extended by delivering it in a microsphere formulation. This slow-release form of exenatide (82) could be administered once weekly (83). This more stable form showed even greater efficacy, with highly significant reductions of HbA1c and fasting blood glucose (84). These early clinical data suggested that the efficacy of GLP-1 agonists is strongly correlated with their stability, a conclusion that has been borne out in numerous subsequent studies. Slow-release exenatide also led to a small but significant weight loss of ~3 kg versus half that in a placebo group.

These initial findings and similar results from different GLP-1R agonists eventually led to the development of even more stable forms with improved glucose control in patients with diabetes. Liraglutide, another stable form of GLP-1 7-37, was developed by scientists at Novo Nordisk. Liraglutide is a derivative of GLP-1 7-37 in which a C16 fatty acid is coupled to a lysine at position 26 via a γ -glutamyl linker that leads to albumin binding, and lysine 34 is additionally mutated to arginine to prevent fatty acid coupling at this site. These modifications stabilize the peptide and decrease degradation by DPP4 (85). The half-life of liraglutide is 11 to 15 hours and, similar to extended release exenatide, shows greater efficacy than exenatide, with an average HbA1c reduction of 1.1 to 1.6 with a slightly greater benefit than that achieved with slow release exenatide. Since the introduction of exenatide and liraglutide, several other GLP-1R agonists have been introduced, including lixisenatide (86) and dulaglutide (87), which all show similar glucose control efficacy. Dulaglutide achieves substantially higher bioavailability and lower renal clearance than native GLP-1 as a result of a fusion to a human immunoglobulin G Fc fragment. Additional chemical modifications protect it from protease-induced inactivation and result in similar clinical benefit (88). In addition to improved glucose control, patients treated with liraglutide also showed weight loss of 5% or greater in 54.3% of patients treated with a 3.0-mg dose (versus 24% receiving placebo), with only 40.4% losing this amount when treated with a lower 1.8-mg dose versus 19.0% treated with placebo (89, 90). In aggregate, these results provided further evidence that increased GLP-1R agonist exposure is associated with small but significant weight loss.

GLP-1 can also be stabilized by inhibiting DPP-4 (76), which led to the development of DPP-4 inhibitors as oral agents for treating diabetes (91–93). Sitagliptin (91, 92) was the first approved drug in this class, and additional versions have since been developed that lower HbA1c by between 0.5 and 1.0%. DPP4 inhibitors generally raise postprandial GLP-1 by ~2- to 3-fold and increase the circulating concentrations of additional substrates, including GIP, PYY, and other circulating peptides. It is unclear to what extent stabilizing these other factors might contribute to the improvements in glycemic control. The safety profile of these agents is favorable, without a significant risk of hypoglycemia, but in contrast to the GLP-1 agonists, the DPP4 inhibitors have no appreciable effect on body weight (64). Although GLP 7-37 can transiently decrease food intake, these results show that increased circulating endogenous GLP-1 in the high physiologic range is insufficient to suppress food intake and reduce body weight and that pharmacologic concentrations would be required to elicit a sustained effect. The lack of an effect of DPP4

inhibitors on weight further reinforced the possibility that higher circulating concentrations of an even more stable GLP-1R agonist would be required to induce clinically significant weight loss.

This possibility was evaluated by using semaglutide, an ultra-stable version of liraglutide, in which a carboxylated fatty acid is coupled to lysine 26 via a polyethylene glycol linker (85). This results in an extension of its half-life to 7 days. It is this agent that ushered in a new era in the medical treatment of obesity because clinical trials with semaglutide resulted in ~8% average weight loss, with 40% of patients losing more than 10% of their weight. An increased half-life of up to 90 to 160 hours in the cases of dulaglutide and semaglutide allowed for weekly instead of daily injections and led to fewer and milder side effects (94). Today, therapy with GLP-1R mono-agonists offers up to 1.9% reduction of HbA1c in diabetes and up to 17% reduction of body weight in patients with obesity, although effects on body weight among individuals who also have diabetes are more modest (up to 7%) (95, 96).

Whereas the antidiabetic effects of liraglutide are predominantly the result of signaling at peripheral sites, including pancreatic β cells and vagal afferents, cell-specific knockouts of GLP-1R and other studies using vagotomy have shown that the durable suppression of food intake is primarily the result of effects on CNS circuits that reduce feeding (16, 97). Although GLP-1 signaling at vagal afferents may contribute to meal termination, expression here is not required for the weight-reducing effects of GLP-1R agonists (98). In contrast, a neuron-specific knockout of GLP-1R abolishes the effect of liraglutide on food intake and body weight while not altering its insulinotropic effect (16). Although effects of GLPRAs on brain-stem and hypothalamus have been reported, their relative contributions, and those of other regions, to the suppression of appetite have not been fully established, with multiple sites potentially contributing to the satiety effect (23, 99, 100).

Within the brain, GLP-1Rs are highly expressed in the hind-brain, lateral septum, hypothalamus, and elsewhere (67). Many of these regions are sites of circumventricular organs, including the area postrema and the median eminence, which lack a blood-brain barrier, allowing neurons in these regions to sense the plasma concentrations of GLP-1 and other circulating signals (101, 102). Consistent with this, an acute injection of semaglutide increases expression of the cFOS activity marker primarily in these brain regions, particularly in the area postrema (100, 103). In rats, knockdown of GLP1R in NTS neurons reduced the effect of liraglutide, and further studies suggested that GABAergic NTS neurons are the target (104). Curiously, knockout of GLP1-R in vGlut2-expressing glutamatergic but not GABAergic neurons in mice eliminates the anorectic and aversive effects of liraglutide (105). The basis for this discrepancy is not clear. Exendin-4 has been shown to reduce food intake via effects on GLP1R-expressing brainstem neurons and to decrease food reward and motivation (99). CCK-expressing neurons in the brainstem have also been reported to help mediate the weight-reducing effects of GLP-1RAs via taste aversion, although these neurons appear to be an indirect target (106). Hindbrain activation of GLP-1R-expressing neurons by exendin-4 has also been shown to reduce food intake via effects on protein kinase A and mitogen-activated protein kinase signaling, and an effect of exendin-4 on feeding is still evident in decerebrate animals, further suggesting that the brainstem mediates some of its anorectic effects (107, 108). Mice with knockout of GLP-1R in the hypothalamus, where it is normally expressed in POMC

neurons of the arcuate nucleus, show diminished weight gain on a high-fat diet (109, 110). GLP-1 also increases neural activity at other sites, including neurotensin-expressing neurons in the lateral septum that reduce feeding in response to stress (103, 111). GLP-1 itself is also made in a subpopulation of nucleus tractus solitarius (NTS) brain stem neurons that project broadly, and activation of these neurons also reduces feeding, although these neurons do not appear to be required for GLP-1RAs to suppress food intake (112, 113). However, it is still possible that GLP-1R agonists also activate the targets of these neurons in deeper brain structures.

The brain stem circuits that respond to GLP-1 are part of an alert system that detects nutrient, mechanical, and noxious stimuli to suppress feeding and convey nausea, a side effect that is often reported in patients receiving semaglutide. In animals, GLP-1RAs are associated with conditioned taste aversion, indicative of an aversive effect (114). According to one recent study, 58% of patients reported nausea and 27% reported vomiting (22% versus 11% in participants on placebo, accordingly) (115). Consistent with their induction of nausea and conditioned taste aversion, activation of GLP-1R-expressing neurons is associated with negative valence in behavioral assays as well as anxiety (106, 116). Other than this, these drugs appear generally safe, although weight loss is not limited to adipose tissue and lean mass is also substantially reduced, the long-term consequences of which are not known (117). There are also reports of pancreatitis in a small number of patients, and these drugs are also contraindicated in patients with multiple endocrine neoplasia (118).

