Novel pathways regulating neuroendocrine function, energy homeostasis and metabolism in humans

Aimilia Eirini Papathanasiou[1], Eric Nolen-Doerr[2], Olivia M Farr[2] and **Christos SMantzoros[2]**

[1]Department of Pediatric Newborn Medicine, Brigham and Women's Hospital/Harvard Medical School and [2]Division of Endocrinology, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, Massachusetts, USA

Abstract

The discovery of leptin, an adipocyte-secreted hormone, set the stage for unraveling the mechanisms dictating energy homeostasis, revealing adipose tissue as an endocrine system that regulates appetite and body weight. Fluctuating leptin levels provide molecular signals to the brain regarding available energy reserves modulating energy homeostasis and neuroendocrine response in states of leptin deficiency and to a lesser extent in hyperleptinemic states. While leptin replacement therapy fails to provide substantial benefit in common obesity, it is an effective treatment for congenital leptin deficiency and states of acquired leptin deficiency such as lipodystrophy. Current evidence suggests that regulation of eating behavior in humans is not limited to homeostatic mechanisms and that the reward, attention, memory and emotion systems are involved, participating in a complex central nervous system network. It is critical to study these systems for the treatment of typical obesity. Although progress has been made, further studies are required to unravel the physiology, pathophysiology and neurobehavioral mechanisms underlying potential treatments for weight-related problems in humans.

Under stable environmental conditions, body weight is under homeostatic control, maintained relatively constant around a set-operating point (1). This weight stability is observed over short and prolonged periods of time (2). The energy homeostatic system is a complex array of central nervous system (CNS) circuitry actively integrating peripheral metabolic feedback signals of either energy abundance or deficit and is much more complicated in humans than in rodents or other animals (1). According to these signals, adaptive changes to

Invited Author's profile

Dr Mantzoros is the Chief of Endocrinology at the VA Boston Healthcare System and Chief of Human Nutrition at Beth Israel Deaconess Medical Center. He serves as Professor of Medicine atHarvard Medical School and the Boston University School of Medicine and has concurrently served as a Professor at Harvard School of Public Health. He has studied physiology and developed novel compounds and has published extensively in the field of metabolism with more than 71,000 citations in Google Scholar, and has received several awards and honorary PhDs. His translational work on leptin, adiponectin and other key molecules in humans has helped elucidate the physiology and develop as therapeutics leptin and other medications, including CHRS-131, currently in Phase III clinical trials.

appetite and energy expenditure maintain body weight around this setpoint (3) (Fig. 1).

One such peripheral signal is leptin. Adipocyte-derived leptin is a feedback signal regulating energy intake and expenditure in mice and lean humans (4). In states of acute energy deficit and/or low body fat mass or leanness, low leptin levels trigger the hypothalamus to increase appetite and decrease energy expenditure in mice (5) and in humans. Our published and unpublished observations in humans indicate that although energy intake may be decreased in response to leptin, energy expenditure is not appreciably altered (6, 7). Although it might be assumed that in states of energy abundance such as obesity, leptin would be equally effective in defending the energy balance by increasing energy expenditure and decreasing appetite, this is typically not the case (8, 9). Other peripheral and/or CNS systems may be enacted to maintain the body weight setpoint (8, 9).

In terms of CNS mechanisms, human studies implicate several cortical and subcortical networks in the eating process, implying that appetite is regulated by higher cognitive functions such as cognitive control, memory, attention and emotions, as well as the reward system (10, 11). Other peripheral signals such as gastrointestinal, adipose tissue and skeletal muscle-derived peptides and hormones (e.g. GLP-1, gastric inhibitory peptide (GIP), adiponectin) also represent feedback signals regarding current energy status (12, 13, 14). Here, we begin discussing

leptin as a well-studied peripheral signal and its role in leanness before discussing typical obesity, which is a state of leptin tolerance or resistance, and the other peripheral/central mechanisms that may be at play in regulating body weight with a focus on human physiology.

Leptin biology

Adipose tissue, once considered to be an inert triglyceride storage organ, may now represent its largest endocrine organ. Circulating leptin, the prototypical adipocyte-derived hormone (15), constitutes a molecular signal to the CNS regarding the currently available amount of energy stored in adipose tissue and acute nutritional status, the circulating concentration of which is directly proportional to total body fat mass (16). Beyond being a longer-term energy signal, abrupt changes in caloric consumption are also reflected by circulating levels of leptin (17). Food restriction results in a sharp decline of leptin levels, at a greater level than would be expected from changes in fat mass, indicating its overall involvement in energy balance and metabolism (17, 18). Leptin also has a circadian rhythm, reaching its maximum level during the early nocturnal sleep period (19).

Leptin receptors are almost ubiquitously expressed in human tissues, highlighting the diversity of its impact on the human body ranging from reproduction to immunity (20). The leptin–leptin receptor complex activates several intracellular signaling pathways such
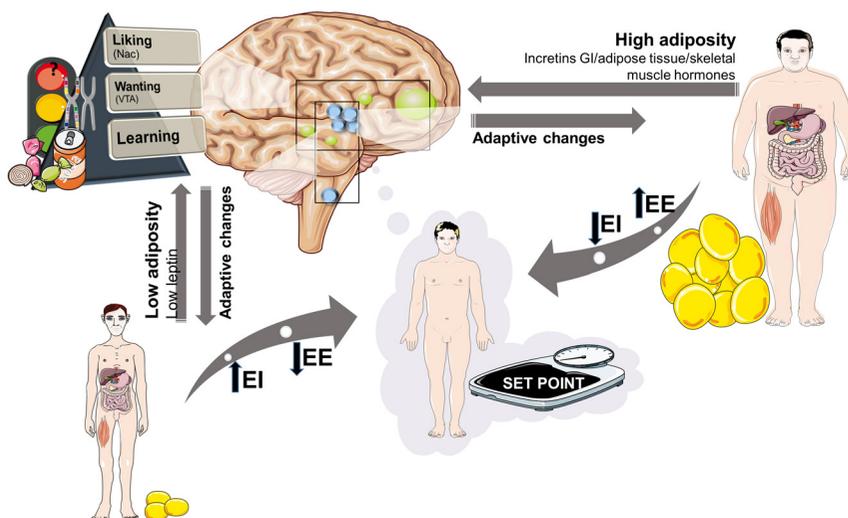

Figure 1

Body weight is determined by a set point, which is individualized based on genetics, hormonal input and neurocognitive changes. When body weight goes above or below this set point, there are certain mechanisms (hormones, energy intake (EI), energy expenditure (EE), brain changes) that will bring body weight back to this point. Treatments now attempt to alter this set point by altering these inputs. Some of the illustrations used have been downloaded from Servier Medical Art Database which provides these illustrations through the Creative Commons license (https://creativecommons.org/licenses/by/3.0/).

as Janus kinase 2-signal transducer and activator of transcription 3 (JAK2-STAT3) (21, 22, 23), insulin receptor substrate (IRS)-phosphatidylinositol 3 kinase (PI3K) (24), SH2-containing protein tyrosine phosphatase 2 (SHP2)-mitogen-activated protein kinase (MAPK) (25) and 5′ adenosine monophosphate activated protein kinase (AMPK) – acetyl-CoA carboxylase (ACC) (26). The aforementioned molecular signaling pathways regulate energy homeostasis, anorectic/orexigenic pathways, glucose and fatty acid metabolism, insulin secretion and activation of cellular proliferation pathways (21, 24, 27, 28).

Central actions

In rat models, leptin receptors are expressed throughout the CNS, in the hypothalamus as well as in cortex, midbrain and hindbrain (29, 30, 31). Within the hypothalamus, a subpopulation of hypothalamic neurons in the arcuate nucleus (ARC) project outside the blood–brain barrier, exhibiting increased accessibility and sensitivity to circulating leptin (32). Two types of leptin receptor-expressing neurons in the ARC, neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons and cocaine- and amphetamine-regulated transcript (CART)/pro-opiomelanocortin (POMC) are responsible for the synthesis of neurotransmitters promoting orexogenic or anorexogenic states, respectively (33, 34, 35, 36, 37, 38, 39). Thus, in states of positive energy balance and higher body fat, the subsequently elevated levels of leptin stimulate the POMC/CART neurons (inhibiting energy intake and promote energy expenditure), while the AgRP/NPY neurons are suppressed (inhibiting energy intake by suppressing appetite) (35, 38). As anticipated, in states of energy deprivation and low body fat levels, low levels of leptin results in the opposite aforementioned trends (40). These data, largely derived from mice, have not yet been confirmed in humans and thus relevant human trials are expected with great anticipation.

