HHS Public Access

Author manuscript

Expert Opin Drug Discov. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Expert Opin Drug Discov. 2021 July ; 16(7): 777–790. doi:10.1080/17460441.2021.1877654.

From Leptin to Lasers: The Past and Present of Mouse Models of Obesity

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Abstract

Introduction.—Obesity is a prevalent condition that accounts for significant morbidity and mortality across the globe. Despite substantial effort, most obesity pharmacotherapies have proven unsafe or ineffective. The use of obese mouse models provides unique insight into the hormones and mechanisms that regulate appetite and metabolism. Paramount among these models are the "obese" and "diabetic" mice that revealed the powerful satiety hormone leptin, revolutionizing obesity research.

Areas Covered.—In this article, we discuss work on leptin therapy, and the clinical response to leptin in humans. The authors describe the use of modern mouse genetics to study targetable mechanisms for genetic forms of human obesity. Additionally, they describe mouse models of neuromodulation and their utility in unraveling neural circuits that govern appetite and metabolism.

Expert opinion.—Combining past and present models of obesity is required for the development of safe, effective, and impactful obesity therapy. Current research in obesity can benefit from repositories of genetically engineered mouse models to discover interactions between appetitive systems and circuits. Combining leptin therapy with other satiety signals comprising the gut-brain axis is a promising approach to induce significant enduring weight loss.

Keywords

animal models; anti-obesity drugs; appetite; gut-brain axis; leptin; leptin resistance; mouse models; neuromodulation; obesity; syndromic obesity

1. Introduction

Obesity, defined as a Body Mass Index (BMI) of 30 or above, is a condition that effects 12% of adults worldwide, and is projected to effect 49% of adults in the United States by 2030

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Declaration of Interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer Disclosures:

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

[1,2]. Obesity-related complications, most commonly coronary heart disease and end-stage renal disease, contributed to 7.1% of deaths worldwide in 2015 [1,3]. The effects of obesity on health have been recognized for centuries, if not millennia [4]. Therapies for obesity date back just as far, beginning with the fundamental concepts of eating less and exercising more. Hippocrates, the "father of medicine", extolled the virtues of healthy diet and prescribed diet and exercise to his patients with obesity [5].

By the mid 18th century, obesity (or "Corpulency") was being discussed at the Royal Society of London, as were its therapies [6]. A case study from this time gives an enthusiastic appeal for soap as a weight-loss supplement due to its "singular power of rendering oil or fat mixable with water" [7]. The study claims that a man who drank a pint of soapy water for two years every night before bed lost "two stone" (12.7 kg), without the assistance of any other medication. Remarkably, the use of soap in weight loss persisted into the 20th century with pseudoscience obesity "cures" like *Fatoff*: a heavily advertised "corpulency reducer" that consisted of 90 percent water and 10 percent soap [8].

Pharmacological cures for obesity in the early-to-mid 20th century were underscored by a fundamental lack of understanding about the regulation of weight gain. Moreover, the weight loss market had been flooded with "curealls" and hoax remedies (like *Fatoff*) for decades, minimizing public and academic perceptions of anti-obesity drugs [9]. At the time, animal models to study obesity in the laboratory setting were not widely used. While the *agouti* mouse was known to have increased weight, the dermatological features interested researchers more than the overweight phenotype [10]. Without preclinical models, weight loss drugs were stumbled upon incidentally in the clinic, with unfortunate results. Amphetamines are a prime example. In 1937, a study using the amphetamine beta-aminopropylbenzene to treat "nervous exhaustion" noted that 25% of patients showed rapid, sustained weight loss [11]. Shortly thereafter, amphetamines became widely developed and prescribed as weight loss drugs with limited understanding of their physiological effects. After years of rampant prescription, amphetamines were removed from the market due to multiple side effects, including addiction and numerous fatalities [12].

The scientific rigor of obesity research arrived with the advent of animal models for obesity. Inbred mouse strains, first developed in the early 20th century, were gaining acceptance as models of human physiology.[13] These inbred strains were bred specifically to reduce genetic variability of previous outbred strains and provide reproducible, translatable data. Two specific mutations in inbred mouse strains: the "obese" (*ob/ob*) and "diabetic" (*db/db*) mouse mutations, were instrumental in legitimizing obesity research and the molecular basis for metabolic regulation. After decades of research, we now know that *ob/ob* mice model deficiency in the hormone leptin, while *db/db* mice model deficiency in the leptin receptor. The discovery of the leptin system changed the zeitgeist for obesity therapy from quackery and happenstance to testable hypotheses, as evidenced by increases in anti-obesity publications (Fig. 1). This article will review some landmark discoveries in the leptin system that form the basis for obesity research, as well as the modern mouse models of obesity research influenced by leptin.

2. Obese Mice, Diabetic Mice, and the Rise and Fall of Leptin

In the summer of 1949, “some very plump young mice” were discovered in C57BL/6J mice at the Jackson Labaratory in Bar Harbor, Maine [14]. These “plump” mice weighed in excess of 90 grams, triple the weight of their littermates, earning them the phenotype designation of “*ob*” for “obese”. Breeding studies revealed that the *ob* phenotype was a recessive mutation, and that homozygotes were not only obese, but also infertile and mildly diabetic.

In 1965 a second metabolic mutation was characterized at Jackson Labs, this time in the mouse strain C57Bl/KsJ [15]. This mutation, in contrast to the *ob* mutation, resulted in comparatively modest weight gain, with females averaging weights of 55 grams. This new mutation caused mild obesity and profound diabetes (blood sugars exceeding 300 mg/100 mL by 10 weeks of age). Aptly named “*db*” for “diabetic”, these mice, like *ob/ob* mice, were infertile and exhibited small ovaries with immature follicles. Interestingly, transplanting ovaries from *ob/ob* mice and *db/db* mice into wild type mice fully rescued ovarian function [16]. Because the mutated ovaries could still respond to endocrine signals from non-mutant animals, the ovarian transplantation studies implied that the *ob* and *db* mutations did not directly produce dysfunction of these peripheral tissues. Instead, the *signals* sent to the ovaries in *ob/ob* and *db/db* mice appeared to be dysregulated. To researchers at Jackson Labs, this implied that *ob* and *db* were responsible for a central signaling defect, possibly shared by both mice [17].

Douglas Coleman and the researchers at Jackson Labs speculated that loss of a “circulating factor” could be the cause of the obesity and diabetes in these mouse models [18]. The strongest evidence for *ob* and *db* mutations causing obesity through a circulating factor came from pioneering parabiosis experiments. Parabiosis is the surgical joining of two animal’s circulatory systems, and began to be used experimentally at the turn of the 20th century [19]. By the late 1960s parabiotic studies were in their heyday, employed by almost every field of biomedical research [20]. Parabiosis between healthy and diseased rodents was used to determine if humoral factors from a healthy mouse could rescue the diseased mouse (for example returning to healthy blood pressure, or recovering from radiation) [20]. Parabiosis was then the natural step to identify the “circulating factor” that linked *ob* and *db* mutations to obesity.

2.1 Parabiosis studies using *Ob/Ob* and *Db/Db*

The first parabiosis experiments on *ob/ob* mice were performed by Franz Hausberger in 1959 at Jefferson Medical College [21]. These experiments, in contrast to conventional parabiosis studies, were used primarily to graft adipose tissue between *ob/ob* mice and wild type mice. Hausberger surgically joined an *ob/ob* mouse and a wild type littermate for 2–5 months, and then surgically separated the mice, leaving adipose tissue from the *ob/ob* mouse in the wild type mouse and adipose tissue from the wild type mouse in the *ob/ob* mouse. He observed that after separation, the *ob/ob* adipose tissue in the wild type mouse regressed, while the wild type adipose tissue in the *ob/ob* mouse took on an *ob/ob* adipose phenotype. This indicated to Hausberger that the *ob/ob* mouse lacked a “secreted factor”, which he

believed acted directly on the adipose tissue. Unfortunately, Hausberger did not report the effects that parabiosis itself had on the *ob/ob* mice.