More recently, an oral version of semaglutide has become available (119) (120, 121). To overcome limitations as an orally administered peptide, it is designed to protect from stomach acidity while increasing lipophilicity to improve bioavailability. However, the doses required after oral administration are still roughly 50 times higher than those used for subcutaneous injections, need to be administered on an empty stomach before breakfast, and still show limited efficacy (119, 121). There are also ongoing efforts to develop oral GLP-1R agonists that would similarly obviate the need for regular injections (122). A recent clinical study showed that a small-molecule GLP-1R agonist led to highly significant weight loss, albeit with many of the same side effects of the peptide GLP-1R agonists (123).

The expanding universe of gut hormones

Although these initial GLP-1R agonists showed unprecedented efficacy, their ability to induce weight loss in patients with diabetes is lower (~7%), and the overall response is not as great as in patients treated with bariatric surgery (124). Bariatric surgery alters gut-brain communication at numerous levels, including vagal afferents, and is also associated with alterations in circulating GLP-1 and several other short-term endocrine signals (27). Curiously, however, a GLP-1R knockout did not alter the response to bariatric surgery in animals (125). Notably, concentrations of other peptide hormones are also changed by bariatric surgery, including the gastric peptide hormone ghrelin (126). Ghrelin is a 28-amino acid peptide that requires a unique fatty acid side chain to activate its target, known as growth hormone secretagogue receptor 1a (127). It was named gh-relin because it was originally isolated as a ligand for the growth hormone-releasing factor. Shortly thereafter, it was discovered that ghrelin can stimulate appetite and antagonize leptin's effects on anorexigenic neurons in the arcuate nucleus of the

hypothalamus (128). Circulating ghrelin increases with fasting and decreases after nutrient ingestion (129) and bariatric surgery (130), suggesting that blocking this pathway could have activity as an agent for treating obesity. However, a loss of endogenous ghrelin function as a result of either knockout of the ghrelin gene or of GOAT, an octanoate conjugating enzyme required for its activity, led to only relatively small effects on body weight and metabolism (131). Moreover, circulating ghrelin concentrations are already low in human obesity (129), and subsequent studies have suggested that its main function may be to maintain plasma glucose concentrations during fasting (132).

Amylin is a peptide produced by pancreatic β cells that is approved for the treatment of type 1 diabetes (133, 134). Administration of this agent also leads to small but significant weight loss in humans and rodents, and animal studies have shown that it can activate neurons in the brain stem and hypothalamus (135). In addition, it has also been shown to restore leptin sensitivity in diet-induced obese mice and, in combination with leptin, substantially reduced body weight in obese humans (136–138). PYY 1–36 and PYY 3–36 are two other peptides produced by enteroendocrine

cells in the ileum and colon in response to nutrient ingestion and can regulate satiety under certain conditions. PYY (1–36), which activates hypothalamic neuropeptide Y receptors, is cleaved by DPP4 into PYY (3–36), which then potently activates Y2 receptors (139, 140). To date, no PYY-based molecules have been developed into an anti-obesity therapeutic. Another peptide, CCK, is released from the intestine in response to lipids to stimulate gall bladder contraction and also acutely reduces food intake (141). A knockout of the CCK receptor somewhat paradoxically confers resistance to a high-fat diet, and CCK agonists have shown only limited effects in reducing weight (142). However, as was the case for a combination of leptin and amylin, leptin and CCK showed synergistic effects in reducing weight in rodents (143). Similarly, bombesin is produced by stomach and reduces food intake, but its long-term effects are minimal (144). Although these and many other short-term signals can transiently reduce food intake, none of the native peptides have been shown to elicit major weight loss by themselves. However, as described below, engineering the GLP-1 hormone sequence in a manner that stimulates other signaling pathways has

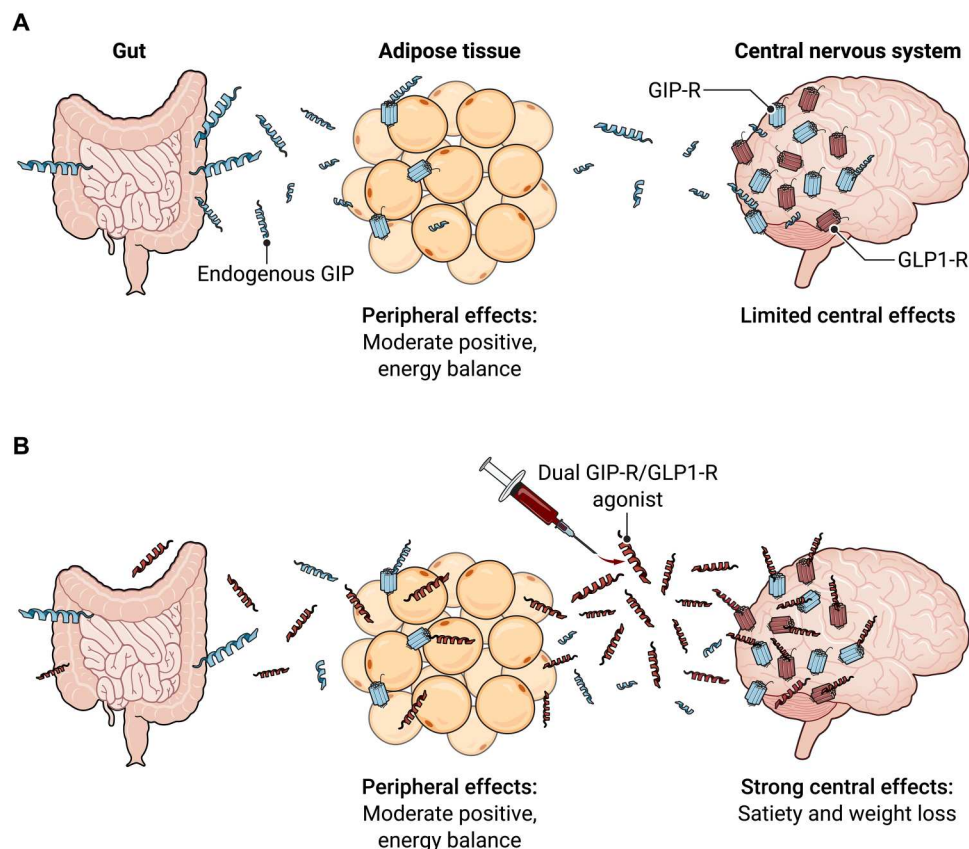


Fig. 2. Targeting the GIP system for obesity therapy. The success of dual agonists combining GIPR agonism with GLP-1R agonism has yet to be mechanistically reconciled with data showing an obese phenotype of mice with genetically deleted GIPRs and recent observations indicating body weight-lowering effects of GIPR antagonism using GIPR antibodies (159). Differences in subpopulations of targeted receptors may offer an explanation. **(A)** Actions of endogenous GIP. Endogenous GIP secreted by the gut has a relatively short half-life and may predominantly reach peripheral organs. Activation of peripheral receptors would be expected to drive a moderately obesogenic phenotype. **(B)** Effects of pharmacological intervention. Dual GIPR/GLP-1R agonists, which have a longer half-life than endogenous GIP, activate GIPRs and GLP-1Rs in the CNS, resulting in strong synergistic effects that promote satiety and lower body weight. These effects likely overwrite direct peripheral effects. GIPR antagonist antibodies, on the other hand, may trigger anti-obesity effects (159) by displacing endogenous GIP from peripheral receptors but are unlikely to reach receptors in the CNS.

been shown to reduce weight by an average of 25%, a response that now approaches bariatric surgery (145).