Our knowledge regarding the specific regions where leptin acts in the CNS is primarily based on behavioral, cognitive and physiology studies in rodents, the brains and metabolism of which are different than in humans. However, the utilization of continuously evolving neurocognitive and neuroimaging techniques, such as MRI, which allow investigators to noninvasively study brain structure and activation in humans (41). Morphologically, congenital leptin deficiency leads to atrophy of gray matter in regions responsible for the regulation of eating in adults, that reverses with leptin administration (42, 43). In addition, leptin administration affects food-reward and homeostatic related brain regions (43, 44). On the other hand, acquired hypoleptinemia in three female patients was not associated with structural brain differences neither pre nor post leptin treatment (45). In these same patients, short-term administration of leptin increased the activation of cortical areas responsible for decision-related evaluative processes while fasting, when food is expected to be more salient (45). With long-term leptin administration, areas related to food saliency and reward processing in response to visual food stimuli were decreased (45). Hypothalamic activity was also investigated, and, with metreleptin, administration paradoxically increases when viewing food compared to controls at baseline, however, decreases after 24 weeks of metreleptin use in comparison to controls (45). This increased sensitivity to leptin may be explained as a response to chronic leptin deficiency with upregulation of leptin receptors and eventually corrects with leptin repletion (45). Additionally, a decrease in the functional connectivity of the hypothalamus with midcingulate, somatosensory and insular cortices was observed after metreleptin therapy in these women, suggesting a decreased saliency of food images (45). This is opposite to patterns observed in patients with obesity that lose weight and then are given leptin at a dose to return leptin to pre-weight loss levels (46). These trends suggest the complexity of leptin's interaction may depend on the initial etiology, but regardless, may help dictate feeding behavior (45). Whether and how leptin alters body weight and fat mass in lean (leptin-sensitive) vs obese (leptin-tolerant) humans remains to a certain extent unknown, and ongoing human studies along these lines are anticipated with great interest.

Leptin – regulation of neuroendocrine axis

The long active leptin receptor isoform is primarily expressed in the hypothalamus including nuclei involved in the regulation of energy balance (47, 48). This location places leptin in position to communicate energy balance but also to influence hypothalamus–pituitary–endocrine organ axes (49). As outlined below, this influence can vary between axes, species and etiology of leptin biology and dysregulation.

Hypothalamus–pituitary–adrenal (HPA) axis

The hormones contributing to this pathway include corticotropin-releasing hormone (CRH) causing adrenocorticotropic hormone (ACTH) release, which in turn stimulates the adrenal gland to release hormones. Leptin and its receptor are both expressed within the hypothalamus and the pituitary gland, while only the leptin receptor is expressed in the adrenal gland (29, 30, 50, 51). *In vitro* studies demonstrate a direct relation of CRH secretion and leptin administration, a phenomenon also witnessed in lean rats after an 18-h fast (52, 53). In acutely starved mice, the stress response HPA axis activation diminishes with leptin administration, which has not been seen in small-scale studies of lean humans (5, 7, 54). Non-randomized investigations of congenital leptin deficiency do not indicate significant impairment of this axis (55, 56, 57). In our large randomized, placebo-controlled trial, leptin administration in women with hypothalamic amenorrhea induced statistically significant decreases in cortisol levels (58). In a similar manner, we demonstrate that fluctuations in circulating leptin levels inversely relate to those in ACTH and cortisol in healthy men (19). It appears that unlike rodent studies in which the effect is more profound, leptin affects the human HPA axis to a small extent and can be detected in larger, randomized studies.

Hypothalamus–pituitary–thyroid (HPT) axis

The HPT axis begins with thyrotropin-releasing hormone (TRH) inducing production of thyroid-stimulating hormone (TSH) which then stimulates assembly of thyroxine (T4) within the thyroid gland. Like TSH, leptin release exhibits a pulsatile pattern and circadian rhythm that becomes disrupted in deficiency (59). Leptin stimulates rat pro-thyrotropin-releasing hormone (proTRH) gene expression, which produces mature TRH (60, 61). Ob/ob mice display striking features consistent with congenital hypothyroidism that contrasts children with congenital leptin deficiency with normal thyroid function tests (TFTs) (56, 57, 62). Fasting-induced hypoleptinemia depresses T4 levels in rats, and this effect is ameliorated by leptin administration (5, 63). To investigate effects leptin has on TSH changes in men and women after acute caloric deprivation, we performed two studies. In lean men but not lean women, leptin administration blunts the decrease of TSH in response to fasting-induced hypoleptinemia (7, 54). Comparing

these results, leptin levels in the lean women studied did not reach the same magnitude as the men and thus, may suggest a threshold which leptin exerts an effect on the HPT axis (7, 54). Leptin may regulate human TSH secretion at higher levels; however, its role in downstream HPT hormone levels may be variable (6, 27, 64). The HPT axis also exhibits variable responses to leptin administration between relative acute hypoleptinemia and complete deficiency (6, 27, 64). Collectively, these data suggest hypoleptinemia downregulates the HPT axis and leptin replacement restores its function uniformly in rodent models; however, this trend is not consistently observed in human models where the effect of leptin to regulate thyroid hormones is rather limited.

Hypothalamus–pituitary–growth hormone (HPGH) axis

This axis begins with growth hormone-releasing hormone (GHRH) causing growth hormone (GH) production at the pituitary, which interacts with the liver, increasing insulin-like growth factor 1 (IGF-1) production. IGF-1 is associated with at least six IGF-binding proteins (IGFBP1–6), which can hinder and/or promote IGF-1 actions; thus, it is important not only to understand the levels of IGF-1 in total or active forms but also to understand the levels of the IGFBPs. GH and IGF-1 play key roles in body composition as well as growth where they increase muscle growth and lipolysis in adipose tissue, leading to weight loss and a leaner physique (65). Fasting-induced hypoleptinemia in rats inhibits GH secretion that reverses with leptin administration (66). Patients with congenital leptin deficiency present with normal linear growth during childhood; however, final adult height is decreased due to absent, sex hormone-driven, pubertal growth spurt (56, 57, 67). In contrast to rodents, humans with fasting-induced hypoleptinemia demonstrate increases in GH pulsatility, enhancement of GH secretion and total GH production and decreased IGF-1 levels and increased IGFBP3 (68, 69, 70). Total levels of IGFBP1, 4 and 6 did not show changes with leptin (70). Leptin administration restores IGF-1 levels in men but not women; however, no amelioration of GH pulsatility is observed in either sex (7, 54, 70). The same is true for states of acquired leptin deficiency, such as hypothalamic amenorrhea and anorexia nervosa (71, 72, 73). The varying IGF-1 response to leptin administration between our studies in men and women may again highlight a threshold for leptin action on

this axis (56, 57). Our group hypothesized that leptin may not directly be involved with GHRH-mediated GH secretion, but rather GH-associated IGF-1 secretion and its binding proteins (70). Aside from those mentioned above, other IGFBPs (total and/or intact) remain to be investigated to unravel the complex relationship between leptin and the HPGH axis in humans and these studies are underway.

Hypothalamus–pituitary–gonadal (HPG) axis

The hypothalamic component of this axis is gonadotropin-releasing hormone (GnRH) which stimulates the pituitary's gonadotropes to produce luteinizing (LH) and follicle-stimulating hormones (FSHs) which interact with sex-specific gonadal structures to produce androgens or estrogens. Human reproductive biology demands substantial energy investments and, therefore, is tightly associated with energy balance (74). In rodent models, leptin demonstrates profound influence over reproductive processes including puberty and ovulation by acting as a gatekeeper, permitting progression when adequate energy reserve exists (75, 76, 77, 78, 79, 80). Humans with congenital leptin deficiency display a similar reproductive phenotype of hypogonadotrophic hypogonadism with axis function restoration after leptin treatment despite absent leptin receptors on GnRH-secreting neurons (56, 57, 81, 82, 83). It has been suggested that leptin does not directly act upon GnRH neurons but rather interacts with neurons afferent to them, such as AGP/NPY, POMC, and arcuate nucleus neurons expressing kisspeptin, bradykinin and dynorphin via KiSS-1 neurons (83, 84, 85, 86).