Ten years later, Coleman's first parabiosis experiments coupled a *db/db* mouse to a wild type C57Bl/KsJ mouse in search of a factor that might sensitize the *db/db* animals to insulin and normalize their blood sugar [22]. These conventional parabiosis studies measured the effect of shared circulation on the mice during parabiosis. Instead of rescuing the diabetic phenotype, parabiosis between *db/db* and wild type mice lead to the starvation and swift death (median survival time 23 days) of the wild type animals. Similar results had been found in a parabiosis study 10 years earlier, where rats with hypothalamic lesions had been surgically fused with uninjured rats [23]. Rats with bilateral ventromedial hypothalamic lesions overeat, gain weight, and become obese. Healthy, lesion-less rats coupled to obese, lesioned rats suffered the same fate as the wild type mice coupled to *db/db* mice: anorexia and eventual death. These studies led to the hypothesis that *db/db* and lesioned mice overproduced a satiety factor to which they were somehow unable to respond. The hypothalamic lesioning experiments implied that receptors for the satiety response were located in the ventromedial hypothalamus.

Further experimentation to test this hypothesis came with parabiosis experiments that included the *ob/ob* mouse [24]. Previous experiments that crossed *ob/ob* and *db/db* mice served a dual purpose: revealing the exact phenotypic overlap of the two mutations on the same strain, and protecting against rejection (or "disharmony") after parabiosis surgery [25]. These experiments revealed that different mechanisms drove the obese and diabetic mutations, one seemingly ligand-based, and the other seemingly receptor-based. When *ob/ob* mice were paired with wild type mice, the obese phenotype was partially rescued. This showed, in classic parabiosis fashion, that the *ob* phenotype resulted from the deficiency in a blood-borne factor. When *db/db* and *ob/ob* mice were paired together, the *ob/ob* mouse would starve and die (median survival time of 26 days), very closely matching the parabiosis experiments of *db/db* and wild type mice. These experiments provided further evidence that the *ob/ob* and *db/db* mutations resulted in the insufficiency of a humoral satiety factor in the former and an inability to respond to the factor in the latter.

2.2 Leptin: a new age of molecular obesity

Search for the humoral satiety factor lasted until the early nineties, when advancements in molecular genetics and painstaking work by the Friedman Laboratory finally elucidated the mutated gene in *ob/ob* mice. Forty-four years after their discovery, positional cloning revealed a nonsense mutation in amino acid 105 of an unnamed gene in *ob/ob* mice [26]. The Friedman lab named the product of this gene "leptin": "derived from the Greek root "leptos" for "thin," the notion being that leptin kept a mouse (and humans) from becoming obese" [27].

The obesity field erupted after the seminal discovery of the "obese gene product". One year later, the causative mutation in *db/db* mice was cloned from a protein with similarities to cytokine receptors (what we now know as leptin receptor; LepR) [28]. The mutation was subsequently found to be a splicing defect that eliminated key signaling domains of the long-form of LepR, rendering it ineffective at regulating satiety and metabolism [29].

Recombinant leptin was shown to induce weight loss by central and peripheral administration in *ob/ob* mice, but not *db/db* mice [30]. Further, even wild type mice were shown to have decreased appetite and weight loss after chronic intraperitoneal (IP) administration of leptin [31]. These experiments demonstrated that leptin is a powerful satiety hormone released by the adipose mass that acts in an endocrine fashion on LepR in the ventromedial hypothalamus, inducing satiety and increasing metabolism. The buzz around leptin had reached a fever-pitch after this string of high-impact publications revealed the molecular mechanisms for obesity.

Since the discovery of leptin and leptin receptor, many of the downstream signaling mechanisms have been revealed. Leptin binding to LepR activates two downstream pathways necessary for satiety: JAK2 phosphorylation of STAT3 and SH2B1 recruitment of IRS proteins to activate PI3K.[32–35] Leptin binding to LepR causes phosphorylation of the transcription factor STAT3, which in the arcuate nucleus increases expression of anorexigenic hormones (CART and POMC) and decreases expression of feeding hormones (AGRP and NPY).[36–38] Activation of PI3K confers the more immediate signaling of LepR, especially by mediating the effect of leptin signaling on both depolarizing and hyperpolarizing neuronal subpopulations.[39–41] Additionally, pSTAT3 forms a negative feedback loop by increasing the transcription of Suppressor of Cytokine Signaling 3 (SOCS3), which inhibits the JAK-STAT activation by LepR.[42,43] SOCS3 and the tyrosine phosphatase PTP1B are negative regulators of LepR, and loss of either of these proteins results in leptin hypersensitivity and resistance to diet-induced obesity in mice.[44–46] Pharmacological inhibitors of SOCS3 and PTP1B are promising approaches to enhance leptin signaling in mice and patients with obesity.

Direct evidence for leptin signaling through the hypothalamus emerged from intracerebroventricular (ICV) cannulation and leptin injection. Initial studies in rats and mice revealed that central administration of leptin produced rapid (<1hr) decreases in food intake and transcription of Neuropeptide Y (NPY).[47,48] Additional studies revealed that peripheral and central administration of leptin also reduced food intake, and that mice deficient in the cytokine receptor IL-1RI were not responsive to central leptin administration.[49] Further, ICV injection of leptin and subsequent staining of Fos (a canonical readout of neuron activation), identified the specific hypothalamic nuclei activated by leptin administration.[50,51] Microinjection of leptin into the arcuate nucleus (ARC), ventromedial hypothalamus (VMH), and lateral hypothalamus (LH), and lesioning studies of the ARC using monosodium glutamate, revealed that the ARC was the primary site of leptin's satiety effect.[52,53] The central effects of leptin in humans has been studied through functional Magnetic Resonance Imaging. When shown pictures of food, subjects had increased activation in the ventromedial striatum in a leptin-deficient state.[54] This implies that leptin signaling decreases "wanting" signals for food through the ventromedial striatum, and provides evidence for a leptin-driven central satiety mechanism in humans.

2.3 Leptin as a therapuetic

Unfortunately, the promising effects of leptin in lean mice did not translate to a universal human obesity therapy. Unlike the *ob/ob* mouse, most individuals with obesity did not

harbor mutations in the leptin gene, so direct replacement of leptin in most of these individuals would not be a simple cureall [55]. Even more damning, human obesity is characterized by a marked *increase* in leptin levels, not a deficiency that could be treated with exogenous leptin like in the *ob/ob* mouse [56]. Even in other obese mouse models like Diet Induced Obese (DIO), Agouti (A^{vy}), and New Zealand Obese (NZO) mice, chronic leptin treatment was ineffective at producing the robust weight loss seen in *ob/ob* and even lean mice [57]. A clinical trial in which 54 lean and 73 patients with obesity administered subcutaneous recombinant leptin daily showed extremely variable effects in both groups [58]. Patients with obesity were concurrently placed on a 500 calorie deficit diet, and only a modest weight loss trend was observed after 24 weeks. Leptin administration in mice and humans with obesity revealed a state of "leptin resistance" where weight-loss signals from leptin are relatively ineffective. The mechanisms behind leptin resistance are contentious and complex. An initial human study found a decreased ratio of leptin in the cerebrospinal fluid compared to plasma leptin in patients with obesity. This implied that leptin resistance in obesity could be caused by impaired transport across the blood brain barrier (BBB).[59] Further investigation of this hypothesis in mice has been technically challenging and resulted in divergent data. [60,61] Subsequent studies have also shown that DIO mice are resistant to both central and peripheral leptin, suggesting that the resistance may be downstream of leptin transport into the brain.[62] Overcoming leptin resistance in obesity is an active area of research, and could allow leptin to re-emerge as a universal obesity therapy [63].

