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The Hunger Genes: Pathways to Obesity

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The global rise in the prevalence of obesity and associated co-morbidities such as type 2 diabetes, cardiovascular disease, and cancer represents a major public health concern. The biological response to increased consumption of palatable foods or a reduction in energy expenditure is highly variable between individuals. A more detailed mechanistic understanding of the molecular, physiological, and behavioral pathways involved in the development of obesity in susceptible individuals is critical for identifying effective mechanism-based preventative and therapeutic interventions.

Introduction

Obesity is defined as an increase in fat mass that is sufficient to adversely affect health (Sperrin et al., 2014; Whitlock et al., 2009). While the absolute quantification of fat mass is usually only performed in the research setting, body mass index (BMI; weight in kg/height in meters^2) is a useful surrogate marker. Using the World Health Organization (WHO) definition of a BMI more than 30 kg/m^2 to define obesity, 30% of Americans and 10%–20% of Europeans are classified as obese, with the prevalence rising in many developing countries (http://www.who.int). As body mass index increases, so does the relative risk of type 2 diabetes, hypertension, and cardiovascular disease (Berrington de Gonzalez et al., 2010). Furthermore, an increase in the prevalence of childhood obesity (11%–17% in Europe and the US) has driven an increase in medical problems such as type 2 diabetes mellitus in adolescents (Fagot-Campagna, 2000). At a societal level, obesity is associated with disability, mortality, and substantial health costs. At an individual level, severe obesity is often associated with a multitude of clinical problems, including sleep disturbance and respiratory difficulties, joint and mobility issues, as well as considerable social stigma, which can affect quality of life as well as educational attainment and job opportunities (Puhl and Brownell, 2001).

In this Review, we provide a perspective on the contribution of environmental, genetic, and other factors to the development of obesity. We discuss how these factors impact the molecular and physiological mechanisms that regulate energy intake and energy expenditure in humans and highlight ongoing strategies to dissect the complex neural circuits and pathways that modulate energy homeostasis and their potential to be targeted by preventative and therapeutic interventions.

Obesity as a Disorder of Energy Homeostasis

Humans, like other mammals, are able to regulate their body weight over long periods of time despite day-to-day variation in the number of calories consumed and in levels of energy expenditure, irrespective of the level of adiposity. Fundamentally, factors that influence changes in body weight must ultimately disrupt the balance between energy intake and expenditure over time, the utilization of substrates (fat, protein, carbohydrate), and/or nutrient partitioning (storage of excess calories). Physiological studies in healthy normal weight individuals have shown that total energy expenditure decreases by an average of 10% with acute caloric restriction and increases with caloric excess (Ravussin et al., 2014). However, in humans, the homeostatic regulation of energy balance is easily overwhelmed by external stimuli. For example, in a study in which people were given free access to food, the average daily intake exceeded 150% of energy requirements. In such experimental settings, and potentially in the free-living environment, some individuals seem more readily able to resist weight change with overeating, possibly due to inter-individual variation in the energy costs of weight gain (Ravussin et al., 2014).

Environmental Factors Drive the Rise in Obesity Prevalence

The increasing prevalence of obesity worldwide (an approximate doubling in the last 30 years), the inverse relationship between obesity and socioeconomic class, and the secular trend toward increasing obesity in developing countries associated with urbanization provide clear evidence of the environmental influences on weight gain (Ogden et al., 2014; Popkin, 2006). The adoption of relatively sedentary lifestyles due to reduced physical activity at work and in leisure time coupled with an abundance of easily available, energy-rich, highly palatable foods represents a nutrition transition that, according to the World Health Organization, is now one of the greatest risk factors for ill health worldwide (http://www.hsph.harvard.edu) (Figure 1). Interestingly, some recent analyses of trends in obesity prevalence have suggested a decline or stabilization of obesity prevalence, especially in children in the US and some European countries, findings that are consistent with dynamic models using prevalence data and birth and death rates (Ogden et al., 2014; Thomas et al., 2014). However, many countries have either increasing (China) or decreasing (European countries) birth rates, so the potential global impact of these estimations is not readily predictable. Recent studies show that second-generation migrants to the US from all ethnic groups are heavier than their parents who migrated but that people from some ethnic groups are more likely to gain weight than others upon