The discovery of multireceptor drugs

The large and expanding portfolio of gut-brain signals (27) has substantially increased the number of combinatorial possibilities for peptide polygonists. The first such polygonist was a glucagon variant that also activated the GLP-1R and showed enhanced efficacy for weight loss relative to GLP-1 alone while preventing the hyperglycemia associated with glucagon administration (146). As mentioned, glucagon is produced by pancreatic α cells in response to hypoglycemia or excess amino acids and normally leads to hyperglycemia, an undesirable outcome in patients with diabetes. However, the action profile of this hormone also includes lipolysis, thermogenesis, and satiety, which could be beneficial for reducing weight (126). Thus, peptide modifications that would cause coactivation of the glucagon and GLP-1Rs were developed with the aim of buffering glucagon's diabetogenic effects while preserving its other metabolic benefits. The first balanced co-agonist was developed with minimal engineering of the human glucagon peptide sequence, thereby limiting risk for antigenicity. This hybrid peptide combining glucagon and GLP-1R agonism—a dual agonist—was first tested in animals and showed superior metabolic and weight loss benefits compared with GLP-1R mono-agonists (146). Importantly, no detrimental effects on plasma blood glucose or insulin concentrations were observed, suggesting that the buffering function of GLP-1 mitigated the potential hyperglycemic effect of glucagon agonism. Several versions of this class of molecules are now being tested in clinical trials for obesity as well as type 2 diabetes, with promising results thus far (28).

The relative success of this first unimolecular gut hormone therapeutic triggered intensive efforts to identify even more potent unimolecular polygonists capable of activating multiple gut hormone receptors (147). This included a dual agonist that had both GIP receptor (GIPR) and GLP-1R agonist activity. Whereas initial evidence suggested that GIPR agonism alone was not a viable strategy for inducing weight loss (Fig. 2A), GIP/GLP-1R co-agonists with an extended half-life exhibited unprecedented metabolic benefits and body weight loss in preclinical models of obesity (Fig. 2B) (148). This effect initially seemed surprising because the phenotype of global GIPR knockout mice showed protection from both obesity and insulin resistance when fed a high-fat diet. In addition, double-homozygous *Gipr*^{-/-} *ob/ob* mice gained less weight and had lower adiposity than wild-type *ob/ob* mice (149). Despite this, testing of the first GIPR/GLP-1R dual agonist (150) confirmed a synergistic interaction yielding both metabolic benefits and weight loss in rodent and primate models that went well beyond the effects of a GLP-1 mono-agonist. Initial clinical studies confirmed its profound weight-reducing and antidiabetic effects (96), and in 2022, a refined version of this molecule class, tirzepatide, was approved for the treatment of diabetes (87, 151). Tirzepatide is a representative of the GIP/GLP-1R co-agonist class favoring GIP over GLP-1 action in a 5:1 ratio. Clinical studies have shown that tirzepatide induces an average of 21.5% weight loss in humans with obesity, with 15% weight loss in obese patients diagnosed with diabetes (152). Animal studies have also shown that GIP-1R is expressed in inhibitory GABAergic brainstem neurons, raising the possibility that this population inhibits the excitatory GLP-1R-expressing brainstem neurons that convey nausea (153). GIP also suppressed the activity of

brainstem CCK neurons that mediate some of the aversive effects of GLP-1 agonism (106). However, the mechanism of action of dual GIPR/GLP-1R co-agonists versus the unimolecular versions remains to be fully elucidated.

The enhanced efficacy of dual glucagon receptor (glucagon-R)/GLP-1R and GIPR/GLP-1R co-agonists has also led to the synthesis of a unimolecular triple co-agonist with activity at the GIP-R, glucagon-R, and GLP-1R that has activity at all three human gut hormone receptors (154). In preclinical studies, this agent outperformed dual and mono-agonist compounds for all endpoints, showing even greater efficacy on body weight, body fat, food intake, glucose tolerance, hepatosteatosis, and blood lipids in diet-induced obese mice. Although it remains to be seen whether phase 3 clinical trials replicate or even surpass this enhanced efficacy, phase 2 trial data of retatrutide, a GIPR/glucagon-R/GLP-1R triagonist version developed by Eli Lilly and Co, have already shown 24% weight loss after 48 weeks of treatment, without reaching a plateau (155, 156). Extrapolating these data to 18 months raises the possibility that weight loss as great as 30% may be possible. Overall, the aggregate data suggest that engagement of different receptors may simultaneously activate multiple signaling pathways, further increasing efficacy. Single-cell RNA sequencing of brainstem neurons has identified numerous distinct clusters, with different populations expressing receptors for GLP1, GIP, and several other short-term signals, including GDNF family receptor alpha like (GFRAL), the receptor for growth differentiation factor 15 (GDF15), the Casr calcium sensor, with yet other neuronal populations responding to signals associated with infection (13, 157). Thus, simultaneous activation of multiple neural populations may provide even more therapeutic opportunities. The most recent example is a combination of the amylin analog cagrilintide with semaglutide, which showed 17% weight loss in a phase 2 trial. This combination, however, is not achieved with a single-molecule approach but rather with the separate entities being concomitantly injected (158). The explosion of information establishing the identity of individual neural populations may thus provide an opportunity to develop classes of peptide drugs that engage defined subsets of brain stem, hypothalamic, and other neurons, perhaps leading to even more effective agents. The downstream neural targets of these brainstem targets of GLP-1 are still emerging, and their elucidation may in time also yield additional therapeutic opportunities.

Nonetheless, many questions remain. The molecular pathways by which GIP augments the satiety effects of GLP-1 in the CNS are not fully understood, and the glucagon targets in the peripheral or CNSs driving energy expenditure remain to be elucidated. Preclinical and early clinical data originally suggested additive benefits of combining GIPR antagonizing antibodies with GLP-1R agonism (159). This result would seem to be at odds with the clinical success of tirzepatide but may be explainable given that the antibody used to antagonize the receptor may predominantly work in the periphery because it is unlikely to reach CNS GIPRs. In contrast, the different unimolecular dual and triple agonists predominantly act on GIP receptors in the brain (160). It is also unclear whether distinct subpopulations of patients respond differently to these different agents. In addition, the effect of mono, dual, and triple gut hormone receptor co-agonists is lost when the drugs are stopped, so they need to be taken continuously, because rebound weight gain is seen when therapy is stopped. The long-term effect of these agents will thus need to be carefully monitored, including,

as mentioned, possible effects on lean body mass. It is possible that additional medicines tailored for weight maintenance rather than weight loss may be necessary. Because weight loss is associated with lower leptin concentrations that in turn increase appetite, leading to recidivism, studies testing the potential of leptin or other agents to prevent weight regain may be informative. Last, as was the case for GLP-1, lack of a physiologic reduction in weight does not preclude the possibility that improved pharmacological strategies harnessing other gut hormones in alternative ways may reveal untapped therapeutic potential. Thus, peptides that have agonist activity for other hormone receptors such as PYY, CCK, or amylin, alone or potentially even combined with long-term signals, could provide additional therapeutic modalities.