Disruptions in LH pulsatility and decreases in testosterone levels occur during fasting-induced hypoleptinemia in lean men, while in lean women LH peak frequency decreases, and in both cases, leptin restores to baseline with leptin administration (7, 54, 87). Women experiencing chronic energy deprivation may develop hypothalamic amenorrhea related to a severe decline in leptin levels, which is amendable to leptin administration. Women with chronic hypoleptinemia caused by hypothalamic amenorrhea have dysfunctional HPG function which is restored with leptin therapy (88, 89). Thus, leptin strongly influences the HPG axis in humans, and this physiological function has important pathophysiological and potentially therapeutic implications.

Hypoleptinemic states

Lipodystrophy: the case of lack of adipose tissue and low leptin

Lipodystrophy can be broadly categorized into generalized or partial forms and can be congenital or acquired. Generalized lipodystrophy indicates whole body subcutaneous adipose tissue loss while partial lipodystrophy describes local loss and redistribution of this tissue (90). While lipodystrophy is considered rare, objective diagnostic criteria are not yet established, often requiring long-term follow-up for confirmation (91). Conversely, acquired forms are more common and are often seen in patients receiving highly active antiretroviral therapy with a prevalence ranging from 13 to 70% (92, 93).

Complication severity with lipodystrophy, including abnormal glucose (insulin resistance, diabetes), hypertriglyceridemia and nonalcoholic fatty liver disease, correlates with the amount of adipose tissue loss and highlights the importance of adipose tissue as an active endocrine organ (93, 94).

Patients with lipodystrophy can also present with neuroendocrine axis abnormalities. While pubertal development is appropriate, these patients still have attenuated LH response to luteinizing hormone-releasing hormone, irregular menses and amenorrhea, all of which can be corrected with leptin treatment (80, 95). The thyroid, adrenal and GH axes do not exhibit profound dysfunctions, and leptin treatment only significantly increases IGF-1 levels (95, 96).

The first studies investigating recombinant human methionyl leptin (metreleptin) as a replacement therapy in lipodystrophy demonstrate dramatic improvement in most metabolic abnormalities, including insulin resistance (80). Given the observed potential development of leptin-neutralizing antibodies, the US Food and Drug Administration approved metreleptin in 2014 for the treatment of congenital and acquired generalized lipodystrophy, and in Japan, it is approved for all types of lipodystrophy by the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) (97). Studies, which supported the approval of metreleptin for lipodystrophy treatment have been open-label in design with small sample sizes (80, 98, 99, 100, 101). Results from much-needed ongoing RCTs will more clearly evaluate metreleptin's safety, efficacy and cost-effectiveness (90).

Hypothalamic amenorrhea

Hypothalamic amenorrhea (HA) represents a chronic energy-deficient state associated with excessive energy expenditure, decreased caloric consumption or excessive stress causing infertility, neuroendocrine abnormalities and osteoporosis (89). Independent of body fat, exercise intensity is inversely related to serum estradiol and directly related to leptin levels (102). Amenorrheic athletes display lower leptin levels than their eumenorrheic counterparts (103).

The dysregulation of ovulatory function results from an absent LH surge, a consequence of impaired pulsatile secretion of GnRH (88). Other hormones, the activin-follistatin system, have been implicated as a largely leptin-independent system that also modulates the HPG axis during acute and chronic hypoleptinemic states (104). Hormones of this system include activin A, activin B, follistatin and follistatin-like 3 (FSTL3) of which all, excluding follistatin, decrease in response to states of energy deprivation with subsequent downregulation of the HPG axis through interactions with the pituitary gland and the gonads to decrease follicular growth, hormone production, Sertoli cell proliferation and germ cell development (104, 105, 106). The development of HA in chronic energy-deficient states illustrates impairment of the HPG axis; however, it appears each of the hypothalamic axes are affected as an adaptive mechanism for homeostatic maintenance. Activation of the HPA axis occurs in response to this stress, identified by increasing levels of CRH, ACTH, cortisol and increased adrenal sensitivity to ACTH (107, 108). Conversely, suppression of the HPT axis occurs with low-normal TRH, decreased T3 and increased reverse T3 (88). Despite increased 24-h GH levels, basal IGF-1 concentration is significantly decreased (70, 109), but the exact effect on free IGF-1, IGFBPs, and total vs intact components of the IGF axis remains to be fully elucidated.

Our group's proof-of-concept pilot study and subsequent randomized, placebo-controlled trial assessing leptin as a possible treatment of for HA demonstrated that leptin resulted in recovery of menstruation and improved neuroendocrine dysfunction with increased fT3 levels, IGF1:IGFBP3 ratio and decreased cortisol levels (5, 6, 54, 70, 73). Regarding the activin-follistatin system, leptin administration improved only levels of activin B, again reinforcing the leptin-independent nature of this system (104). These results illustrate that chronic hypoleptinemia influences neuroendocrine abnormalities associated with HA primarily in the HPG, HPT, HPA and to a lesser extent

the hypothalamic–pituitary–GH axis (6) – the effect on which remains to be fully elucidated by ongoing studies.

Another consequence of ovulatory dysfunction and decreased estrogen levels in HA is impairments in bone health. In these models, leptin acts to positively regulate bone metabolism (110). Leptin treatment for 4 weeks in HA women results in increased markers of bone formation which remained significantly elevated during the study period without significant effects on bone resorption markers (6, 73). Two-year metreleptin treatment significantly increases bone mineral density at the lumbar spine by 4–6% (58). Intact parathyroid hormone (iPTH) and receptor activator of nuclear factor kappa-B ligand (RANKL) to osteoprotegerin (OPG) ratio levels were significantly decreased after 36 weeks of leptin treatment (111). These trends of iPTH and RANKL:OPG are postulated in being beneficial to bone metabolism (111, 112, 113).

According to the 2017 Endocrine Society Clinical Practice guidelines, leptin is not recommended for the improvement of bone metabolism or fertility treatment in adolescents and women with functional HA. The small yet significant weight loss, development of leptin antibodies during treatment with the currently available compound and alternative therapeutic options such as cognitive behavioral therapy limit the use of the current formulation of leptin as a treatment for HA (97, 114, 115). We have shown, however, that with leptin dose adjustments one could avoid weight loss while still maintaining the desired hormonal (6) and metabolic and bone metabolism outcomes (58) without any decreases in lean body mass (116). In other words, when achieved leptin levels remain within a range of physiological leptin levels, neuroendocrine abnormalities and fertility are restored, and weight loss occurs only when leptin levels increase above the upper physiological level. Moreover, the weight lost was primarily fat mass whereas lean mass remained unchanged or increased. Larger studies for longer study periods with careful metabolic monitoring and dose adjustments need to be conducted so as safety and efficacy could be assessed (114). Moreover, investigations into alternative leptin analogs not triggering anti-leptin antibodies could potentially alleviate immunogenic concerns.

Common obesity

Obesity is a worldwide health problem directly related to leading causes of preventable death including

cardiovascular disease, type 2 diabetes and cancer (3). In an effort to address the complexity of this chronic medical problem, obesity has been declared as a disease by several scientific organizations (3).

Evidence from adoption, twin and family studies estimate heritability of BMI between 40 and 70%, highlighting the role of genetic contribution in obesity (117). Seventy-nine syndromes associated with obesity are reported in the literature with a substantial portion following Mendelian patterns of inheritance stressing the role genetics has in obesity (118). The most common cause of monogenic obesity results from mutations in the leptin–melanocortin pathway encoding the MC4R receptor (119). However, these mutations account for less than 10% extreme obesity cases (3). Genome-wide association studies support a polygenic nature for common obesity by identifying hundreds of SNPs associated with this pathology (120). Modifiable epigenetic changes from *in utero* and postnatal exposures have also been associated with obesity and may be targets to prevent future adverse metabolic outcomes (121). Looking at this modifiable factor, two pregnancy cohorts suggested that maternal adherence to a Mediterranean diet in early stages of pregnancy resulted in lower child adiposity and improved cardiometabolic outcomes (122).

Environmental factors related to diet, food intake and physical activity have also been linked to obesity. Larger portions of low-nutrient, high-caloric, palatable food consumed at irregular meal patterns by sedentary individuals are all factors promoting obesity (123). With the use of functional MRI (fMRI), internal factors including post-prandial decreased activation in reward-related putamen and salience- and reward-related insula in fasting subjects with obesity and prediabetes are hypothesized to underle caloric intake, which leads to these cnditioms (124). Altogether, these environmental and central mechanisms in combination with genetic, epigenetic and other mechanisms create a complex disease in need of effective therapies.