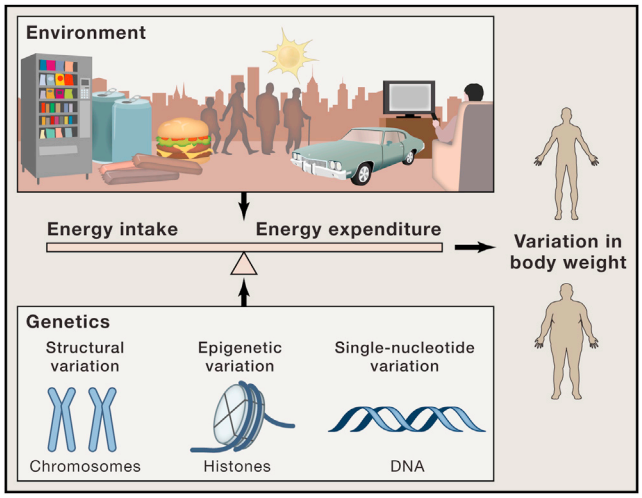

Figure 1. Contribution of Genes and Environmental Factors to Weight Gain

Human adiposity is influenced by complex interactions between genetic and environmental influences. The current environment potently facilitates the development of obesity. Abundance of highly processed food has a major impact on energy intake, whereas numerous other environmental factors, such as television watching, leisure activities, and transport, negatively affect energy expenditure. In any environment, there is a variation in body fat and BMI in large part influenced by genetic variation disrupting energy homeostasis by either decreasing energy expenditure or increasing energy intake.

transitioning to a more obesogenic environment (Singh and Lin, 2013), suggesting that, in addition to strong environmental drivers, genetic factors play a role in influencing obesity susceptibility.

Individual Susceptibility to Weight Gain Is Highly Variable—Role of Genetic Factors

In any environment, whether energy rich or energy lacking, there is considerable individual variation in body weight and fat mass, suggesting that human adiposity is influenced by complex interactions between genetic, developmental, behavioral, and environmental influences. Evidence for genetic contributions to body weight comes from family, twin, and adoption studies, which cumulatively demonstrate that the heritability (fraction of the total phenotypic variance of a quantitative trait attributable to genes in a specified environment) of BMI is between 0.71 and 0.86 (Silventoinen et al., 2008). Heritability estimates can change over time and can differ between populations. Recent studies in a UK sample of 5,092 twin pairs aged 8–11 years growing up during a time of dramatic rises in obesity confirmed substantial heritability for BMI and waist circumference (77% for both), while there was a very modest shared-environment effect, and the remaining environmental variance was unshared (Wardle et al., 2008b). Interestingly, similar heritability estimates have been found when studying monozygotic and dizygotic twins who were reared together and apart (Allison et al., 1996) and in adoption studies in which adopted children were discovered to have body sizes that were more similar to those of their biological parents than their adopted parents (Sørensen et al., 1989).

The high heritability of phenotypes related to obesity supports the contribution of genetic factors but does not indicate the number of genes or how those genes interact with environmental factors. The "thrifty gene hypothesis" suggests that we harbor genetic variants that favor efficient food collection and fat deposition to survive periods of famine and that, in the face of the easy availability of food, these genes/variants are disadvantageous. However, an alternative hypothesis is that obesity is selected against by the risk of predation. This hypothesis suggests that random mutations and genetic drift, rather than directed selection, have influenced changes in the population distribution of fat mass that may be more readily reconcilable with the findings that, even in Western societies, most people are not obese (Speakman, 2007).

Evidence for the interaction of inherited factors with changes in energy intake and expenditure was provided by landmark experimental overfeeding studies by Bouchard and colleagues, who showed that weight gain induced by overfeeding mono- and dizygous twin pairs under direct supervision was highly correlated within twin pairs but varied widely among pairs of twins (Bouchard et al., 1990). Similarly, the response to negative energy balance via an exercise regime was also heritable (Bouchard et al., 1996). Notably, the inter-twin correlations were greater for weight loss than for weight gain, suggesting tighter biological control of the response to negative energy balance.