THE LONG-TERM SYSTEM

Subsequent to Lavoisier's revolutionary demonstration that combustion and respiration are chemically equivalent, he and Laplace fabricated the first calorimeter and showed that the same bioenergetic principles apply to living organisms and inanimate systems (161). This pivotal finding was followed by the formulation of the first law of thermodynamics, which asserts that in closed systems, including living organisms if defined as such, energy can neither be created nor destroyed and that a change in the amount of stored energy must be the result of a change in either energy input or output. Together, these findings require that any sustained deviation in adipose tissue mass, the principal site of energy storage, by necessity, has to be the result of either altered food intake or energy expenditure. Yet, despite frequent periods of overeating (such as during the holidays) or food restriction (by dieting), in patients not taking anti-obesity medications or undergoing bariatric surgery, the size of the adipose tissue mass is remarkably stable over the long-term despite the more than a million calories that are consumed annually by most adults. Similarly, in animal studies, body weight invariably returns to that of control animals after periods of either food restriction or overfeeding. In aggregate, these findings reveal that despite the consumption of an enormous number of calories each year, energy intake is precisely balanced against the number of calories that are burned (8). Hervey noted that despite wide fluctuations in daily food intake, a stable weight is maintained and that this is unlikely to be the result of chance but rather set by chemical signals that function as part of a negative feedback loop. However, maintaining stability of adipose tissue mass, ~15% of total weight, even in lean individuals, is a formidable biologic challenge, and he and others hypothesized that this stability is maintained by signals emanating from fat that regulate energy balance.

In a classic 1953 paper, Kennedy (161) set forth the lipostasis theory hypothesizing that chemical signals act on satiety centers in the brain to maintain constancy of fat mass. A putative satiety center had been previously identified by Hetherington and Ranson who, in a set of pioneering studies, showed that lesions of the ventromedial nucleus of the hypothalamus (VMH) cause obesity, whereas lesions of the lateral hypothalamus reduce food intake and cause leanness (162, 163). In seminal studies suggesting that signals from adipose tissue regulate appetite, Hervey showed that obese rats with VMH lesions placed in parabiotic union with normal rats overproduce an appetite-suppressing factor. In parabiosis experiments, surgical union leads to an exchange of blood

between two animals, allowing one to determine whether circulating factors exert effects on animals exposed to it. In this case, the VMH-lesioned animal overproduced an appetite-suppressing factor that normal but not lesioned animals were capable of responding to. These data suggested that neurons in the ventromedial regulated appetite in responses to a change in the concentration of a circulating factor(s). Kennedy and later Hervey speculated that the factor was fat soluble, possibly a metabolite or steroid hormone that could partition in fat, thereby buffering its effects as adipose tissue mass increased (8). They proposed that because the factor could be concentrated in fat, much the same as partitioning of water in the interstitial space alters blood volume, its plasma concentrations would decrease when fat mass expanded and increase when fat mass diminished. However, neither Kennedy nor Hervey was able to establish the chemical nature of the factor(s) or its site of synthesis.

The identification of leptin

The identification of the factor 40 years later devolved from studies of genetically obese mice. In 1953, the same year that Kennedy set forth the lipostasis theory, Snell and colleagues (164) used genetic crosses to show that a genetically obese strain of mice, named *ob/ob* (for obese), mapped to mouse chromosome 6. Snell had identified this fully penetrant spontaneous recessive mutation in the course of brother-sister matings and found that mutant animals weighed three times that of littermate controls, with five times as much adipose tissue as part of a complex syndrome that also included diabetes, infertility, hypothermia, immune defects, and many other abnormalities. The cause of these associated abnormalities, which are not generally seen in patients with obesity, was unclear, and this discrepancy raised the question of whether the *ob* gene product would inform a deeper understanding of the pathogenesis of human obesity. However, the subsequent cloning of the *ob* gene product provided an explanation for this conundrum and provided an impetus for the development of therapies for several human diseases.

In 1968, Coleman and colleagues identified a second recessive mutation that caused a less extreme form of obesity than that of *ob* mice but with a more severe diabetic phenotype (165). He then showed that the differences between *ob* mice and this new mutant that he named diabetes (*db*) were a result of the background strain on which they were carried and that the phenotype of *ob* and *db* mice was identical when carried on the a C57/Bl6J background strain (166). The *db* mice also showed the same set of pleiotropic abnormalities as *ob* mice, suggesting that both genes functioned in the same pathway. Coleman confirmed this in another classic set of parabiosis experiments that paired *ob* and *db* mice to wild-type mice and one another (166). Wild-type or *ob* mice in parabiosis with *db* mice ate less, lost weight, and died of apparent starvation. In contrast, the *db* mice continued to gain weight. Coleman concluded that *ob* mice lack a hormone that normally suppresses food intake and body weight and that *db* mice lack the receptor for that factor. Subsequent parabiosis experiments by Harris (167) pairing fatty (*fa/fa*) rats to wild-type animals yielded the same result seen in *db* mice. Thus, the aggregate data from the Hervey, Coleman, and Harris laboratories, in studies performed over the course of two decades, suggested the parsimonious hypothesis that *ob* encoded a circulating factor regulating weight, *db* and *fa* encoded its receptor, and, based on Hervey's studies more than a decade earlier, the

receptor was expressed in the hypothalamus. However, neither the chemical nature of the circulating factor nor its site of synthesis was known. Contemporaneous studies had also shown that when adipose tissue was transplanted from *ob* mice into wild-type mice (or vice versa), the adipocytes assumed the morphology of the host and that transplantation of small amounts of wild-type fat into *ob* mice did not alter the obese phenotype (168). These initial findings suggested that the signal was not synthesized in adipose tissue, but this did not vitiate the possibility that its abundance was buffered by fat as suggested by Kennedy. It was thus somewhat unexpected that 20 years later when the parabiotic factor was shown to be encoded by the *ob* gene, it was found to be expressed almost exclusively in adipose tissue.

The *ob* gene was identified by positional cloning in 1994 and found to encode a predicted 162-amino acid protein with an N-terminal signal sequence, suggesting that it was secreted (169). The RNA was expressed exclusively in adipose tissue, although subsequent studies have also shown expression in placenta, with much lower abundance in other sites. In one of the two known mutations (*ob*^{2j}), a viral integration inserted between exons 1 and 2 presents a polyadenylation site 5' of the coding sequence leading to a hybrid RNA that no longer encodes the wild-type protein (170). In the original *ob* mutation identified by Snell and colleagues, a nonsense mutation at position 105 truncates the protein and renders it non-functional (169). In this second allele, the mutant RNA was markedly overexpressed, which suggested that, consistent with the parabiosis experiments, the protein is under feedback control and increases as adipose tissue mass expands (8). In this case, however, a protein coding defect leads to an absence of the hormone and the development of obesity alongside a secondary increase of gene expression. The aggregate data suggested that the mutant gene encoded an adipocyte polypeptide that functioned as the afferent signal in a negative feedback loop that maintains homeostatic control of adipose tissue mass. If true, on the basis of these data and the prior parabiosis experiments, then the following predictions needed to be satisfied: The protein should circulate in plasma (171); its abundance should increase with increased adiposity and decrease with weight loss (172); the recombinant protein should reduce weight in wild-type and *ob* mice but not in *db* animals (171); the receptor for the recombinant protein should be expressed in hypothalamus (173–177); and last, the *db* and *fa* mutations should be allelic. All of these predictions were borne out, confirming leptin's role as a critical component of a long-term system that maintains homeostatic control of adipose tissue mass. In addition, subsequent parabiosis experiments performed by Harris (167) confirmed that leptin was the parabiotic factor that was responsible for the loss of weight in lean or *ob* mice paired to *db* mice.