Despite it's disappointing efficacy in treating obesity, leptin monotherapy has utility in patients with dysregulated adipose signaling. Metreleptin, recombinant human leptin, is clinically effective at treating hypothalamic amenorrhea and lipodystrophy (especially lipodystrophy caused by anti-retroviral therapy for HIV).[64,65] Further, metreleptin ameliorated hyperactivity and improved cognitive, emotional, and behavioral symptoms in three patients with anorexia.[66] Metreleptin also improved insulin sensitivity and decreased de novo lipogenesis (a contributor to hepatic steatosis) in eleven patients with lipodystrophy. [67] Moreover, leptin dysregulation in obesity plays a role in atherosclerosis and cardiovascular disease, possibly through immune-mediated and inflammatory pathways.[68–70]

2.4 Adipokines

The discovery of leptin not only revolutionized obesity research, but also emphasized the role of adipose tissue as and endocrine organ. When leptin was cloned, adipose tissue was beginning to be recognized as more than just a depot of triglycerides. Adipsin (complement factor D), and tumor necrosis factor alpha (TNFa) were first revealed to be secreted by adipose tissue and mediate inflammation and insulin resistance in distal tissues.[71] Since the discovery of these initial bioactive molecules released by adipose ("adipokines"), over 600 adipokines have been identified, revealing the complex role of adipose in maintaining homeostasis.[72] Of these adipokines, many have therapeutic promise, improving insulin resistance in rodent models.[73] Adiponectin is a particularly attractive therapeutic candidate, as its levels decrease in obesity and administration of adiponectin decreases body weight and increases insulin sensitivity and release.[74,75] Dipeptidyl peptidase 4 (DPP-4)

is an adipokine that has been targeted therapeutically since 2006.[76] DPP-4 inhibitors such as sitagliptin, saxagliptin, and linagliptin are used in the management of type 2 diabetes by increasing the activity of the incretins that DPP-4 degrades.[77] A multitude of adipokines like resistin, visfatin, chemerin, and omentin reinforce the link between adipokine dysregulation, inflammation, insulin resistance, cardiovascular disease, and metabolic syndrome.[78–81] Investigation of adipokine signaling is revealing novel obesity pathophysiology, and leading towards therapeutics to treat the sequalae of obesity.

3. Modern Mouse Models: Reverse Translation from Human to Mouse

In light of the disappointing outcomes of leptin monotherapy, the utility of mouse models in designing human obesity therapies needed to be rethought. The original mouse models of obesity were spontaneous mutations resulting in remarkable phenotypes, requiring decades of research to pinpoint their genomic origin. Modern mouse models reverse this paradigm by targeting a gene of interest and subsequently observing a phenotype. This, coupled with advances in uncovering human genetic causes of obesity, allows researchers to investigate relevant genes for human obesity in mouse models.

3.1 Mouse models of human syndromic obesity

Mouse models of rare forms of human congenital obesity have provided insights into the mechanisms of human satiety and weight gain. Though less generalizable, mice engineered in this way model actual human mutations, and therefore are directly applicable to treating clinical obesity. “Syndromic obesity” refers to severe congenital obesity that occurs with other stereotypical phenotypes (e.g. dysmorphic features and developmental abnormalities) [82]. The two most common forms of syndromic obesity are Bardet-Beidel syndrome (BBS) and Prader Willi syndrome (PWS) [82]. Mouse models engineered to study these diseases have helped to reveal specific genes implicated in obesity in these syndromes, while answering broader questions about regulation of appetite and satiety.

3.1.1 Bardet Beidel syndrome—Bardet Beidel Syndrome is a heterogenous genetic disorder whose primary features include retinal dystrophy, obesity, polydactyly, and hypogonadism [83]. The incidence of obesity in the BBS population is reported to be 72–86% [84]. Molecularly, BBS mutations lead to ciliary defects, which lead to dysfunction in receptor trafficking and cytoskeletal abnormalities [85]. Mutations in 19 different genes have been associated with BBS (BBS1-BBS19). Of these genes, 8 are associated with the “BBSome”, a complex that effects trafficking to cilia and the cell membrane [86].

Like humans, mice with mutated BBSome proteins become obese. *Bbs2*$^{-/-}$, *Bbs4*$^{-/-}$, and *Bbs6*$^{-/-}$ mice are hyperphagic, and gain significantly more weight than WT mice [87]. Interestingly, all 3 knockout mice exhibit leptin resistance, and show no feeding or body weight changes to leptin administration [87]. The BBSome was found to directly interact with LepR in the hypothalamus, and mice with mutant BBSome proteins showed decreased hypothalamic leptin receptor signaling [88]. A knockin mouse model of the most common mutation in BBS (*Bbs1*$^{M390R/M390R}$) also exhibits obesity, in addition to sperm flagellar defects (male infertility), and hydrocephaly [89]. The *Bbs1*$^{fl/fl}$ mouse has been useful in creating conditional BBS knockout models to decipher which tissues require the BBSome to

regulate satiety. Crossing the Bbs1$^{fl/fl}$ mouse with a neuron-specific cre (*Nestin^{Cre}/Bbs1$^{fl/fl}$*) and an adipose-specific cre (*Adip^{Cre}/Bbs1$^{fl/fl}$*) resulted in obesity in only the *Nestin^{Cre}/Bbs1$^{fl/fl}$* mice, indicating that neural Bbs1 is necessary for maintaining normal weight. Furthermore, eliminating Bbs1 in only LepR+ cells (*LepR^{Cre}/Bbs1$^{fl/fl}$*) also lead to obesity in mice [90]. *In vitro* experiments revealed that the BBSome is necessary for leptin receptor trafficking to the cell surface [90]. Gene therapy using Adeno-Associated virus (AAV) has been successful in treating the retinal degeneration of *Bbs4$^{-/-}$* mice, however similar approaches have not yet been attempted in the hypothalamus to treat obesity [91].

3.1.2 Prader Willi syndrome—Prader Willi Syndrome is caused by deficient expression of the Prader Willi Critical Region (PWCR) of chromosome 15q11.2-q13 [92]. PWS usually is caused by the loss of a paternally imprinted region of chromosome 15 by paternal deletion, maternal uniparental disomy of chromosome 15, or imprinting defect. Angelman Syndrome is caused by the loss of the maternally imprinted region on the q arm of chromosome 15, and is phenotypically similar to PWS until two years of age, when hyperphagia and obesity become apparent in PWS [92]. Feeding behaviors in PWS are classically divided into two phases: poor feeding and hypotonia in infancy, with a reversal to hyperphagia and obesity that occurs between 18 and 36 months [93]. More recently, the nutritional phases of PWS have been divided into 4 main phases, with phase 3 (8 years old – adulthood) characterized by insatiable hunger [93]. During this phase, individuals with PWS seem unable to feel full. The compulsion to eat can be so severe that individuals with PWS have been reported to gain up to 20lbs in one weekend, and can eat to the point of life-threatening gastric rupture [94]. Complications from obesity account for the majority of mortality in individuals with PWS [95].