Hypothalamic Circuits Regulating Energy Homeostasis

Ultimately, signals from cumulative genetic and environmental influences that reflect changing energy status have to be detected and integrated by brain circuits that can, through their projections, regulate energy balance. In the early 1900s, clinical reports of patients with tumors involving hypothalamo-pituitary structures associated with food-seeking behavior and obesity suggested that the hypothalamus may be involved in the regulation of body weight. Chemical and electrolytic lesioning experiments in animals in the 1930s and 1940s established the key role of the hypothalamus in the regulation of energy homeostasis. The degree of weight gain/weight loss seen in these experiments was, in part, determined by the size and precise location of the lesions, which suggested that there were specific hypothalamic circuits that promote or suppress feeding behavior (Anand and Brobeck, 1951; Hetherington and Ranson, 1940).

The hypothalamus is essentially a hub for key circuits that integrate sensory inputs; compare those inputs to basic "set points," or parameters for body temperature, electrolyte balance, sleep-wake cycle, circadian rhythms, and energy homeostasis; and then initiate a set of responses by activating autonomic, endocrine, and behavioral outputs that aim to maintain these set points (homeostasis). The hypothalamus regulates autonomic nervous system activation via neurons that directly innervate parasympathetic and sympathetic preganglionic neurons, as well as neurons in the brainstem that control autonomic reflexes. Individual pre-autonomic neurons project to multiple levels of the spinal cord, where they selectively innervate end organs such as the heart, kidney, and adipose tissue. Autonomic innervation of the pancreas contributes to the regulation of insulin and glucagon secretion.

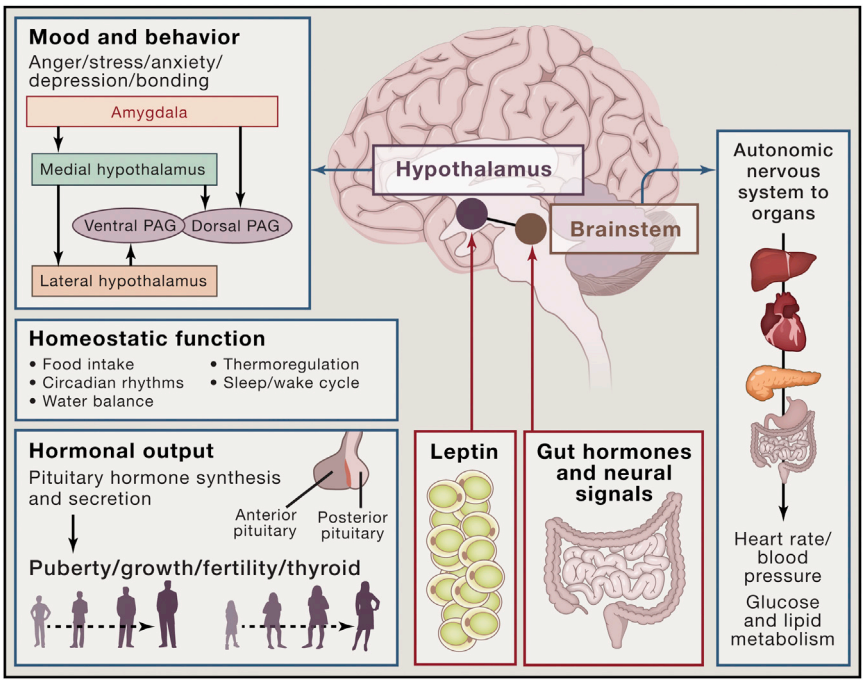

Figure 2. Leptin: A Master Regulator of Human Energy Homeostasis

The adipocyte-derived hormone leptin signals nutritional depletion and initiates a series of changes in energy intake, energy expenditure, autonomic nervous system tone, and neuroendocrine function in order to maintain energy homeostasis. The hypothalamus primarily coordinates many of these processes and also regulates circadian rhythms, temperature, and sleep. Through neuronal connections to the amygdala and periaquaductal gray (PAG) the hypothalamus also modulates a range of behaviors and moods such as stress, anger, anxiety, and aggression. Via its connections to the brainstem—direct and indirect via the cortex—neurons in the hypothalamus modulate autonomic nervous system tone which, in turn, influences many metabolic processes in peripheral tissues, such as the liver, pancreas, heart, and gut. Beyond energy homeostasis, leptin also has important effects on immune function and puberty.