The function of the hormone is as follows (178). At baseline, leptin concentrations are stable, and the number of calories consumed is precisely balanced against the number of calories that are expended. When weight is lost, leptin concentrations fall, which stimulates appetite, leading to the return of body weight (fat mass) to the starting point (Fig. 3A). A decreased leptin concentration is also associated with a broad set of physiologic responses normally associated with starvation, including alterations of energy expenditure, reproductive capacity, thermoregulation, insulin signaling, and immune function, among others (179–181). Thus, leptin treatment of fasted animals suppresses the physiologic

responses to food restriction (179). In addition, graded increases of leptin concentrations in lean, chow-fed mice using osmotic pumps lead to a robust dose-dependent reduction of food intake and fat mass, showing that in lean animals, increasing circulating leptin prevents weight gain and that a physiologic increase acts to return weight to the starting point (182). However, despite reducing body weight and fat mass, physiologic increases of leptin concentration in fed animals do not alter these other physiologic systems (182). Consistent with its reduction of weight in lean animals, leptin treatment of non-obese patients, including those with low baseline concentrations of the hormone, also reduces food intake and body weight (183–187). *ob/ob* mice eat larger meals, indicative of reduced satiety, suggesting that leptin acts at least in part by modulating the short-term system (188, 189). Overall, changes in leptin concentration enable animals to maintain weight in a relatively narrow range, thus avoiding the selective disadvantages of marked deviations in fat mass.

Some mouse strains such as C57/BL6J, but not others, become obese when fed a palatable high-fat diet (189), and this is associated with an increased plasma leptin concentration and a diminished response to exogenous leptin (172, 190). Whereas lean adults and hypoleptinemic patients lose weight when receiving leptin, the majority of patients with obesity do not, with only a small fraction responding (172, 183, 191). A high hormone concentration and diminished response to it are hallmarks of a hormone resistance syndrome, suggesting that much human obesity may be the result of leptin resistance (Fig. 3B), analogous to how insulin resistance causes type 2 diabetes. In some instances, the pathogenesis of leptin resistance is known and includes patients with leptin receptor mutations or defects in melanocortin signaling, which is normally activated by leptin treatment (24). However, the cause of obesity and leptin resistance in the general human population is not known, although studies of knockout mice have identified several genes that alter leptin sensitivity (see below). A recent study has also shown that leptin resistance in diet-induced obese mice is the result of increased mTor activity in POMC neurons (192). These findings are consistent with a prior finding that elevated mTor in these neurons also contributes to obesity in aged mice (193).

Decreased leptin sensitivity would be expected to reset weight to a higher set point, with increased leptin production as fat mass increased. If true, then inhibiting leptin signaling further in leptin-resistant animals would be expected to increase food intake and body weight. Consistent with this, administration of a leptin antagonist to mice fed a high-fat diet led to further weight gain (194). However, similar to other hormones, it has also been shown that increased leptin can cause a secondary decrease in the response (tachyphylaxis) (195). This has led to the suggestion that in some cases, lowering leptin concentrations in leptin-resistant animals could act to improve leptin action and reduce weight (196). In addition, even in obese humans, weight is stably maintained, albeit at a higher baseline, and additional data also suggest that there likely to be other signals in addition to leptin that act to resist further weight gain. Given that unchecked weight gain has potentially severe consequences, it is possible that there is a series of signals, each of which is activated at different points as weight increases, thus restricting further weight gain once obesity has developed (197). The nature of these other putative signals is largely unknown, and their identification could provide new avenues for treatment.

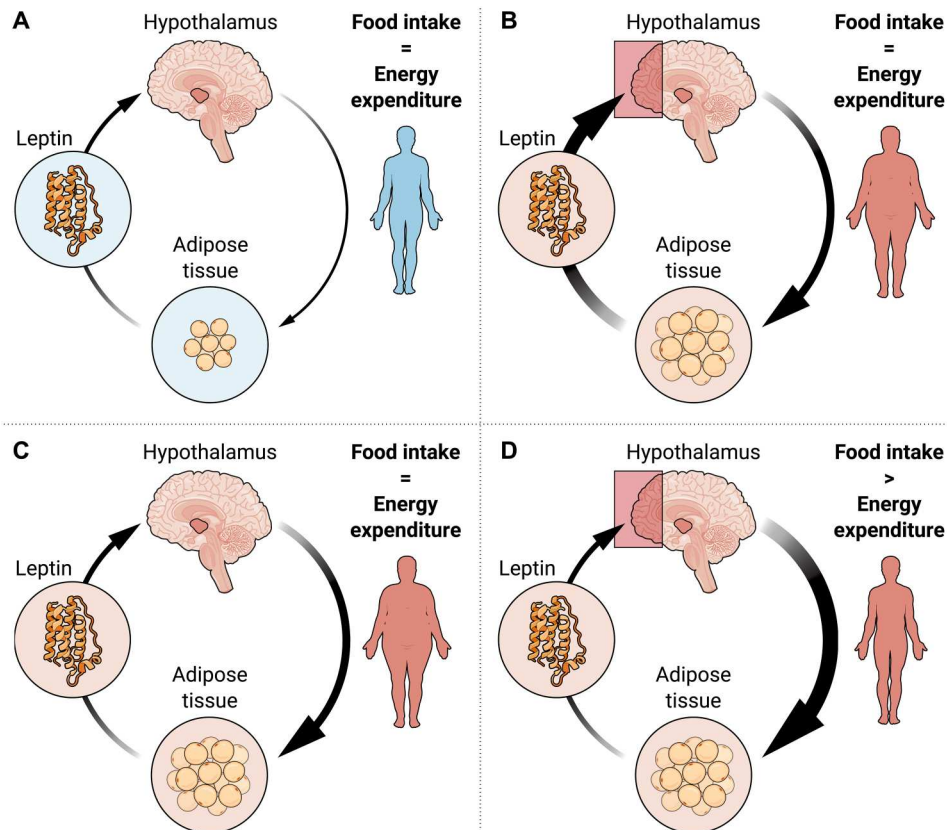


Fig. 3. Leptin regulation of fat mass. (A) Leptin is the afferent signal in a negative feedback loop that maintains homeostatic control of adipose tissue mass by regulating energy balance. Lean individuals are sensitive to leptin and precisely balance food intake and energy expenditure to maintain relatively small amounts of adipose tissue. (B) Most obese individuals (~90%) have high circulating leptin and a decreased response to the endogenous hormone. These are the hallmarks of a hormone resistance syndrome, suggesting that most obesity is a result of leptin resistance. As a result of a block in leptin action, individuals initially consume increased amounts of food, ultimately reaching a stable weight and maintaining energy balance with increased adipose tissue mass and circulating leptin. (C) A subset of obese individuals have relatively normal leptin concentrations. These individuals hyposecrete leptin, leading to a transient increase in food intake until a stable weight is achieved but with relatively normal leptin concentrations. These individuals can show substantial weight loss on leptin therapy. The threshold leptin concentration that reliably predicts a response is not well established. (D) After dieting, bariatric surgery, or medically induced weight loss, leptin concentrations fall substantially. Reduced leptin concentrations generate a potent stimulus to eat more and could contribute to recidivism. Evidence suggests that leptin supplementation might assist with weight maintenance, although this is not confirmed and will require further investigation.

THERAPEUTIC POTENTIAL OF LEPTIN IN HYPOLEPTINEMIA-ASSOCIATED CONDITIONS

Leptin is highly effective at reducing food intake in mice and humans with leptin mutations, and both show great decreases in weight after treatment (171, 198). Leptin mutations in humans are extremely rare, and most patients with them fail to express the mature protein. Recently, however, loss-of-function variants that bind to the receptor without activating it or that bind but fail to signal have been identified (199, 200).