The Prader Willi Critical Region encodes five protein-coding genes, three of which have been made into knockout mouse models: MKRN3, MAGEL2, and NECDIN [92]. Knockout mouse models of these genes have helped to unravel the specific role of each of these genes in the clinical presentation of PWS. In addition to altered feeding behavior, PWS is also characterized by developmental delay, hypogonadism and infertility, and oppositional behavioral problems [96]. Necdin-deficient mice exhibit cognitive behaviors reminiscent of PWS, including skin-scraping activity that mirrors skin-picking seen in individuals with PWS [97]. Additionally, loss of oxytocin- and luteinizing hormone releasing hormone neurons in Necdin-deficient mice implicates Necdin in the disordered pubertal development characteristic of PWS. MKRN3 also is involved in pubertal development, as evidenced by the Mkrn3 knockout mouse, which exhibits precocious puberty [98]. *Mkrn3$^{-/-}$* mice do not model the obesity associated with PWS. However, *Magel2$^{-/-}$* mice exhibit decreased weight at birth, and a subsequent increase in weight gain and adiposity at 5–6 weeks of age, matching the nutritional phases of PWS [99]. By 16 weeks, *Magel2$^{-/-}$* mice have 18% body fat, double that of wild-type littermates [99]. Surprisingly, at 16 weeks *Magel2$^{-/-}$* mice eat 10% less than wild-type littermates, which contrasts with the insatiable appetites of individuals with PWS. Similar to the other mouse models of PWS, *Magel2$^{-/-}$* mice also have reproductive defects [100]. Thus, the obesity phenotype seen in PWS appears to be driven by decreased MAGEL2 expression, while the reproductive phenotype appears to involve genes throughout the Prader Willi Critical Region.

The mechanism behind the increased adiposity of the *Magel2*$^{-/-}$ mouse is similar to that of BBS mouse models: decreased surface-expression of leptin receptor in the hypothalamus [101]. Like BBS mice, *Magel2*$^{-/-}$ mice are resistant to leptin administration, and show no decrease in food intake after leptin treatment [102]. Moreover, this leptin de-sensitization occurs between week 4 and 6, overlapping with the switch from low weight and adiposity to increased weight and adiposity in these mice [103]. Magel2 is a MAGE protein, a family of proteins that inhibit the activity of E3 protein ligases, thereby protecting proteins from degradation. Lack of Magel2 in *Magel2*$^{-/-}$ mice leads to decreased expression of LepR in the hypothalamus, likely through increased lysosomal degradation of LepR, and decreased trafficking of LepR to the cell surface [101].

3.1.3 Leptin receptor trafficking, therapeutic implications—

Mouse models of BBS and PWS underscore the importance of LepR localization on the cell surface of hypothalamic neurons. The effect of LepR trafficking on appetite and metabolism also can be appreciated in mouse models with silenced Endospanin-1 (Endo1, also known as OB-RGRP or LEPROT). Endo1 is encoded on the same gene as LepR via alternative splicing, and directly interacts with LepR by trafficking the receptor inside the cell and targeting LepR for lysosomal degredation [104]. Thus, Endo1 opposes Magel2 and BBSome proteins, which increase LepR trafficking to the cell surface and decrease LepR degradation. Though an Endo1 knockout mouse has not been reported, stereotactic injection of Endo1-specific shRNA has been used to silence Endo1 in the mouse hypothalamus [105]. Endo1 knockdown in the mouse hypothalamus imparts resistance to diet-induced obesity (DIO) [105]. Mice injected with Endo1 shRNA showed significantly decreased body weight and fat mass after being fed high fat diet when compared to mice injected with sham RNA. In mice that are already obese, Endo1 knockdown increases weight loss when mice are switched to a lean diet, and decreases weight gain when obese mice are maintained on a high fat diet [106]. Whether or not knockdown of Endo1 can overcome decreased LepR surface expression in BBS and PWS mouse models has yet to be investigated.

3.2 TBC1D1, an obesity variant

While syndromic obesities clearly link certain genetic loci to obesity, other loci implicated in human obesity have been discovered through linkage analysis. One such locus was discovered on chromosome 4p (4p15.1) using data from the San Antonio Family Diabetes Study [107]. Subsequent studies revealed that the variant R125W in the gene *TBC1D1* within 4p15.1 correlated with obesity in females [108]. *TBC1D1* is a gene expressed primarily in skeletal muscle and is important for regulating glucose and lipid uptake by muscle cells. Using these human data, mouse models that manipulate TBC1D1 were engineered to investigate the role of this gene in obesity. Coincidentally, it was revealed that a Tbc1d1 variant in Swiss Jim Lambert (SJL) mice conferred resistance to DIO in this mouse strain [109]. This mouse model implied that variations in the Tbc1d1 gene could alter weight gain in two directions, either protecting against or exacerbating obesity. In order to study the R125W variant that predisposes humans to obesity, Tbc1d1 with the R125W mutation was transfected into mouse skeletal muscle [110]. Introduction of the R125W variant of TBC1D1 into the tibialis anterior muscle in mice impaired insulin-stimulated glucose transport, providing a possible mechanism for obesity in individuals with the

variant. Germline knockin models of *Tbc1d1* variants also produce obesity in mice on lean diets, lending further evidence that this gene regulates energy uptake and storage [111]. Like mouse models of BBS and PWS, Tbc1d1 mice are examples of "reverse translation": using clinical phenotypes to derive basic research models that investigate mechanisms underlying human disease.

3.3 Melanocortin receptor 4, the most common cause of human monogenic obesity

Other mouse models of obesity have shown promising correlation to human obesity. Research on the melanocortin 4 receptor (*MC4R*) is a prime example of the modern interplay between mouse models, the genetic basis for human obesity, and development of effective anti-obesity pharmaceuticals. The viable yellow Agouti mouse (A^{vy}) was one of the original mouse models of obesity, characterized by its late-onset obesity (Agouti obesity syndrome) and unique coloring [10]. The Agouti gene was the first obesity factor cloned (2 years before leptin), and cloning revealed that the mutation in the A^{vy} mouse caused ectopic expression of Agouti peptide [112]. Unlike *ob* and *db* mice, the A^{vy} mouse at the time did not have a reciprocal receptor knockout mouse. This changed in 1997, when researchers developed a MC4R knockout mouse, which recapitulated the phenotype of Agouti obesity syndrome and implicated MC4R as the receptor that mutant Agouti antagonized [113]. Like LepR, MC4R is a hypothalamic receptor that induces satiety when stimulated by ligands from neurons in the arcuate nucleus (ARC) [114]. Using these data from mouse models, it was revealed that mutations in MC4R produce the most common monogenic form of human obesity [115]. The strong evidence that MC4R receptor regulates mouse and human body weight led to the development of additional mouse models that modulate MC4R function. One such model disrupts Melanocortin 2 Receptor Accessory Protein 2 (MRAP2), a protein that interacts directly with MC4R, leading to profound obesity in mice [116]. Human studies revealed that loss-of-function mutations in *MRAP2* lead to hyperphagic obesity associated with hyperglycemia and hypertension in children and adults [116,117]. Notably, a second generation MC4R agonist, setmelanotide, has shown promising results in reducing hyperphagia and obesity in patients with rare genetic obesities, and is currently in a phase III clinical trial for patients with BBS (Clinical trial # NCT03746522) [118].

4. Mouse Models of Neuromodulation to Treat Obesity

Rodent models are instrumental in revealing not only the genetic basis of obesity, but also the neuroanatomic basis for obesity. Rodent models are useful for manipulating neuronal populations that modulate appetite and satiety. "Neuromodulation" of appetitive circuits in mice through various techniques has revealed complex neuronal circuits that interact to modulate feeding behavior. One of the first neuromodulation experiments in rodent models was electrolytic ablation of the hypothalamus, which lead to marked adiposity in lesioned rats [119]. Ablation studies, along with research on hormone receptors revealed the role of the hypothalamus in integrating appetite and satiety cues.