Following observations of dramatic weight reducing effects of leptin administration in rodents and humans with congenital leptin deficiency, leptin was assumed to be an anti-obesity hormone (125). Although the discovery of leptin generated hopes of successfully treating obesity, results from clinical trials in typical obesity demonstrate significant weight loss only with supraphysiologic doses not well tolerated, thus limiting its application as an anti-obesity medication in clinical practice (126, 127). While leptin treatment alone may not be feasible in typical obesity, other effective medications have been developed. However, downstream effectors of leptin may still be effective at treating obesity, and some, such as melanocortin receptor agonists, are currently being investigated for their potential use in obesity (128, 129, 130, 131).

Pointed investigation of pharmaceutical targets for obesity treatment largely distills from observational peripheral hormone changes following bariatric surgery (132). These hormones include, but are not limited to, leptin, gastrin, ghrelin, cholecystokinin, GLP-1, oxyntomodulin, GIP and peptide YY (PYY) (133, 134). Many of these molecules have been (GLP-1 as liraglutide) or are being developed (oxyntomodulin, GIP, etc.) for the treatment of obesity. Other medications approved for obesity target the brain directly, instead of the periphery, to alter appetite (e.g. lorcaserin, phentermine/topiramate, bupropion/naltrexone).

Liraglutide is a GLP-1 receptor agonist that acts peripherally and centrally to induce weight loss through its interplay with POMC/CART and AgRP/NPY neurons while also displaying interaction with cortical areas in mice (135). Our group identified presence of GLP-1 receptors in the hypothalamus, medulla and parietal cortex of humans (136). In rodent models, GLP-1 suppresses the excitatory input destined for the mesolimbic dopamine neurons of reward pathways causing decreases in food intake (137). This pathway highlights just one of the many neural circuits where GLP-1 acts; however, there is minimal interaction with cortical centers in rodents (138). In humans, there appears to be additional GLP-1 action more peripherally in cortical areas of the CNS, as we recently demonstrated. We performed a randomized, controlled trial to demonstrate that GLP-1 receptors are expressed in several brain areas including the parietal cortex (136). Liraglutide treatment decreases brain activation of this and similar cortical areas, and therefore, the attention and reward networks, in response to highly desirable food cues (136). Interestingly, the amplification of fasting-induced hypoleptinemia in response to liraglutide treatment, increased activation of reward-related brain regions and decreased activation of attention-related brain areas (11). This highlights the compensatory role of low leptin levels in appetite and provides a theoretical rationale for combination therapies of GLP-1 with leptin analogs (11).

Lorcaserin is a medication which acts centrally as a 5-hydroxytryptamine 2C (5HT2C) receptor agonist possibly through stimulation of POMC neurons (139, 140). Our group demonstrated in a RCT that lorcaserin decreased food cue activation of brain networks associated with attention and saliency, again, highlighting more

interactions with cortical systems in humans (10, 11). In contrast to rodent studies with lorcaserin, hypothalamic activation was not observed and highlights interspecies differences of brain complexity/function (10, 141). Our study further determined that baseline activation of the amygdala (a component of the emotional/limbic system) was directly correlated to weight loss success with lorcaserin, suggesting it decreases food intake by decreasing the emotional significance of highly palatable food cues (10). Future research will continue to delineate the brain areas which are impacted by anti-obesity therapies. Defining the brain areas in which different medications work to treat obesity work in humans, using fMRI and/or PET imaging, can lead to the elucidation of underlying mechanisms in humans, which have brains very different from the brains in rodents that have served as the experimental model to date, and thus, could lead to effective single or combination therapies leveraging complementary mechanisms.

Conclusions

Ultimately, experimental data from rodents generate hypotheses and provide mechanisms that may or may not directly translate to human physiology, making it difficult to directly translate hypotheses raised by studies in rodents into findings and data in humans indicating the necessity for novel translational methods and efforts that would be performed in an ethical manner but apparently overcoming the inconsistencies associated with historical methods. Rapid advancement and availability of neuroimaging, such as fMRI, PET scans or other novel techniques, allow for in-depth investigation of the neurophysiological underpinnings of weight balance in human brains, and in the future, may help further elucidate pathways responsible for weight regulation and obesity. Treating underlying pathology with novel medical therapies to address the problem of dysregulated energy homeostasis proves daunting given the complexity of pathways contributing to the body's response toward energy balance.

While metreleptin shows clear evidence of efficacy for treating obesity and leptin-deficient states, the side effect profile and immunogenicity of the currently available formulation limits widespread use and endorsement for disease states other than complete leptin deficiency. Further investigation into compounding leptin with other substances for synergistic and/or development of novel compounds with less immunogenic effects are ongoing.

Ultimately, in order to further investigate leptin's role in the future, more RCTs with greater number of participants and thus power are needed and appropriate use of databases as well as real-world trials and/or the leverage of patient registries worldwide may prove to be more effective.

Appetite and body weight regulation in humans is complicated, involving the integration of massive amounts of data reflecting the status of our internal and external environments. A seemingly infinite interplay of hormones, receptors, neurons and cells all coordinate to shape our body and its response in an ever-changing environment. Analysis of these data will require a combination of efforts of scientists working not only in human physiology and therapeutics but also in biology and neurosciences and the vast amounts of data to be collected would require novel mathematical modeling for their analysis and reduction to practice in order for us to be able to provide tangible benefits to suffering humans in the not so distant future (142, 143).

References

1 Woods SC & D'Alessio DA. Central control of body weight and appetite. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** (11 Supplement 1) S37–S50. (https://doi.org/10.1210/jc.2008-1630)

2 Bray GA. Weight homeostasis. *Annual Review of Medicine* 1991 **42** 205–216. (https://doi.org/10.1146/annurev.me.42.020191.001225)

3 Upadhyay J, Farr O, Perakakis N, Ghaly W & Mantzoros C. Obesity as a disease. *Medical Clinics of North America* 2018 **102** 13–33. (https://doi.org/10.1016/j.mcna.2017.08.004)

4 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994 **372** 425–432. (https://doi.org/10.1038/372425a0)

5 Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E & Flier JS. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996 **382** 250–252. (https://doi.org/10.1038/382250a0)

6 Chou SH, Chamberland JP, Liu X, Matarese G, Gao C, Stefanakis R, Brinkoetter MT, Gong H, Arampatzi K & Mantzoros CS. Leptin is an effective treatment for hypothalamic amenorrhea. *PNAS* 2011 **108** 6585–6590. (https://doi.org/10.1073/pnas.1015674108)

7 Chan JL, Matarese G, Shetty GK, Raciti P, Kelesidis I, Aufiero D, De Rosa V, Perna F, Fontana S & Mantzoros CS. Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. *PNAS* 2006 **103** 8481–8486. (https://doi.org/10.1073/pnas.0505429103)

8 Flier JS & Maratos-Flier E. Leptin's physiologic role: does the emperor of energy balance have no clothes? *Cell Metabolism* 2017 **26** 24–26. (https://doi.org/10.1016/j.cmet.2017.05.013)

9 Ravussin Y, Leibel RL & Ferrante AW Jr. A missing link in body weight homeostasis: the catabolic signal of the overfed state. *Cell Metabolism* 2014 **20** 565–572. (https://doi.org/10.1016/j.cmet.2014.09.002)

10 Farr OM, Upadhyay J, Gavrieli A, Camp M, Spyrou N, Kaye H, Mathew H, Vamvini M, Koniaris A & Kilim H. Lorcaserin administration decreases activation of brain centers in response to food cues and these emotion- and salience-related changes correlate with weight loss effects: a 4-week-long randomized, placebo-controlled, double-blind clinical trial. *Diabetes* 2016 **65** 2943–2953. (https://doi.org/10.2337/db16-0635)

11 Farr OM, Tsoukas MA, Triantafyllou G, Dincer F, Filippaios A, Ko BJ & Mantzoros CS. Short-term administration of the GLP-1 analog liraglutide decreases circulating leptin and increases GIP levels and these changes are associated with alterations in CNS responses to food cues: a randomized, placebo-controlled, crossover study. *Metabolism: Clinical and Experimental* 2016 **65** 945–953. (https://doi.org/10.1016/j.metabol.2016.03.009)

12 Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes* 2004 **53** (Supplement 1) S143–S151. (https://doi.org/10.2337/diabetes.53.2007.S143)

13 Wren AM & Bloom SR. Gut hormones and appetite control. *Gastroenterology* 2007 **132** 2116–2130. (https://doi.org/10.1053/j.gastro.2007.03.048)

14 Karras S, Goulis DG, Mintziori G, Katsiki N & Tzotzas T. The effects of incretins on energy homeostasis: physiology and implications for the treatment of type 2 diabetes mellitus and obesity. *Current Vascular Pharmacology* 2012 **10** 781–791. (https://doi.org/10.2174/157016112803520909)

15 Lonnqvist F, Arner P, Nordfors L & Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nature Medicine* 1995 **1** 950–953. (https://doi.org/10.1038/nm0995-950)

16 Klein S, Luu K, Gasic S & Green A. Effect of weight loss on whole body and cellular lipid metabolism in severely obese humans. *American Journal of Physiology* 1996 **270** E739–E745.