Leptin concentrations are highly correlated with adipose tissue mass, and most patients with obesity have elevated leptin. In contrast to patients with leptin deficiency, hyperleptinemic individuals with obesity as well as diet-induced obese mice do not lose weight on leptin therapy (171, 173, 183, 190, 198). However, a subset of patients with obesity, up to 10%, have relatively low leptin concentrations similar to those of lean individuals. This has suggested that the pathogenesis of obesity in this subgroup might be different from those with leptin resistance and that, in some instances, obesity is caused by inappropriately low leptin production (Fig. 3C). Were

this the case, an initially low leptin concentration would lead to an increased appetite and increased fat mass until "normal" hormone concentrations were achieved (201). If true, then one might expect this subgroup to respond robustly to leptin therapy, and several mouse and human studies are consistent with this possibility.

This possibility was first tested in animals by generating mice that constitutively express a small amount of leptin in fat from a poorly expressed *aP2-leptin* transgene (201). These mice were then mated to *ob/ob* mice such that the only source of leptin was from the transgene. *ob/ob* mice carrying the transgene had a plasma leptin concentration about one-half that found in normal, nontransgenic mice. *ob/ob* animals expressing the leptin transgene were markedly obese, although not as obese as *ob/ob* mice without the transgene. Leptin treatment of these *ob/ob* transgenic mice resulted in marked weight loss, with efficacy similar to that seen after treatment of wild-type mice, demonstrating that subnormal leptin production can cause a hormone-responsive form of obesity. Consistent with this, patients with heterozygous leptin mutations have

been shown to have significantly increased weight (202, 203). More recently, studies of the transcription of the leptin gene revealed that it is regulated by a long noncoding RNA expressed exclusively in fat (204). Mutations in this long noncoding RNA reduce plasma leptin RNA and protein abundance and worsen obesity in diet-induced obese mice. In contrast to wild-type diet-induced obese mice, these obese animals do lose weight after leptin treatment, whereas control diet-induced obese mice do not. In addition, point mutations in the human lncOb RNA are associated with a low leptin obese phenotype.

These data thus suggested that the subset of obese patients with inappropriately low leptin concentrations might lose weight on leptin therapy. This possibility was first tested in a single study in which obese patients with extremely low endogenous hormone concentrations in the lowest decile received daily leptin injections. Patients with low leptin lost significant amounts of weight at 6 months without an evident plateau in the effects, although the full magnitude of the response was not determined (187). In another study, nine male patients with relative leptin deficiency [defined as patients in the lowest quartile of leptin concentration indexed to body mass index (BMI)] showed substantial weight loss, as well as improvements in nonalcoholic steatohepatitis (NASH) score (205). However, the response of these patients was not as robust as in the prior study, although the nine patients in this cohort had higher baseline leptin and were not as obese at baseline. The possibility that patients with low baseline leptin will lose weight on leptin therapy is also supported by the previously mentioned findings that non-obese patients also lost weight on leptin therapy, as do patients with hypoleptinemia resulting from lipodystrophy and HA (see below) (183–186). However, the precise baseline circulating leptin concentration that predicts a robust response has not yet been determined, and the number of patients with obesity who might benefit is likely to be only a small fraction of the total obese population.

Overall, these data indicate that the pathogenesis of obesity is heterogeneous and that, similar to the setting of diabetes, some patients with obesity show hormone resistance, whereas in others, there is a complete or relative hormone deficiency that may be treatable with hormone replacement. To date, leptin is administered as a recombinant protein and, in a relatively small number of cases, has been reported to induce neutralizing antibodies (206). In most instances, this can be addressed by increasing the dose of the hormone or suspending its use, but it is likely that this complication has limited leptin's utilization as an anti-obesity therapeutic. However, the development of leptin antibodies in treated patients could in the future be mitigated by using monoclonal antibody agonists to the leptin receptor that would not cross-react with the endogenous hormone (207). These agents show potent weight-reducing effects in animals as well as, according to anecdotal reports, reducing weight and improving glucose tolerance in humans.

Leptin is a highly effective treatment for several relatively uncommon conditions associated with low endogenous amounts of the hormone. This includes lipodystrophy, a severe metabolic disorder associated with complete or partial loss of adipose tissue and a secondary decrease in leptin (184). The pathogenesis of lipodystrophy is heterogeneous and can be inherited or acquired (208). Patients with generalized lipodystrophy often have recessive mutations in genes required for adipogenesis and have little or no

adipose tissue, leading to a severe, sometimes life-threatening metabolic disorder. This syndrome includes uncontrolled diabetes, markedly elevated plasma triglycerides sometimes requiring plasmapheresis, and hepatic steatosis. Generalized lipodystrophy can also be acquired, and in both forms, leptin is a highly effective treatment that has been approved in the United States, the European Union, and Japan. Patients with generalized lipodystrophy also show substantial hyperphagia, owing to their low leptin, and similar to patients with leptin mutations, leptin treatment markedly reduces their food intake and body weight (187). The suppression of hyperphagia in these patients may account for some of its therapeutic effects, although careful dose-response analyses in lipodystrophic and *ob* mice have indicated that leptin also has antidiabetic effects independent of its effects on food intake or body weight (209). Consistent with this, leptin has also been shown to improve glucose tolerance in animals with type 1 diabetes, although this effect has not yet been replicated in humans (209–211).

Partial lipodystrophy is associated with regional fat loss, often in the extremities, and is typically less severe than the generalized form. It is most often inherited as an autosomal dominant, and there are also acquired forms (208). The response to leptin among patients with partial lipodystrophy is less profound relative to those with generalized lipodystrophy, in part because the metabolic disease is less severe. The response appears to be most pronounced in those patients with low leptin, and the response among those patients with higher leptin is more variable (212). There are also polygenic forms of lipodystrophy, but the efficacy of leptin in this setting has not been evaluated (213). Leptin treatment is approved for the treatment of partial lipodystrophy in the European Union, and further clinical studies are underway in the United States.

HA is also associated with low plasma leptin (214). HA is an infertility syndrome resulting from low concentrations of gonadotrophins that develops among women with little body fat, including ballet dancers, gymnasts, and long-distance runners, and accounts for a substantial proportion of patient visits to infertility clinics. Its pathogenesis was described by Frisch, who noticed that females with less body fat often enter puberty later than those with a higher BMI and that menstruation sometimes ceases when women lose substantial amounts of weight (215). On the basis of this, she speculated that a factor from adipose tissue is necessary for the onset of puberty. The finding that patients with leptin or leptin receptor mutations do not enter puberty at the expected age and that leptin induces puberty in these patients suggested that the factor predicted by Frisch is leptin (216). However, leptin does not induce premature puberty if administered to pre-adolescents, suggesting that additional factors are also required for the onset of menarche. Consistent with Frisch's hypothesis, leptin also restores reproductive function in patients with HA, some of whom became pregnant on leptin therapy (217). These patients also show alterations of several other endocrine and metabolic functions generally associated with undernutrition, and leptin also improves these associated abnormalities (214). Leptin treatment also reduces food intake and body weight in patients with HA, further confirming its potential for reducing weight in patients with low endogenous leptin. Many patients with HA also develop severe osteoporosis despite being young, and leptin has also been shown to significantly improve bone mineral density relative to the current standard of care, estrogen plus Vitamin D, possibly by increasing insulin-like growth factor 1, which is low in these patients (218).

However, despite the great unmet medical need, leptin is not approved for this indication.

Low leptin is also associated with alterations of immune function, and patients with leptin mutations show a shift from T helper cell 1 (T_H1) to T_H2 immunity (217, 219). In addition, there have been reports showing increased deaths from infectious disease in mutant mice and in families with segregating mutations in the leptin receptor (220). These same immune abnormalities are also seen after starvation, and leptin treatment of food-restricted animals normalizes immune function (219). This raises the possibility that low circulating leptin may contribute to the development of severe infections in patients with malnutrition, possibly including cancer cachexia, although the possibility that leptin could enhance immune function in these settings has not been evaluated.