The hypothalamus is a complex brain region composed of at least 11 major nuclei, each regulating multiple homeostatic functions. Adding to this complexity is the heterogeneity of hypothalamic cell types, recently tallied at 34 distinct neuronal cell types in a single cell

RNAseq study [120]. In order to study these and other heterogeneous neuronal populations, modern mouse models of obesity have evolved beyond simple ablation studies and conventional knockout models. In the ARC nucleus, for example, two intermingled neuronal populations communicate opposing appetitive cues to MC4R receptors. One population, Proopiomelanocortin positive (POMC+) neurons, are activated by leptin and induce satiety. This was discovered using a transgenic mouse model expressing enhanced Green Fluorescent Protein (eGFP) in POMC neurons, which allowed the excitability of POMC neurons to be specifically probed in brain slices after leptin administration [121]. The other population, Agouti-Related Peptide positive (AGRP+) neurons, send hunger signals, necessary for feeding. Though AGRP injections were known to induce feeding, the conventional AGRP knockout mouse had no dysregulation in feeding behavior [122]. To further investigate this circuit, a Cre-lox system was employed in mice to more specifically ablate AGRP and POMC neurons in the hypothalamus [123]. Diphtheria toxin receptor (DTR) controlled by a loxP-flanked stop cassette was selectively expressed in AGRP+ cells by Cre recombinase under the control of the AGRP promoter (AGRP-cre). IP injection of diphtheria toxin in these mice killed AGRP neurons in the ARC, leading to decreases in food intake and body weight within 48 hrs, and eventual starvation. This implied that AGRP+ ARC neurons are necessary for regulation of appetite. Moreover, when DTR was expressed in POMC neurons and mice were given diphtheria toxin, mice became hyperphagic and gained weight. Thus, the push and pull of appetite by AGRP+ and POMC+ neurons in the ARC was established using Cre-lox mouse models.

Current mouse models can manipulate neural circuits that control appetite within milliseconds. These highly engineered mouse models modulate neural activity with direct electrical currents, light, designer chemicals, and electromagnetic waves.

4.1 Deep brain stimulation

Deep brain stimulation (DBS) is a direct method of electrical neuromodulation used in mice to influence appetite and satiety. In DBS, current is passed between surgically implanted electrodes in the brain to stimulate neural firing. DBS was first shown to decrease feeding when mice were presented with highly palatable food [124]. In these experiments, electrodes were implanted into the nucleus accumbens (NA), the projection target of the brain's reward pathway. Stimulation of the NA decreased "binge eating" in these animals, and lead to significant weight loss after 4 days of chronic stimulation. Further studies revealed that stimulation of the NA shell inhibited neuronal firing in the Lateral Hypothalamic Area (LHA), providing a possible link between stimulation of the NA and canonical hypothalamic regulation of feeding behavior [125]. DBS as a therapy for human obesity has been attempted with a wide range of results. Numerous case studies have noted success from DBS of the NA, LHA, and Ventromedial Nucleus (VMN) of the hypothalamus, and have been recently reviewed [126]. DBS of the LHA in 4 patients with PWS was ineffective at inducing weight loss or decreasing food cravings, instead causing stimulation-induced manic symptoms in 2 patients [127]. In contrast, DBS of the LHA in 3 patients with refractory obesity was safe, and possibly effective as 2 of the patients had more than a 10% decrease in body weight after 2.5 years of stimulation [128]. More robust studies of DBS in the general

population are necessary to fully understand the safety and efficacy of this therapy in treating obesity.

4.2 Optogenetics

DBS, like ablation studies, lacks the specificity to target specific neuronal subtypes. To simulate particular neuronal populations, the Cre-lox system had to be adapted to express modulatory receptors controlled by cell-type-specific Cre drivers. Ideally, these modulatory receptors would be responsive only to external stimuli applied by the experimenter, to eliminate confounding endogenous signaling from the mice. Studies on the phototaxis responses of the green algae *Chlamydomanas reinhardtii* revealed a unique class of opsin-related proteins called channelrhodopsins that produced light-gated ion conductance in the eye spot of these organisms [129]. When packaged into a lentiviral vector, Channelrhodopsin-2 (ChR2) evoked blue-light-dependent spike chains in rat hippocampal neurons *in vitro*, establishing channelrhodopsins as a tool for selectively stimulating rodent neurons [130].

Stimulation and inhibition of appetitive circuits by light-sensitive channels (optogenetics), is in the forefront in the study of obesity over the last decade. Many studies use stereotactic injection of Adeno-Associated viruses (AAV) to infect neurons with channelrhodopsin. To further dissect the functionality of AGRP and POMC neurons in the ARC, AGRP-Cre and POMC-Cre mice were again used to discriminate between the two neuronal populations [131]. These mice were stereotactically injected with AAV-FLEX-*rev*-ChR2:tdtomato, a virus encoding a fluorescently-labelled ChR2 that would only be expressed in neurons that also expressed Cre. Stimulation of AGRP+ ARC neurons resulted in an 8-fold increase in food consumption over 1 hr in mice with high levels of ChR2 expression. POMC stimulation decreased food consumption over a longer time course, resulting in a cumulative decrease in food intake and body weight after 24hrs.

Optogenetic mouse models have also shed light on novel circuits that regulate appetite and body weight. One such circuit projects from the dorsal raphe nucleus (DRN) of the brainstem to canonical feeding centers in the hypothalamus like the LHA [132]. Like the ARC, the DRN is comprised of 2 populations of neurons that have opposite effects on feeding [133]. Unlike AGRP+ and POMC+ neurons in the ARC which are characterized by unique neuropeptides, the DRN subpopulations are characterized by general inhibitory (Vgat +) or excitatory (VGLUT3+) markers. Injection of Cre-dependent AAV5-EF1a-DIO-ChR2-EYFP into the DRN of Vgat-IRES-Cre mice lead to an increase in food consumption that scaled with photostimulation. Conversely, optogenetic stimulation of the ChR2-infected DRN of VGLUT3-IRES-Cre mice induced the opposite effect, a significant decrease in feeding. In addition to ChR2, this study employed Arch3.0, a light-induced proton pump that inhibits neuronal firing by hyperpolarizing the cell (the opposite effect of ChR2 stimulation). Inhibition of the respective DRN circuits with light-activated Arch3.0 produced the converse outcome of stimulation with ChR2, providing evidence that these circuits can independently regulate appetite bi-directionally.

Optogenetic studies in mice have been valuable in studying obesity outside of the brain as well. Through a combination of techniques, including optogenetics, it was discovered that

leptin acts through the sympathetic nervous system to induce lipolysis in white adipose tissue (WAT) [134]. This was a remarkable finding, indicating that leptin induces weight loss not only through appetite suppression and a general increase in metabolic rate, but also through a direct neural pathway back to the adipose tissue that initially secretes leptin. This study used a Cre-dependent fluorescent reporter (Rosa26-LSL-Tdtomato) crossed with tyrosine hydroxylase Cre (TH-Ccre) to visualize sympathetic innervation of WAT. Then, using this same TH-Cre mouse, ChR2 was expressed in sympathetic neurons by crossing these animals to a transgenic Cre-dependent ChR2 mouse line (Rosa26-LSL-ChR2-YFP). Unilateral optogenetic stimulation of these sympathetic neurons targeted at the inguinal fat pad decreased fat mass to 23% of the untreated side. More recently, it was revealed that the sympathetic stimulation of lipolysis in WAT is dependent on leptin signaling in the ARC through both AGRP+ and POMC+ neurons [135].