17 Boden G, Chen X, Mozzoli M & Ryan I. Effect of fasting on serum leptin in normal human subjects. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3419–3423. (https://doi.org/10.1210/jcem.81.9.8784108)

18 Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D & Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 1730–1736. (https://doi.org/10.1210/jc.2002-021604)

19 Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, Bongiorno PB, Chrousos GP, Karp B, Allen C, Flier JS *et al*. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nature Medicine* 1997 **3** 575–579. (https://doi.org/10.1038/nm0597-575)

20 Cottrell EC & Mercer JG. Leptin receptors. In *Appetite Control*, pp 3–21. Ed H-G Joost. Berlin, Heidelberg: Springer, 2012.

21 Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E *et al*. STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 2003 **421** 856–859. (https://doi.org/10.1038/nature01388)

22 Moon HS, Huh JY, Dincer F, Schneider BE, Hasselgren PO & Mantzoros CS. Identification and saturable nature of signaling pathways induced by Metreleptin in humans: comparative evaluation of in vivo, ex vivo, and in vitro administration. *Diabetes* 2015 **64** 828–839. (https://doi.org/10.2337/db14-0625)

23 Chan JL, Moschos SJ, Bullen J, Heist K, Li X, Kim YB, Kahn BB & Mantzoros CS. Recombinant methionyl human leptin administration activates signal transducer and activator of transcription 3 signaling in peripheral blood mononuclear cells in vivo and regulates soluble tumor necrosis factor-alpha receptor levels in humans with relative leptin deficiency. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 1625–1631.

24 Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG Jr & Schwartz MW. Intracellular signalling. Key enzyme in leptin-induced anorexia. *Nature* 2001 **413** 794–795. (https://doi.org/10.1038/35101657)

25 Mauvoisin D, Prevost M, Ducheix S, Arnaud MP & Mounier C. Key role of the ERK1/2 MAPK pathway in the transcriptional regulation of the stearoyl-CoA desaturase (SCD1) gene expression in response to leptin. *Molecular and Cellular Endocrinology* 2010 **319** 116–128. (https://doi.org/10.1016/j.mce.2010.01.027)

26 Chen PC, Kryukova YN & Shyng SL. Leptin regulates KATP channel trafficking in pancreatic beta-cells by a signaling mechanism involving AMP-activated protein kinase (AMPK) and cAMP-dependent protein kinase (PKA). *Journal of Biological Chemistry* 2013 **288** 34098–34109. (https://doi.org/10.1074/jbc.M113.516880)

27 Kelesidis T, Kelesidis I, Chou S & Mantzoros CS. Narrative review: the role of leptin in human physiology: emerging clinical applications. *Annals of Internal Medicine* 2010 **152** 93–100. (https://doi.org/10.7326/0003-4819-152-2-201001190-00008)

28 Robertson SA, Leinninger GM & Myers MG Jr. Molecular and neural mediators of leptin action. *Physiology and Behavior* 2008 **94** 637–642. (https://doi.org/10.1016/j.physbeh.2008.04.005)

29 Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J *et al*. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995 **83** 1263–1271. (https://doi.org/10.1016/0092-8674(95)90151-5)

30 Elmquist JK, Bjorbaek C, Ahima RS, Flier JS & Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. *Journal of Comparative Neurology* 1998 **395** 535–547. (https://doi.org/10.1002/(SICI)1096-9861(19980615)395:4<535::AID-CNE9>3.0.CO;2-2)

31 Leshan RL, Bjornholm M, Munzberg H & Myers MG Jr. Leptin receptor signaling and action in the central nervous system. *Obesity* 2006 **14** (Supplement 5) 208S–12S. (https://doi.org/10.1038/oby.2006.310)

32 Faouzi M, Leshan R, Bjornholm M, Hennessey T, Jones J & Munzberg H. Differential accessibility of circulating leptin to individual hypothalamic sites. *Endocrinology* 2007 **148** 5414–5423. (https://doi.org/10.1210/en.2007-0655)

33 Zhu P, Zhang Z, Huang X, Liang S, Khandekar N, Song Z & Lin S. RANKL reduces body weight and food intake via the modulation of hypothalamic NPY/CART expression. *International Journal of Medical Sciences* 2018 **15** 969–977. (https://doi.org/10.7150/ijms.24373)

34 Elias CF, Lee CE, Kelly JF, Ahima RS, Kuhar M, Saper CB & Elmquist JK. Characterization of CART neurons in the rat and human hypothalamus. *Journal of Comparative Neurology* 2001 **432** 1–19. (https://doi.org/10.1002/cne.1085)

35 Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB & Elmquist JK. Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* 1998 **21** 1375–1385. (https://doi.org/10.1016/S0896-6273(00)80656-X)

36 Hahn TM, Breininger JF, Baskin DG & Schwartz MW. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nature Neuroscience* 1998 **1** 271–272. (https://doi.org/10.1038/1082)

37 Erickson JC, Hollopeter G & Palmiter RD. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science* 1996 **274** 1704–1707. (https://doi.org/10.1126/science.274.5293.1704)

38 Mizuno TM & Mobbs CV. Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. *Endocrinology* 1999 **140** 814–817. (https://doi.org/10.1210/endo.140.2.6491)

39 Krashes MJ, Shah BP, Koda S & Lowell BB. Rapid versus delayed stimulation of feeding by the endogenously released AgRP neuron mediators GABA, NPY, and AgRP. *Cell Metabolism* 2013 **18** 588–595. (https://doi.org/10.1016/j.cmet.2013.09.009)

40 Mantzoros CS, Qu D, Frederich RC, Susulic VS, Lowell BB, Maratos-Flier E & Flier JS. Activation of beta(3) adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. *Diabetes* 1996 **45** 909–914. (https://doi.org/10.2337/diab.45.7.909)

41 Glover GH. Overview of functional magnetic resonance imaging. *Neurosurgery Clinics of North America* 2011 **22** 133–9, vii. (https://doi.org/10.1016/j.nec.2010.11.001)

42 Matochik JA, London ED, Yildiz BO, Ozata M, Caglayan S, DePaoli AM, Wong ML & Licinio J. Effect of leptin replacement on brain structure in genetically leptin-deficient adults. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 2851–2854. (https://doi.org/10.1210/jc.2004-1979)

43 Frank S, Heni M, Moss A, von Schnurbein J, Fritsche A, Haring HU, Farooqi S, Preissl H & Wabitsch M. Leptin therapy in a congenital leptin-deficient patient leads to acute and long-term changes in homeostatic, reward, and food-related brain areas. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E1283–E1287. (https://doi.org/10.1210/jc.2010-2713)

44 Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S & Fletcher PC. Leptin regulates striatal regions and human eating behavior. *Science* 2007 **317** 1355. (https://doi.org/10.1126/science.1144599)

45 Farr OM, Fiorenza C, Papageorgiou P, Brinkoetter M, Ziemke F, Koo BB, Rojas R & Mantzoros CS. Leptin therapy alters appetite and neural responses to food stimuli in brain areas of leptin-sensitive subjects without altering brain structure. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E2529–E2538. (https://doi.org/10.1210/jc.2014-2774)

46 Hinkle W, Cordell M, Leibel R, Rosenbaum M & Hirsch J. Effects of reduced weight maintenance and leptin repletion on functional connectivity of the hypothalamus in obese humans. *PLoS ONE* 2013 **8** e59114. (https://doi.org/10.1371/journal.pone.0059114)

47 Gorska E, Popko K, Stelmaszczyk-Emmel A, Ciepiela O, Kucharska A & Wasik M. Leptin receptors. *European Journal of Medical Research* 2010 **15** (Supplement 2) 50–54.

48 Huang Q, Rivest R & Richard D. Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (ob/ob) mice. *Endocrinology* 1998 **139** 1524–1532. (https://doi.org/10.1210/endo.139.4.5889)

49 Khan SM, Hamnvik OP, Brinkoetter M & Mantzoros CS. Leptin as a modulator of neuroendocrine function in humans. *Yonsei Medical Journal* 2012 **53** 671–679. (https://doi.org/10.3349/ymj.2012.53.4.671)

50 Glasow A, Haidan A, Hilbers U, Breidert M, Gillespie J, Scherbaum WA, Chrousos GP & Bornstein SR. Expression of Ob receptor in normal human adrenals: differential regulation of adrenocortical and adrenomedullary function by leptin. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 4459–4466.