Starvation is also associated with emotional sequelae, raising the possibility that decreased leptin might contribute to this. This possibility has been explored in a very small study of three patients with anorexia nervosa who were treated with leptin (221). These patients had extremely low leptin at baseline, and this study reported a marked improvement in the sense of well-being in two of the three patients receiving leptin injections, both of whom also showed reduced anxiety. Although it might seem paradoxical to treat these patients with a weight-reducing hormone, these highly preliminary data suggest that the negative emotional sequelae associated with leptin deficiency might blunt what should otherwise be a markedly increased appetite in individuals with anorexia nervosa.

Leptin resistance

Although high-dose leptin treatment was reported to cause small but statistically significant weight loss in patients with obesity, the reduction is not clinically relevant (183). However, in several small studies, leptin treatment has been shown to potentiate weight loss during dieting, although the effect was variable in hyperleptinemic patients with obesity and was not effective in patients after gastric bypass (222, 223). An even greater effect has been seen among patients treated with leptin after weight had been lost by dieting (222). Weight loss after dieting is associated with a high recidivism rate with substantial weight regain, which can also be seen in patients after bariatric surgery (19). These data suggest that decreasing leptin may drive weight regain after weight has been lost and that leptin could potentially be used for weight maintenance (Fig. 3D). However, although several small studies are consistent with this possibility, larger studies will be necessary to draw a meaningful conclusion about leptin's potential to prevent weight regain (222). Further studies will also be necessary to track the recidivism rate among patients receiving current-generation incretin-based therapies, and pending these results, there could be a possible role for leptin as an adjunct during or after treatment with these agents.

Whereas leptin monotherapy is largely ineffective for most patients with obesity, several studies in patients and animals have suggested that combinations of leptin and short-term signals may have synergistic effects. For the reason outlined above, some have speculated that an optimal anti-obesity therapy would target both the short-term and long-term systems, because the activity of one might compensate for counter-regulatory changes in the other. Consistent with this, combinations of leptin and exendin-4, CCK, Fgf21, a GLP-1/glucagon dual agonist, or other short-term signals all induced marked weight loss among diet-induced obese animals to a greater extent than treatment with either agent alone, and one of

these combinations has been tested in humans (224, 225). A combination of leptin and amylin, an approved treatment for type 1 diabetes, showed highly significant, synergistic effects in obese humans (138, 226). Amylin is a peptide secreted from pancreatic β cells that reduces gastric emptying, digestive enzymes, and food intake (138). Further studies have suggested that amylin, which induces modest weight loss of ~5% in obese humans, resensitizes leptin-resistant animals. In a subsequent clinical study, a leptin-amylin combination led to substantial weight loss similar to that seen with newer incretin therapies. In aggregate, these studies raise the possibility that adding leptin to incretin-based treatments might increase the magnitude of the response or help to maintain weight loss. Concerns about the development of neutralizing antibodies led to the cessation of the leptin/amylin trials, although this could potentially be addressed by using less immunogenic forms of the hormone or leptin antibody agonists.

Modulation of leptin-regulated neural circuits

The hypothalamus is a principal site of leptin action, where it regulates the activity of several neural populations, including those expressing AGRP or POMC neuropeptides (227). AGRP and POMC neurons express the leptin receptor and also respond to other interoceptive signals to regulate appetite, including ghrelin, GLP-1, and insulin (228). These neurons are components of key appetitive neural circuits, and modulation of these and other neural pathways presents therapeutic opportunities. AGRP neurons are GABAergic and are activated by acute food restriction and chronic weight loss. They are inhibited by a chronic increase in leptin (228). POMC neurons are mainly glutamatergic and reduce food intake and are activated by leptin. Both populations project broadly throughout the brain, where they antagonize one another in the paraventricular hypothalamus, VMH, and a number of other sites. Leptin and other key signals thus act to maintain homeostasis by altering the relative balance between these orexigenic and anorexigenic pathways.

POMC is a peptide precursor that in mice is posttranslationally processed by prohormone convertases, including proprotein convertase subtilisin/kexin type 1 (PCSK1), into several peptides, including α -melanocyte-stimulating hormone (α MSH), β -endorphin, and adrenocorticotrophic hormone. α MSH reduces food intake and weight by engaging the G protein-coupled melanocortin 4 receptor (MC4R). AGRP is an endogenous antagonist of the MC4R, and both signaling pathways converge on key effector neurons in a number of brain regions (228). The MC4R also activates sympathetic neurons to increase blood pressure and heart rate as well as regulate linear skeletal growth, glucose homeostasis and other functions (228, 229). Although mice do not generate β MSH, genetic studies indicate that this peptide can also activate MC4R to regulate food intake in humans.

In humans, loss-of-function mutations of *POMC*, *PCSK1*, and other enzymes required for POMC processing and *MC4R* all cause severe obesity (24, 228, 230). Heterozygous mutations in *MC4R* are the most common genetic cause of severe obesity, accounting for ~10% of the population with type 3 obesity (24). Patients with homozygous *MC4R* mutations are rare, but these patients are even more obese (230). These genetic data thus suggest that stimulation of melanocortin signaling could represent a therapeutic strategy in this setting. Consistent with this, gain-of-function mutations in *MC4R* that augment β -arrestin signaling were shown to be associated with a lean phenotype in humans (231).

The therapeutic benefit of enhancing melanocortin signaling has been tested using setmelanotide, an injectable circularized peptide agonist of the MC4 receptor (229). Setmelanotide injections have been reported to reduce weight by more than 10% in a substantial proportion of patients with mutations in genes encoding the leptin receptor, PCSK1, or POMC (232, 233). Setmelanotide also reduced hunger scores and was approved for patients with these mutations in 2020. It has also shown to induce ~8% weight loss in patients with Bardet-Biedl syndrome, a syndromic form of obesity associated with abnormal hypothalamic leptin signaling (234). Setmelanotide has additionally shown potential for treating other forms of obesity, including patients with heterozygous mutations in MC4R, as well as leading to a modest but statistically significant weight loss in patients even without the aforementioned mutations (235). These results suggest that in addition to peptide mimetics, an oral MC4R agonist could have considerable efficacy as an anti-obesity treatment. However, despite preclinical efficacy, these agents have failed in the clinic because of a lack of efficacy or on-target side effects that include increased sympathetic tone with elevated blood pressure and increased sexual arousal (229). Some of these side effects are also seen in patients treated with setmelanotide, although it is unclear why there is an apparent difference in efficacy between the peptide and oral melanocortin receptor agonists.

Lorcaserin is a medication approved for obesity that acts by increasing melanocortin signaling. It is a selective serotonergic receptor 2C receptor agonist that was developed after the finding that a serotonin 2C receptor knockout causes weight gain in mice (236). POMC neurons are also activated by dexfenfluramine (237), a serotonin reuptake inhibitor and a component of the fen-fen drug combination that showed some efficacy for weight loss before being withdrawn because it caused valvular disease (238, 239). Serotonergic signaling increases the activity of POMC neurons, and a POMC-specific knockout of the HT-2c receptor causes obesity (238). In humans, lorcaserin elicited an average placebo-corrected weight loss of 3.6%, with 47% of patients losing greater than 5% compared to 20.5% in the control group after a year (240). Patients who continued lorcaserin for a second year also maintained weight loss to a greater extent than did patients who stopped the drug. The weight loss in patients receiving lorcaserin was associated with a reduction of HbA1c of 0.9% compared with a 0.4% reduction with placebo. However, the relatively modest weight loss associated with lorcaserin treatment has limited its utilization, and it was subsequently withdrawn because of the possibility that there is an increased cancer risk (241). However, as suggested by the efficacy of setmelanotide, it is possible that other modulators of POMC neurons could show efficacy.