Though conventional optogenetics is a powerful tool for neuromodulation, it is not without drawbacks. Notably, the need for chronic cannulation and attachment of a wired device can be limiting to long-term studies and behavioral assays. Recent innovation in lightweight, wireless optogenetic devices can alleviate some of the experimental strain imposed by hardware issues.[136] Wireless optogenetics has been used in mouse models of DIO, preventing weight gain in mice fed a high fat diet and inducing weight loss in DIO mice by stimulating “beige fat” [137].

4.3 Chemogenetics

Other methods of neuromodulation eliminate the need for light-producing hardware altogether. “Chemogenetics” uses designer compounds to activate engineered G protein-coupled receptors (GPCRs) that modulate neuronal excitability and transmission [138]. These GPCRs, also known as designer receptors exclusively activated by designer drug (DREADDs), can be genetically encoded and expressed in particular tissues and cell types through viral or transgenic methods. Clozapine-N-oxide (CNO) is the designer drug used to activate DREADDs, as it is an inert, bioavailable compound that interacts solely with DREADDs [138]. Expression of an inhibitory DREADD (hM4D), in ARC^{AGRP+} neurons using the AGRP-Cre mouse, reduces feeding by 40% after injection of CNO [139]. These appetite-inducing ARC^{AGRP+} neurons were known to act on paraventricular hypothalamus SIM1+ neurons (PVH^{SIM1+}), but the rest of the circuit was not known. Expression of the same inhibitory DREADD in PVH^{SIM1+} neurons revealed a new appetitive circuit from the PVH to the DRN in the brainstem. Injection of CNO into mice expressing hM4D in PVH^{SIM1+} neurons decreased synaptic transmission to the DRN and recapitulated the 40% feeding reduction produced by inhibiting ARC^{AGRP+} neurons [139].

Chemogenetic study of the DRN has extended our knowledge of this circuit. In addition to optogenetic stimulation and inhibition of DRN^{Vgat+} neurons, chemogenetic neuromodulation has revealed the role of these neurons in the regulation of appetite and obesity. Crossing Vgat-IRES-Cre mice to *ob/ob* mice permitted evaluation of this circuit in an obese mouse model. Expression of the DREADD hM4D in the DVN revealed weight loss and feeding reduction over 24 days of CNO injection that was reversed after CNO was withdrawn [133]. In a follow-up study, the DRN of Vgat-IRES-Cre mice was injected with

AAV5-hSyn-DIO-hM3D(Gq)-mCherry, a virus that induced expression of the excitatory hM3D DREADD in DRN^{Vgat+} neurons of these mice [140]. Activation of DRN^{Vgat+} neurons with CNO decreased thermogenesis in mice, indicating that DRN^{Vgat+} neurons both increase appetite and decrease energy expenditure. Further, these neurons were shown to exert their effect on feeding through projections to the Bed Nucleus of the Stria Terminalis (BNST), and the Dorsomedial Hypothalamus (DMH), while regulating temperature chiefly through descending projections to the raphe palladius (RPa).

4.4 Magnetogenetics

An emerging method of neuromodulation used in obesity research is termed "magnetogenetics". As the name implies, magnetogenetics involves genetically-encoded channels that can be controlled by magnetic fields [141]. These genetically encoded channels fuse the cation channel TRPV4 (or TRPV1) to ferritin, an iron-storing protein. The opening of the TRP channel can be controlled by applying a magnetic field to the fusion protein and changing its conformation, allowing ions to flow through the channel and depolarize or hyperpolarize the cell (depending on the channel). This concept was first shown to be effective in vivo by controlling tactile responses in zebrafish, and reward preferences in mice [142]. Magnetogenetics subsequently was used to regulate food intake in mice. Ad-FLEX-anti-GFP-TRPV1/GFP-ferritin was injected into the VMH of glucokinase-Cre (GK-Cre) mice to target the glucose-sensing neurons in the brain [143]. Stimulation of these neurons with a magnetic field increased feeding to levels comparable to optogenetic stimulation of the same neurons with ChR2. This method boasts advantages over optogenetics (notably the lack of hardware on the mice). However, the original TRPV1-based magnetogenetics model (Magneto2.0) recently has come under scrutiny [144]. Though the technique is still in its infancy, magnetogenetics provides a hardware-free approach to neuromodulation that maintains the temporal control of optogenetics.

5. Conclusions

Mouse models of obesity are central to our current understanding of appetite and weight regulation. The discovery of *ob/ob* and *db/db* mice at Jackson Labs paved the way for mice to become an essential model organism for obesity research. Since the cloning of leptin using the *ob/ob* mouse mutation in 1994, enthusiasm for discovering successful obesity therapies has boomed. Current mouse models have moved away from spontaneous mutations like *ob/ob* and *db/db* towards more targeted approaches. Insights into the genetic basis for human obesity coupled with modern methods for genetically engineering mice facilitates the development of models that more accurately reflect human disease. Using these new mouse models, promising therapies are being developed with efficacy in treating human obesity. Further, modern methods of neuromodulation have provided insights into the signals and circuits modulating feeding in real time. Indeed, transgenic mice have powered the ability to dissect individual neural circuits that regulate feeding, metabolism, energy expenditure, and food-based reward (Fig 2). Modulating these circuits with electrodes, light, and designer drugs produces profound and highly specific changes in feeding and body weight. Though clinical therapies that can target appetitive circuits with the precision of transgenic mouse

models are still a long way off, they could be the solution for safe, effective, and personalized obesity therapy.

6. Expert Opinion

Monogenic mouse models have been invaluable for elucidating the molecular mechanisms underlying obesity and metabolic disease. By 2015, mouse models of 221 distinct genes had been studied for obesity-related phenotypes [145]. This list is continually expanding, as are the tools that allow researchers to interrogate the mechanisms behind obesity. Large-scale projects like The GENSAT Project at Rockefeller University have developed hundreds of Bacterial Artificial Chromosome (BAC)-driven GFP and Cre mouse lines that have been used in over 1000 publications [146]. Multi-institutional, international projects have developed suites of neuron specific Cre-drivers and toolboxes of transgenic mice with Cre-dependent optogenetic channels for dissecting the neural circuits that underlie mammalian behavior [147,148]. These herculean efforts provide obesity researchers with thousands of combinations of transgenic mice that can study the interactions between appetitive pathways. While individual circuits are being rigorously mapped and stimulated, discovery of how these pathways work in concert to evoke feeding responses is a crucial next step to precisely modulating satiety in human obesity.

Combining pharmaceuticals that regulate appetite and metabolism can synergistically enhance the effect either signal alone exerts on body weight. This concept has produced a resurgent focus on leptin therapy for obesity. In particular, coupling leptin with gastrointestinal hormones that regulate satiety has proven to increase weight loss over either therapy alone (Table 1).[149] These gastrointestinal hormones are thought to act distally on receptors in the brain and vagus nerve, thus forming a gut-brain axis of satiety. Leptin's interaction with gut hormones in the nervous system could overcome leptin resistance in obesity, similar to the possible effects of endospanin-1 inhibition [104]. Combining these approaches with calorie restriction could lead to increased efficacy in treating weight loss, as well as benefiting longevity and aging [69].