51 Roubos EW, Dahmen M, Kozicz T & Xu L. Leptin and the hypothalamo-pituitary-adrenal stress axis. *General and Comparative Endocrinology* 2012 **177** 28–36. (https://doi.org/10.1016/j.ygcen.2012.01.009)

52 Uehara Y, Shimizu H, Ohtani K, Sato N & Mori M. Hypothalamic corticotropin-releasing hormone is a mediator of the anorexigenic effect of leptin. *Diabetes* 1998 **47** 890–893. (https://doi.org/10.2337/diabetes.47.6.890)

53 Costa A, Poma A, Martignoni E, Nappi G, Ur E & Grossman A. Stimulation of corticotrophin-releasing hormone release by the obese (ob) gene product, leptin, from hypothalamic explants. *NeuroReport* 1997 **8** 1131–1134. (https://doi.org/10.1097/00001756-199703240-00014)

54 Chan JL, Heist K, DePaoli AM, Veldhuis JD & Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *Journal of Clinical Investigation* 2003 **111** 1409–1421. (https://doi.org/10.1172/JCI200317490)

55 Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998 **392** 398–401. (https://doi.org/10.1038/32911)

56 Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA & O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *New England Journal of Medicine* 1999 **341** 879–884. (https://doi.org/10.1056/NEJM199909163411204)

57 Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *Journal of Clinical Investigation* 2002 **110** 1093–1103. (https://doi.org/10.1172/JCI0215693)

58 Sienkiewicz E, Magkos F, Aronis KN, Brinkoetter M, Chamberland JP, Chou S, Arampatzi KM, Gao C, Koniaris A & Mantzoros CS. Long-term Metreleptin treatment increases bone mineral density and content at the lumbar spine of lean hypoleptinemic women. *Metabolism: Clinical and Experimental* 2011 **60** 1211–1221. (https://doi.org/10.1016/j.metabol.2011.05.016)

59 Mantzoros CS, Ozata M, Negrao AB, Suchard MA, Ziotopoulou M, Caglayan S, Elashoff RM, Cogswell RJ, Negro P, Liberty V et al. Synchronicity of frequently sampled thyrotropin (TSH) and leptin concentrations in healthy adults and leptin-deficient subjects: evidence for possible partial TSH regulation by leptin in humans. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 3284–3291. (https://doi.org/10.1210/jcem.86.7.7644)

60 Legradi G, Emerson CH, Ahima RS, Flier JS & Lechan RM. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. *Endocrinology* 1997 **138** 2569–2576. (https://doi.org/10.1210/endo.138.6.5209)

61 Sanchez VC, Goldstein J, Stuart RC, Hovanesian V, Huo L, Munzberg H, Friedman TC, Bjorbaek C & Nillni EA. Regulation of hypothalamic prohormone convertases 1 and 2 and effects on processing of prothyrotropin-releasing hormone. *Journal of Clinical Investigation* 2004 **114** 357–369. (https://doi.org/10.1172/JCI21620)

62 van der Kroon PH, Boldewijn H & Langeveld-Soeter N. Congenital hypothyroidism in latent obese (ob/ob) mice. *International Journal of Obesity* 1982 **6** 83–90.

63 Seoane LM, Carro E, Tovar S, Casanueva FF & Dieguez C. Regulation of in vivo TSH secretion by leptin. *Regulatory Peptides* 2000 **92** 25–29. (https://doi.org/10.1016/S0167-0115(00)00145-2)

64 Dardeno TA, Chou SH, Moon HS, Chamberland JP, Fiorenza CG & Mantzoros CS. Leptin in human physiology and therapeutics. *Frontiers in Neuroendocrinology* 2010 **31** 377–393. (https://doi.org/10.1016/j.yfrne.2010.06.002)

65 Vijayakumar A, Yakar S & Leroith D. The intricate role of growth hormone in metabolism. *Frontiers in Endocrinology* 2011 **2** 32.

66 Carro E, Senaris R, Considine RV, Casanueva FF & Dieguez C. Regulation of in vivo growth hormone secretion by leptin.

Endocrinology 1997 **138** 2203–2206. (https://doi.org/10.1210/endo.138.5.5238)

67 Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, Lank E, Bottomley B, Lopez-Fernandez J, Ferraz-Amaro I *et al*. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *New England Journal of Medicine* 2007 **356** 237–247. (https://doi.org/10.1056/NEJMoa063988)

68 Ho KY, Veldhuis JD, Johnson ML, Furlanetto R, Evans WS, Alberti KG & Thorner MO. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *Journal of Clinical Investigation* 1988 **81** 968–975. (https://doi.org/10.1172/JCI113450)

69 Hartman ML, Veldhuis JD, Johnson ML, Lee MM, Alberti KG, Samojlik E & Thorner MO. Augmented growth hormone (GH) secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 757–765. (https://doi.org/10.1210/jcem.74.4.1548337)

70 Chan JL, Williams CJ, Raciti P, Blakeman J, Kelesidis T, Kelesidis I, Johnson ML, Thorner MO & Mantzoros CS. Leptin does not mediate short-term fasting-induced changes in growth hormone pulsatility but increases IGF-I in leptin deficiency states. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2819–2827. (https://doi.org/10.1210/jc.2008-0056)

71 Laughlin GA, Dominguez CE & Yen SS. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 25–32.

72 Misra M, Miller KK, Bjornson J, Hackman A, Aggarwal A, Chung J, Ott M, Herzog DB, Johnson ML & Klibanski A. Alterations in growth hormone secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5615–5623. (https://doi.org/10.1210/jc.2003-030532)

73 Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A & Mantzoros CS. Recombinant human leptin in women with hypothalamic amenorrhea. *New England Journal of Medicine* 2004 **351** 987–997. (https://doi.org/10.1056/NEJMoa040388)

74 Mathew H, Castracane VD & Mantzoros C. Adipose tissue and reproductive health. *Metabolism: Clinical and Experimental* 2018 **86** 18–32. (https://doi.org/10.1016/j.metabol.2017.11.006)

75 Mounzih K, Lu R & Chehab FF. Leptin treatment rescues the sterility of genetically obese ob/ob males. *Endocrinology* 1997 **138** 1190–1193. (https://doi.org/10.1210/endo.138.3.5024)

76 Chehab FF, Mounzih K, Lu R & Lim ME. Early onset of reproductive function in normal female mice treated with leptin. *Science* 1997 **275** 88–90. (https://doi.org/10.1126/science.275.5296.88)

77 Chehab FF, Lim ME & Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics* 1996 **12** 318–320. (https://doi.org/10.1038/ng0396-318)

78 Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK & Steiner RA. Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology* 1997 **138** 855–858. (https://doi.org/10.1210/endo.138.2.5054)

79 Barkan D, Hurgin V, Dekel N, Amsterdam A & Rubinstein M. Leptin induces ovulation in GnRH-deficient mice. *FASEB Journal* 2005 **19** 133–135. (https://doi.org/10.1096/fj.04-2271fje)

80 Oral EA, Ruiz E, Andewelt A, Sebring N, Wagner AJ, Depaoli AM & Gorden P. Effect of leptin replacement on pituitary hormone regulation in patients with severe lipodystrophy. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 3110–3117. (https://doi.org/10.1210/jcem.87.7.8591)

81 Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S *et al*. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus,

hypogonadism, and behavior in leptin-deficient adults. *PNAS* 2004 **101** 4531–4536. (https://doi.org/10.1073/pnas.0308767101)

82 Strobel A, Issad T, Camoin L, Ozata M & Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nature Genetics* 1998 **18** 213–215. (https://doi.org/10.1038/ng0398-213)

83 Quennell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, Herbison AE, Grattan DR & Anderson GM. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. *Endocrinology* 2009 **150** 2805–2812. (https://doi.org/10.1210/en.2008-1693)

84 Hill JW, Elmquist JK & Elias CF. Hypothalamic pathways linking energy balance and reproduction. *American Journal of Physiology. Endocrinology and Metabolism* 2008 **294** E827–E832. (https://doi.org/10.1152/ajpendo.00670.2007)

85 Lehman MN, Coolen LM & Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology* 2010 **151** 3479–3489. (https://doi.org/10.1210/en.2010-0022)