Molecular Characterization of the Circuits Involved in Energy Homeostasis

While the location of the neural circuits regulating energy homeostasis was apparent from the early 1930s, a critical advance came as a result of parabiosis experiments in inbred strains of mice with severe obesity (*ob*/*ob* and *db*/*db*), which suggested the existence of a circulating factor that regulated weight (Coleman, 1973; Coleman and Hummel, 1969). The identification of this hormone, leptin, through positional cloning of the *ob* gene that was mutated in severely obese *ob*/*ob* mice (Zhang et al., 1994) paved the way for the identification and characterization of the neural circuits regulating energy homeostasis. Normalization of the phenotype of severely obese leptin-deficient *ob*/*ob* mice (characterized by increased food intake, reduced energy expenditure, hypogonadism, low thyroid hormone levels, elevated levels of corticosterone, and low blood pressure), by central leptin administration proved that leptin is a key regulator of energy homeostasis (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995).

Leptin—A Master Regulator of Human Energy Homeostasis

Early human studies showing that leptin mRNA concentrations in adipose tissue and serum leptin concentrations correlated positively and very closely with the amount of fat mass (Considine et al., 1996; Maffei et al., 1995) led to the notion that leptin's primary role was to signal increasing energy stores. However, it rapidly became clear that most people are relatively resistant to rising endogenous or exogenously administered leptin (Heymsfield et al., 1999). Instead, leptin's physiological role in humans, as in mice (Ahima et al., 1996), appears to be to signal nutritional depletion, such that fasting or weight loss results in a fall in leptin levels (Chan et al., 2003), which then triggers a series of changes in energy intake, energy expenditure, and neuroendocrine function in order to maintain energy homeostasis.

Evidence supporting leptin's role in human physiology emerged from the identification and characterization of severely obese people with homozygous loss-of-function mutations that reduce the production, secretion, or biological activity of leptin (Montague et al., 1997; Strobel et al., 1998; Wabitsch et al., 2015) or disrupt signaling through the leptin receptor (Clément et al., 1998; Farooqi et al., 2007b). While these disorders are rare, being found in 1%–5% of patients with severe obesity, their characterization has demonstrated that leptin regulates energy balance, neuroendocrine pathways, and the autonomic nervous system (Figure 2). These genetic findings have been supported and extended by elegant studies by many investigators in normal weight in the context of fasting or the weight-reduced state (Rosenbaum et al., 2002, 2005; Welt et al., 2004) and in patients with lipodystrophic syndromes characterized by relative leptin deficiency due to a loss of adipose tissue mass (Oral et al., 2002).

Impaired leptin signaling in humans is characterized by an intense drive to eat (hyperphagia), reduced sympathetic tone, mild hypothyroidism, hypogonadism, and impaired T-cell-mediated immunity, features that are reversed with the administration of recombinant human leptin in people with mutations in the leptin gene (Farooqi et al., 1999, 2002; Licinio et al., 2004; Ozata et al., 1999). Leptin also appears to be a major driver of the increase in blood pressure seen with weight gain, as blood pressure is low in obese mice and humans with disrupted leptin signaling (in contrast to diet-induced obesity in rodents/more common forms of obesity in humans) (Simonds et al., 2014).