One such gastrointestinal, leptin-sensitizing hormone is amylin, a hormone secreted along with insulin from pancreatic β-cells. Amylin and its analogue, pramlintide, increase leptin responsiveness in rats with diet-induced obesity and overweight humans [150]. Importantly, weight loss was synergistic, and administration of both amylin and leptin elicited greater weight loss than either alone [151]. Amylin's effect on leptin is mediated by IL-6 in the VMH, which enhances LepR signalling [152]. Amylin Pharmaceuticals Inc and Takeda Pharmaceutical Co. Ltd. were developing pramlintide/metreleptin combination treatment for obesity, but discontinued this project in 2011 citing safety concerns [153].

Glucagon-Like Peptide 1 (GLP-1) is an anorexigenic hormone secreted by intestinal enteroendocrine cells that induces satiety by binding its receptor (GLP-1R) in neurons of the hypothalamus and vagus nerve. Specifically, GLP-1, and its analogue, exendin-4, stimulate GLP-1R in POMC+ ARC neurons, which also express LepR [154]. However, GLP-1 also modulates leptin's action through receptors on the vagus nerve [155]. Treating DIO mice with exendin-4 restores leptin sensitivity, and accentuates leptin-induced weight loss and

satiety [156]. A glucagon receptor/GLP-1R co-agonist showed even greater efficacy when coupled with leptin therapy in DIO mice [157]. Treating DIO mice with leptin alone did not decrease weight. However, GLP-1/glucagon produced 26% weight loss after 33 days, while both GLP-1/glucagon and leptin produced 44% weight loss after 33 days. Combination therapy using GLP-1 and amylin analogues together also induces synergistic, long-lasting effects on weight loss in rats [158].

Uroguanylin (UGN) is yet another gut hormone that has shown efficacy in dual therapy with leptin. Uroguanylin is a hormone released by intestinal cells postprandially to induce satiety through its receptor, guanylyl cyclase C (GUCY2C) in the hypothalamus [159]. Central uroguanylin administration induces weight loss by stimulating thermogenesis in brown adipose tissue and increasing peripheral metabolism [160]. GUCY2C protein and mRNA is co-expressed in hypothalamic LepR+ neurons, providing a possible mechanism for UGN's effect on appetite and metabolism [161,162]. Central administration of UGN re-sensitizes DIO mice to leptin, and co-administration of leptin and UGN in the brain induced greater weight loss than administration of either hormone alone [163].

DIO rodent models provided the first insights into these leptin-sensitizing agents and their possible efficacy in human weight loss. FDA approved analogues for each of these hormones are currently used in the clinic. The amylin analog pramlintide (Symlin, Astrazeneca) is currently approved for use to boost the effectiveness of mealtime insulin in diabetes [164]. Various GLP-1R agonists are currently used for inducing weight loss and boosting glucose-dependent insulin secretion in diabetes [165]. The uroguanylin analogue plecanatide (Trulance, Salix) and GUCY2C agonist linaclotide (Linzess, Allergan and Ironwood) are approved for to treat Chronic Idiopathic Constipation (CIC), and Irritable Bowel Syndrome with Constipation (IBS-C) [166]. Combining these therapies with leptin has yet to be proven safe and effective in humans. The promising animal data, coupled with approved therapies that simulate these appetitive gut hormones, presents a tractable approach for combination leptin therapy in the treatment of obesity.

Acknowledgments

Funding:

The authors are supported by the National Institutes of Health, via grants P30 CA56036 R01 CA204881 and R01 CA206026 from the National Cancer Institute to S Waldman and from National Institute of Diabetes and Digestive and Kidney Diseases via grant 1F30DK127639 to J Barton, the Pharmaceutical Research and Manufacturers of America (PhRMA) Foundation, the Courtney Ann Diacont Memorial Foundation, the Defense Congressionally Directed Medical Research Program via grants W81XWH-17-1-0299, W81XWH-17-PRCRP-TTSA, W81XWH-19-1-0067 and W81XWH-19-1-0263 and Targeted Diagnostics and Therapeutics Inc.

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Article highlights

- Obesity is a prevalent, high-risk condition that has benefitted from mice as pre-clinical models.
- Mice with mutations in leptin and the leptin receptor laid the groundwork for our current understanding of the regulation of appetite and metabolism.
- Genetic loci that are implicated in human obesity have been targeted in mouse models to provide attractive pre-clinical models for drug discovery.
- Many current mouse models of obesity focus on dissecting neurological circuits implied in appetite and the regulation of metabolism.
- Modern obesity studies in mouse models take advantage of large repositories of transgenic mice to modulate specific cell types in the brain using optogenetics, chemogenetics, and magnetogenetics.
- Combining gut hormones that signal satiety with leptin therapy reverses “leptin resistance” in rodent models of obesity and could prove to be a safe and effective obesity therapy in humans.

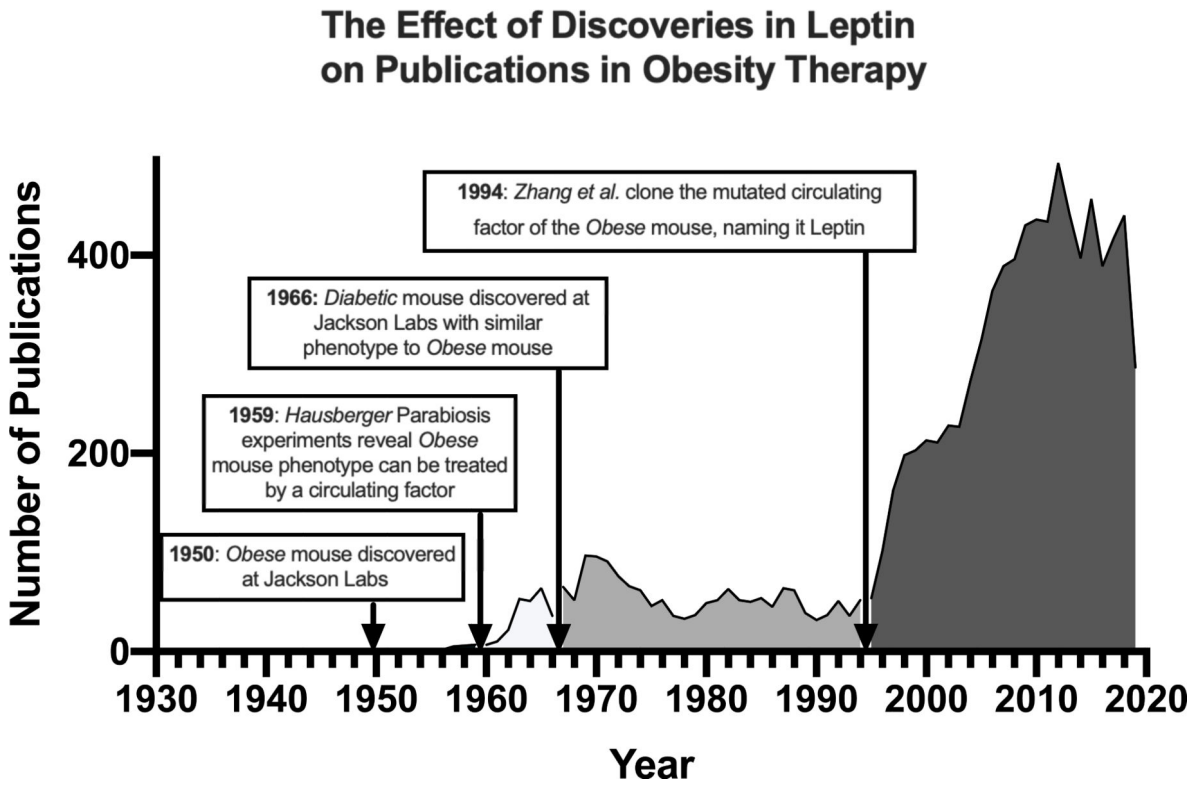

Figure 1: The association of landmark papers using leptin mouse models with the volume of publications in obesity drug discovery.