86 Smith JT, Acohido BV, Clifton DK & Steiner RA. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *Journal of Neuroendocrinology* 2006 **18** 298–303. (https://doi.org/10.1111/j.1365-2826.2006.01417.x)

87 Chan JL, Wong SL & Mantzoros CS. Pharmacokinetics of subcutaneous recombinant methionyl human leptin administration in healthy subjects in the fed and fasting states: regulation by gender and adiposity. *Clinical Pharmacokinetics* 2008 **47** 753–764. (https://doi.org/10.2165/00003088-200847110-00006)

88 Berga SL, Mortola JF, Girton L, Suh B, Laughlin G, Pham P & Yen SS. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. *Journal of Clinical Endocrinology and Metabolism* 1989 **68** 301–308. (https://doi.org/10.1210/jcem-68-2-301)

89 Chan JL & Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet* 2005 **366** 74–85. (https://doi.org/10.1016/S0140-6736(05)66830-4)

90 Polyzos SA & Mantzoros CS. Lipodystrophy: time for a global registry and randomized clinical trials to assess efficacy, safety and cost-effectiveness of established and novel medications. *Metabolism: Clinical and Experimental* 2017 **72** A4–A10. (https://doi.org/10.1016/j.metabol.2017.06.003)

91 Rother KI & Brown RJ. Novel forms of lipodystrophy: why should we care?. *Diabetes Care* 2013 **36** 2142–2145. (https://doi.org/10.2337/dc13-0561)

92 Domingo P, Estrada V, Lopez-Aldeguer J, Villaroya F & Martinez E. Fat redistribution syndromes associated with HIV-1 infection and combination antiretroviral therapy. *AIDS Reviews* 2012 **14** 112–123.

93 Fiorenza CG, Chou SH & Mantzoros CS. Lipodystrophy: pathophysiology and advances in treatment. *Nature Reviews. Endocrinology* 2011 **7** 137–150. (https://doi.org/10.1038/nrendo.2010.199)

94 Nolis T. Exploring the pathophysiology behind the more common genetic and acquired lipodystrophies. *Journal of Human Genetics* 2014 **59** 16–23. (https://doi.org/10.1038/jhg.2013.107)

95 Musso C, Cochran E, Javor E, Young J, Depaoli AM & Gorden P. The long-term effect of recombinant methionyl human leptin therapy on hyperandrogenism and menstrual function in female and pituitary function in male and female hypoleptinemic lipodystrophic patients. *Metabolism: Clinical and Experimental* 2005 **54** 255–263. (https://doi.org/10.1016/j.metabol.2004.08.021)

96 Lungu AO, Zadeh ES, Goodling A, Cochran E & Gorden P. Insulin resistance is a sufficient basis for hyperandrogenism in lipodystrophic women with polycystic ovarian syndrome. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 563–567. (https://doi.org/10.1210/jc.2011-1896)

97 Meehan CA, Cochran E, Kassai A, Brown RJ & Gorden P. Metreleptin for injection to treat the complications of leptin deficiency in patients with congenital or acquired generalized lipodystrophy. *Expert Review of Clinical Pharmacology* 2016 **9** 59–68. (https://doi.org/10.1586/17512433.2016.1096772)

98 Chan JL, Lutz K, Cochran E, Huang W, Peters Y, Weyer C & Gorden P. Clinical effects of long-term Metreleptin treatment in patients with lipodystrophy. *Endocrine Practice* 2011 **17** 922–932. (https://doi.org/10.4158/EP11229.OR)

99 Diker-Cohen T, Cochran E, Gorden P & Brown RJ. Partial and generalized lipodystrophy: comparison of baseline characteristics and response to Metreleptin. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 1802–1810. (https://doi.org/10.1210/jc.2014-4491)

100 Park JY, Javor ED, Cochran EK, DePaoli AM & Gorden P. Long-term efficacy of leptin replacement in patients with Dunnigan-type familial partial lipodystrophy. *Metabolism: Clinical and Experimental* 2007 **56** 508–516. (https://doi.org/10.1016/j.metabol.2006.11.010)

101 Simha V, Subramanyam L, Szczepaniak L, Quittner C, Adams-Huet B, Snell P & Garg A. Comparison of efficacy and safety of leptin replacement therapy in moderately and severely hypoleptinemic patients with familial partial lipodystrophy of the Dunnigan variety. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 785–792. (https://doi.org/10.1210/jc.2011-2229)

102 Puder JJ, Monaco SE, Sen Gupta S, Wang J, Ferin M & Warren MP. Estrogen and exercise may be related to body fat distribution and leptin in young women. *Fertility and Sterility* 2006 **86** 694–699. (https://doi.org/10.1016/j.fertnstert.2006.02.085)

103 Ackerman KE, Slusarz K, Guereca G, Pierce L, Slattery M, Mendes N, Herzog DB & Misra M. Higher ghrelin and lower leptin secretion are associated with lower LH secretion in young amenorrheic athletes compared with eumenorrheic athletes and controls. *American Journal of Physiology. Endocrinology and Metabolism* 2012 **302** E800–E806. (https://doi.org/10.1152/ajpendo.00598.2011)

104 Perakakis N, Upadhyay J, Ghaly W, Chen J, Chrysafi P, Anastasilakis AD & Mantzoros CS. Regulation of the activins-follistatins-inhibins axis by energy status: impact on reproductive function. *Metabolism: Clinical and Experimental* 2018 **85** 240–249. (https://doi.org/10.1016/j.metabol.2018.05.003)

105 Makanji Y, Zhu J, Mishra R, Holmquist C, Wong WP, Schwartz NB, Mayo KE & Woodruff TK. Inhibin at 90: from discovery to clinical application, a historical review. *Endocrine Reviews* 2014 **35** 747–794. (https://doi.org/10.1210/er.2014-1003)

106 Wijayarathna R & de Kretser DM. Activins in reproductive biology and beyond. *Human Reproduction Update* 2016 **22** 342–357. (https://doi.org/10.1093/humupd/dmv058)

107 Kondoh Y, Uemura T, Murase M, Yokoi N, Ishikawa M & Hirahara F. A longitudinal study of disturbances of the hypothalamic-pituitary-adrenal axis in women with progestin-negative functional hypothalamic amenorrhea. *Fertility and Sterility* 2001 **76** 748–752. (https://doi.org/10.1016/S0015-0282(01)02000-3)

108 Bomba M, Gambera A, Bonini L, Peroni M, Neri F, Scagliola P & Nacinovich R. Endocrine profiles and neuropsychologic correlates of functional hypothalamic amenorrhea in adolescents. *Fertility and Sterility* 2007 **87** 876–885. (https://doi.org/10.1016/j.fertnstert.2006.09.011)

109 Genazzani AD, Petraglia F, Gastaldi M, Gamba O, Corazza F, D'Ambrogio G & Genazzani AR. Growth hormone (GH)-releasing hormone-induced GH response in hypothalamic amenorrhea: evidence of altered central neuromodulation. *Fertility and Sterility* 1996 **65** 935–938. (https://doi.org/10.1016/S0015-0282(16)58264-8)

110 Chou SH & Mantzoros C. Bone metabolism in anorexia nervosa and hypothalamic amenorrhea. *Metabolism: Clinical and Experimental* 2018 **80** 91–104. (https://doi.org/10.1016/j.metabol.2017.10.009)

111 Foo JP, Polyzos SA, Anastasilakis AD, Chou S & Mantzoros CS. The effect of leptin replacement on parathyroid hormone, RANKL-osteoprotegerin axis, and Wnt inhibitors in young women with hypothalamic amenorrhea. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E2252–E2258. (https://doi.org/10.1210/jc.2014-2491)

112 Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ & Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *Journal of Bone and Mineral Research* 2000 **15** 2–12. (https://doi.org/10.1359/jbmr.2000.15.1.2)

113 Ma YL, Cain RL, Halladay DL, Yang X, Zeng Q, Miles RR, Chandrasekhar S, Martin TJ & Onyia JE. Catabolic effects of continuous human PTH (1-38) in vivo is associated with sustained stimulation of RANKL and inhibition of osteoprotegerin and gene-associated bone formation. *Endocrinology* 2001 **142** 4047–4054. (https://doi.org/10.1210/endo.142.9.8356)

114 Gordon CM, Ackerman KE, Berga SL, Kaplan JR, Mastorakos G, Misra M, Murad MH, Santoro NF & Warren MP. Functional hypothalamic amenorrhea: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 1413–1439. (https://doi.org/10.1210/jc.2017-00131)