Leptin-responsive neurons, including POMC and AGRP cells, project broadly in the brain, and modulation of these downstream circuits could also form the basis of anti-obesity therapies. Output nodes downstream of the site of leptin resistance may represent potential targets for therapies that would bypass leptin resistance. Recently, opposing populations of GABAergic and glutamatergic neurons in the dorsal raphe nucleus (DRN) have been shown to regulate food intake and locomotor activity (242). Chemogenetic and optogenetic activation of DRN Vglut3 neurons or inhibition of Vgat-expressing cell populations in the DRN has been shown to reduce body weight in diet-induced obese and *ob* mice, confirming that these neurons reduce weight independently of leptin. Subsequent studies revealed that the Vglut3 subpopulation expresses

the hypocretin 1 receptor and that a specific inhibitor of this receptor also significantly reduced the body weight of diet-induced obese mice (243). Thus, pharmacologic modulation of the activity of these and other neural populations represents another potential avenue for the medical treatment of obesity.

Leptin sensitizers

Although several gene products have been shown to alter leptin sensitivity, the biochemical alterations that cause leptin resistance in diet-induced obesity are not known. Similarly to other hormones, increases in leptin can down-regulate the hormone response (as in tachyphylaxis), and the underlying mechanism is known in some cases. Leptin activates suppressor of cytokine signaling 3 (SOCS3), and a brain-specific SOCS3 knockout decreased the weight of diet-induced obese animals (244). Similarly, a knockout of protein-tyrosine phosphatase 1B (PTP1B) also reduced the weight of high-fat diet-fed animals (245). These genetic data thus suggest that inhibitors of SOCS3 or PTP1B could act as leptin sensitizers, although none have been developed thus far. Mutations in histone deacetylase 6 (HDAC6), a cytosolic enzyme, also sensitize animals to leptin, and treatment with the HDAC6 inhibitor tubastatin A reduced weight in diet-induced obese mice (246). This response required intact leptin and melanocortin signaling because the drug had little to no effect in *db* mice with a mutant leptin receptor or in MC4R knockout mice. The data further showed that peripheral but not central inhibition of HDAC6 is responsible for the weight-reducing effect of HDAC6 inhibition, suggesting that it might regulate the production of an unknown adipokine or other signaling molecule that affects leptin sensitivity. The authors thus conclude that HDAC6 is a potential drug target for obesity. Computational analyses of gene expression have also suggested that increased endoplasmic reticulum stress in hypothalamic neurons may contribute to leptin resistance. Subsequent *in silico* drug screens further suggested that celastrol, a component of thundergod vine, can also act as a leptin sensitizer (247). Similarly to HDAC6 inhibition, celastrol reduces weight in diet-induced obese mice but not in *ob/ob* or *db/db* mice. The precise mechanism is unknown, although its effects are attenuated in animals lacking the interleukin-1 receptor (248). Studies of the clinical safety of celastrol are underway, and its potential efficacy in humans awaits further trials. Rapamycin also reduces weight in diet-induced obese and aged mice but not *ob* or *db* mice by down-regulating mTor in POMC neurons, with data suggesting that it restored leptin signaling in these neurons (192) (193). In aggregate, these studies have identified several biochemical mechanisms that cause leptin resistance and suggest potential therapeutic approaches.

OTHER CNS DRUGS

Several other orally administered drugs have been approved for the treatment of obesity, and they too reduce weight by modulating CNS circuits that regulate appetite (249). However, none of these oral medications has achieved the efficacy of the incretin-based therapeutics. A combination of naltrexone, a μ opiate receptor antagonist, and bupropion, an inhibitor of dopamine and norepinephrine uptake (249, 250), has been reported to induce 4.1% placebo-corrected weight loss (250). In 2012, a second drug combination of phentermine, a sympathomimetic agent, and topiramate, an antiseizure medication, was also approved for the treatment

obesity (249, 251). Two doses (7.5/46 and 15/92 mg phentermine and topiramate, respectively) were tested, resulting in weight loss of 4.8% at the low dose and 7.1% at the high dose, whereas the placebo group gained an average of 3.3%. Both phentermine/topiramate and naltrexone/bupropion were approved for weight reduction in combination with a reduced-calorie diet and physical activity among patients with a BMI greater than 30 or a BMI of greater than 27 with a comorbidity of hypertension diabetes or dyslipidemia. Both drugs have potential safety issues, however, and this together with their relatively limited efficacy (less than 10% weight loss) compared with the more recent incretin-based therapies has limited their use. Although it is assumed that both drugs act on appetitive circuits in the CNS, their precise site of action is unknown. Monoamines including dopamine and neurepinephrine as well as serotonin (see above) can modulate feeding but have broad effects on a number of pathways. Topiramate was first developed as an antiseizure medication, and although it can inhibit carbonic anhydrase, its molecular target and the site of action responsible for its effect on weight are not known (248). A fuller understanding of the mechanism of action of each of these drugs could provide still new therapeutic approaches. Brain-clearing methods combined with cFos mapping could potentially be used to identify brain regions that are activated by each of these drugs (242).

CONCLUSION

The identification of a set of short- and long-term signals that regulate food intake has transformed our understanding of the neural and physiologic mechanisms regulating appetite and body weight. These advances have provided the basis for several incretin-based therapies that induce profound weight loss that few believed would be possible. Other studies have led to the development of treatments for a series of less common disorders such as genetic disorders associated with obesity, lipodystrophy, and HA, with potential for others. The emerging basic science has also yielded several oral agents that reduce weight and identified additional drug targets and therapeutic approaches that could be of benefit for either inducing weight loss or maintaining it.

It is impossible to overstate the public health implications of these developments. Obesity is an independent risk factor that greatly increases mortality risk and is estimated to carry a \$173 billion burden for the American health care system alone. It is also a significant problem in the rest of the developed world and a growing one in the developing world. Although concerns about the cost of some of these emerging, more effective drugs have been raised, their potential health benefits make it imperative to develop means for ensuring that they find their way to patients who need them.

In addition to their impact on the treatment of metabolic disease, the substantial weight loss that is now achievable should help mitigate the personal and societal consequences of this disorder. The identification of mutant genes that cause human obesity and the current availability of effective treatments vitiates the notion that food intake and body weight can be controlled by volition alone. Obesity is arguably the most stigmatized human condition, and in addition to their health benefits, this generation of treatments should lay to rest the unfair treatment that many people with obesity endure.

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projects with and has signed/signs contracts for the centers institute(s) related to research collaborations worldwide, including but not limited to pharmaceutical corporations like Boehringer Ingelheim, Novo Nordisk, Roche Diagnostics, Arbormed, Eli Lilly, SCG Cell Therapy, and others. As the CEO of Helmholtz Munich, he was/is further overall responsible for commercial technology transfer activities. M.H.T. confirms to the best of his knowledge that none of the above funding sources or collaborations were involved in or had an influence on the preparation of this manuscript. M.H.T. is a former member of the scientific advisory board of ERX, which is developing celastrol but has no current competing interests. J.M.F. also serves on the scientific advisory board of ERX. As an inventor on the leptin patent and as per Rockefeller University policy, J.M.F. receives a portion of the royalties for the sale of leptin (myalept).

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