Pubmed search using mesh search terms: drugs, anti-obesity (search performed May, 2020).

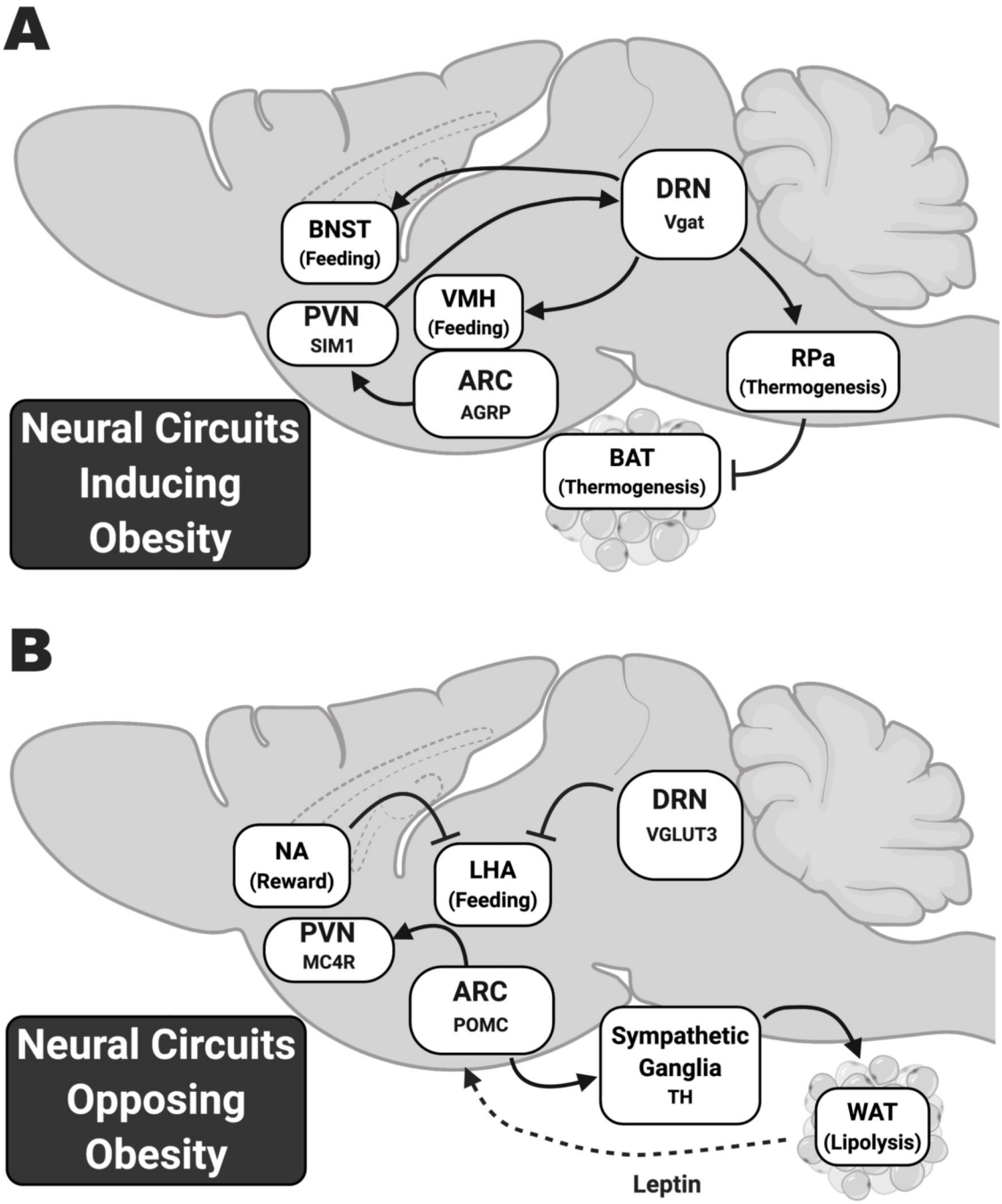

Figure 2: Neural circuits that induce or oppose obesity revealed by mouse models of neuromodulation.

A) Circuits that induce feeding or inhibit thermogenesis. In the Arcuate Nucleus (ARC), neurons that send positive feeding cues are characterized by Agouti Related Peptide (AGRP) and their downstream targets in the Dorsal Raphe Nucleus (DRN) are characterized by Vesicular GABA Transporter (Vgat). **B)** Circuits that induce satiety or lipolysis. ARC neurons that send these signals are characterized by Proopiomelanocortin (POMC), while DRN neurons that oppose feeding are characterized by Vesicular Glutamate Transporter 3 (VGLUT3). While most of these circuits have been investigated with optogenetics or

chemogenetics, Deep Brain Stimulation (DBS) of the Nucleus Accumbens (NA) shows that these neurons reduce feeding by inhibiting the Lateral Hypothalamic Area (LHA). AGRP, Agouti Related Peptide; ARC, Arcuate nucleus; BNST, Bed Nucleus of the Stria Terminalis; BAT, Brown Adipose Tissue; DRN, Dorsal Raphe Nucleus; LHA, Lateral Hypothalamic Area; MC4R, Melanocortin 4 Receptor; NA, Nucleus Accumbens; PVN, Paraventricular Nucleus; RPa, Raphe Palladius; POMC, Proopiomelanocortin; TH, Tyrosine Hydroxylase; VMH, Ventromedial Hypothalamus; Vgat, Vesicular GABA Transporter; VGLUT3, Vesicular Glutamate Transporter 3; WAT, White Adipose Tissue. *Created with* BioRender.com

Table 1:

Leptin-sensitizing gut hormones.

Satiety-signaling gut hormones combined with leptin therapy can induce greater effects on weight loss than either hormone alone in rodent models. Exogenous administration of these hormones with leptin can re-sensitize leptin resistant animals to leptin signaling. The therapeutic potential of these hormones in combination with leptin is highlighted by the current production and use of analogues to many of these hormones in clinical practice. GLP-1, Glugagon-Like Peptide 1; IL-6, Interleukin 6; LepR, Leptin Receptor; ICV, Intracerebroventricular.

Hormone	Hormone Analogue	Efficacy with Leptin	References
Amylin (pancreatic B cells)	**Pramlintide (Symlin, Astrazeneca)**	Synergistic weight loss effect with leptin, mediated by IL-6 in the Ventromedial Hypothalamus, also enhances GLP-1 weight loss	[150–152]
GLP-1 (intestinal L-cells)	**Exendin-4/Exenatide (Byetta, Astrazeneca)**	Restores leptin sensitivity, along with glucagon receptor agonist greatly enhances weight loss over leptin alone, also enhances Amylin weight loss	[157,158]
Uroguanylin (intestinal epithelium)	**Plecanatide (Trulance, Salix), Linaclotide (Linzess, Allergan & Ironwood)**	Re-sensitizes obese mice to leptin, synergistic effect with central co-administration with leptin, induces thermogenesis in brown adipose tissue	[160,161,163]
Cholecystokinin (Intestinal I-cells)	**Sincalide (Kinevac, Bracco Diagnostics)**	Synergistic effect with low-dose ICV leptin, only peripheral Cholecystokinin coupled with ICV leptin was effective	[167,168]
Peptide YY (Intestinal L-cells)	**N/A**	Leptin therapy coupled with Peptide YY increased weight loss over either alone, and extended anorectic effect of Peptide YY	[169]