115 Michopoulos V, Mancini F, Loucks TL & Berga SL. Neuroendocrine recovery initiated by cognitive behavioral therapy in women with functional hypothalamic amenorrhea: a randomized, controlled trial. *Fertility and Sterility* 2013 **99** 2084–2091.e1. (https://doi.org/10.1016/j.fertnstert.2013.02.036)

116 Brinkoetter M, Magkos F, Vamvini M & Mantzoros CS. Leptin treatment reduces body fat but does not affect lean body mass or the myostatin-follistatin-activin axis in lean hypoleptinemic women. *American Journal of Physiology. Endocrinology and Metabolism* 2011 **301** E99–E104. (https://doi.org/10.1152/ajpendo.00146.2011)

117 Stryjecki C, Alyass A & Meyre D. Ethnic and population differences in the genetic predisposition to human obesity. *Obesity Reviews* 2018 **19** 62–80. (https://doi.org/10.1111/obr.12604)

118 Kaur Y, de Souza RJ, Gibson WT & Meyre D. A systematic review of genetic syndromes with obesity. *Obesity Reviews* 2017 **18** 603–634. (https://doi.org/10.1111/obr.12531)

119 Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T & O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *New England Journal of Medicine* 2003 **348** 1085–1095. (https://doi.org/10.1056/NEJMoa022050)

120 Pigeyre M, Yazdi FT, Kaur Y & Meyre D. Recent progress in genetics, epigenetics and metagenomics unveils the pathophysiology of human obesity. *Clinical Science* 2016 **130** 943–986. (https://doi.org/10.1042/CS20160136)

121 van Dijk SJ, Molloy PL, Varinli H, Morrison JL, Muhlhausler BS & Members of EpiSCOPE. Epigenetics and human obesity. *International Journal of Obesity* 2015 **39** 85–97. (https://doi.org/10.1038/ijo.2014.34)

122 Chatzi L, Rifas-Shiman SL, Georgiou V, Joung KE, Koinaki S, Chalkiadaki G, Margioris A, Sarri K, Vassilaki M, Vafeiadi M *et al.* Adherence to the Mediterranean diet during pregnancy and offspring adiposity and cardiometabolic traits in childhood. *Pediatric Obesity* 2017 **12** (Supplement 1) 47–56. (https://doi.org/10.1111/ijpo.12191)

123 Heymsfield SB & Wadden TA. Mechanisms, pathophysiology, and management of obesity. *New England Journal of Medicine* 2017 **376** 1492.

124 Farr OM & Mantzoros CS. Obese individuals with more components of the metabolic syndrome and/or prediabetes demonstrate decreased activation of reward-related brain centers in response to food cues in both the fed and fasting states: a preliminary fMRI study. *International Journal of Obesity* 2017 **41** 471–474. (https://doi.org/10.1038/ijo.2016.231)

125 Flier JS. Clinical review 94: what's in a name? In search of leptin's physiologic role. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1407–1413.

126 Shetty GK, Matarese G, Magkos F, Moon HS, Liu X, Brennan AM, Mylvaganam G, Sykoutri D, Depaoli AM & Mantzoros CS. Leptin

administration to overweight and obese subjects for 6 months increases free leptin concentrations but does not alter circulating hormones of the thyroid and IGF axes during weight loss induced by a mild hypocaloric diet. *European Journal of Endocrinology* 2011 **165** 249–254. (https://doi.org/10.1530/EJE-11-0252)

127 Paz-Filho G, Mastronardi CA & Licinio J. Leptin treatment: facts and expectations. *Metabolism: Clinical and Experimental* 2015 **64** 146–156. (https://doi.org/10.1016/j.metabol.2014.07.014)

128 Chen KY, Muniyappa R, Abel BS, Mullins KP, Staker P, Brychta RJ, Zhao X, Ring M, Psota TL, Cone RD *et al*. RM-493, a melanocortin-4 receptor (MC4R) agonist, increases resting energy expenditure in obese individuals. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 1639–1645. (https://doi.org/10.1210/jc.2014-4024)

129 Kievit P, Halem H, Marks DL, Dong JZ, Glavas MM, Sinnayah P, Pranger L, Cowley MA, Grove KL & Culler MD. Chronic treatment with a melanocortin-4 receptor agonist causes weight loss, reduces insulin resistance, and improves cardiovascular function in diet-induced obese rhesus macaques. *Diabetes* 2013 **62** 490–497. (https://doi.org/10.2337/db12-0598)

130 Kumar KG, Sutton GM, Dong JZ, Roubert P, Plas P, Halem HA, Culler MD, Yang H, Dixit VD & Butler AA. Analysis of the therapeutic functions of novel melanocortin receptor agonists in MC3R- and MC4R-deficient C57BL/6J mice. *Peptides* 2009 **30** 1892–1900. (https://doi.org/10.1016/j.peptides.2009.07.012)

131 Srivastava G & Apovian C. Future pharmacotherapy for obesity: new anti-obesity drugs on the horizon. *Current Obesity Reports* 2018 **7** 147–161. (https://doi.org/10.1007/s13679-018-0300-4)

132 Ribeiro-Parenti L, Cavin JB & Le Gall M. Intestinal adaptations following bariatric surgery: towards the identification of new pharmacological targets for obesity-related metabolic diseases. *Current Opinion in Pharmacology* 2017 **37** 29–34. (https://doi.org/10.1016/j.coph.2017.08.002)

133 Meek CL, Lewis HB, Reimann F, Gribble FM & Park AJ. The effect of bariatric surgery on gastrointestinal and pancreatic peptide hormones. *Peptides* 2016 **77** 28–37. (https://doi.org/10.1016/j.peptides.2015.08.013)

134 Sweeney TE & Morton JM. Metabolic surgery: action via hormonal milieu changes, changes in bile acids or gut microbiota? A summary of the literature. *Best Practice and Research. Clinical Gastroenterology* 2014 **28** 727–740. (https://doi.org/10.1016/j.bpg.2014.07.016)

135 Patel DK & Stanford FC. Safety and tolerability of new-generation anti-obesity medications: a narrative review. *Postgraduate Medicine* 2018 **130** 173–182. (https://doi.org/10.1080/00325481.2018.1435129)

136 Farr OM, Sofopoulos M, Tsoukas MA, Dincer F, Thakkar B, Sahin-Efe A, Filippaios A, Bowers J, Srnka A, Gavrieli A *et al*. GLP-1 receptors exist in the parietal cortex, hypothalamus and medulla of human brains and the GLP-1 analogue liraglutide alters brain activity related to highly desirable food cues in individuals with diabetes: a crossover, randomised, placebo-controlled trial. *Diabetologia* 2016 **59** 954–965. (https://doi.org/10.1007/s00125-016-3874-y)

137 Wang XF, Liu JJ, Xia J, Liu J, Mirabella V & Pang ZP. Endogenous glucagon-like Peptide-1 suppresses high-fat food intake by reducing synaptic drive onto mesolimbic dopamine neurons. *Cell Reports* 2015 **12** 726–733. (https://doi.org/10.1016/j.celrep.2015.06.062)

138 Kanoski SE, Hayes MR & Skibicka KP. GLP-1 and weight loss: unraveling the diverse neural circuitry. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 2016 **310** R885–R895. (https://doi.org/10.1152/ajpregu.00520.2015)

139 Burke LK & Heisler LK. 5-Hydroxytryptamine medications for the treatment of obesity. *Journal of Neuroendocrinology* 2015 **27** 389–398. (https://doi.org/10.1111/jne.12287)

140 Pasqualetti M, Ori M, Castagna M, Marazziti D, Cassano GB & Nardi I. Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. *Neuroscience* 1999 **92** 601–611. (https://doi.org/10.1016/S0306-4522(99)00011-1)

141 Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H *et al*. Activation of central melanocortin pathways by fenfluramine. *Science* 2002 **297** 609–611. (https://doi.org/10.1126/science.1072327)

142 Perakakis N, Yazdani A, Karniadakis GE & Mantzoros C. Omics, big data and machine learning as tools to propel understanding of biological mechanisms and to discover novel diagnostics and therapeutics. *Metabolism: Clinical and Experimental* 2018 **87** A1–A9. (https://doi.org/10.1016/j.metabol.2018.08.002)

143 DeGregory KW, Kuiper P, DeSilvio T, Pleuss JD, Miller R, Roginski JW, Fisher CB, Harness D, Viswanath S, Heymsfield SB *et al*. A review of machine learning in obesity. *Obesity Reviews* 2018 **19** 668–685. (https://doi.org/10.1111/obr.12667)

European Journal of Endocrinology