Leptin mediates its effects by binding to the long form of the leptin receptor expressed on hypothalamic neuronal populations in the arcuate nucleus of the hypothalamus and other brain regions (Münzberg and Myers, 2005). While homozygous mutations that disrupt the expression, binding activity, and signaling

of the LEPR have been reported (Clément et al., 1998; Farooqi et al., 2007b), mutations that disrupt the downstream signaling cascade have not as yet been clearly associated with obesity. One possible exception is the adaptor molecule, Src homology 2 (SH2) B adaptor protein 1 (SH2B1), which is a key endogenous positive regulator of leptin sensitivity (Maures et al., 2007). However, *SH2B1* mutations have not been shown to disrupt leptin sensitivity, and SH2B1 modulates signaling by a variety of receptor tyrosine kinases, which may explain the additional phenotypes, including severe insulin resistance and behavioral abnormalities, reported in mutation carriers (Doche et al., 2012).

Leptin as a Therapeutic Agent

Recombinant human leptin (metreleptin) is highly effective in patients with no circulating or bioinactive leptin and in those with low endogenous levels with exercise-induced amenorrhea and lipodystrophy. Recombinant leptin has been administered successfully to patients with congenital leptin deficiency for more than 15 years on a named patient basis and was recently approved by the Food and Drug Administration (FDA) for the treatment of generalized lipodystrophy. In contrast, metreleptin has minimal efficacy for more common forms of obesity, which may represent a leptin-tolerant or leptin-resistant state (Heymsfield et al., 1999). In a recent clinical trial, leptin administered in combination with another weight loss agent, pramlintide, a synthetic analog of the pancreatic peptide amylin, had beneficial effects on weight loss, although the precise mechanisms underlying these effects are not entirely clear (Smith et al., 2007). A number of intervention studies have shown that some of the counter-regulatory responses to caloric restriction can be modified by leptin administration, including changes in skeletal muscle and autonomic and neuroendocrine adaptation (Rosenbaum et al., 2002, 2005). This form of intervention could be a useful adjunct in weight-loss maintenance, an area that merits further exploration.

Melanocortin Peptides and Their Receptors

Leptin stimulates primary neurons in the arcuate nucleus of the hypothalamus, which express pro-opiomelanocortin (POMC), which is posttranslationally processed to yield the melanocortin peptides (alpha, beta, and gamma MSH), which are agonists at melanocortin 3 and 4 receptors (Mc3r and Mc4r) expressed on second-order neurons. Leptin signaling modulates energy balance through a combination of melanocortin-dependent/independent pathways. These hypothalamic pathways interact with other brain centers to coordinate energy intake and energy expenditure (Morton et al., 2014).

Several lines of evidence support the critical role of melanocortin signaling in human energy balance. Homozygous null mutations in *POMC* result in severe obesity (Krude et al., 1998), while heterozygous loss-of-function mutations in α- and β-melanocyte-stimulating hormone (α- and β-MSH) significantly increase obesity risk (Biebermann et al., 2006; Lee et al., 2006). Targeted genetic disruption of *Mc4r* in mice leads to increased food intake, increased lean mass, and linear growth (Huszar et al., 1997), phenotypes that overlap entirely with those seen in humans with loss-of-function mutations in *MC4R* (Farooqi et al., 2003). Heterozygous *MC4R* mutations are found in 2%–5% of people with childhood-onset obesity, making this the commonest gene in which highly penetrant variants contribute to obesity (Farooqi et al., 2000; Vaisse et al., 2000). Most disease-causing *MC4R* mutations disrupt the expression and trafficking of MC4R to the cell surface (Lubrano-Berthelier et al., 2006; Xiang et al., 2006). In cells, pharmacological chaperones can increase cell surface expression and signaling of mutant GPCRs, which represents a potentially rational therapeutic approach for this condition (René et al., 2010).

As complete loss-of-function *MC4R* mutations are associated with a more severe form of obesity than partial loss-of-function mutations (Farooqi et al., 2003), modulation of melanocortinergic tone has been the focus of drug development strategies for some time. However, despite promising pre-clinical studies, the first generation of MC4R agonists were small molecules that failed primarily due to safety issues (Van der Ploeg et al., 2002), particularly increases in blood pressure. Loss-of-function *MC4R* mutations are associated with a reduced prevalence of hypertension, low systolic blood pressure, lower urinary noradrenaline excretion, and reduced peripheral nerve sympathetic nervous system activation, revealing that MC4R-expressing neurons represent a key circuit linking changes in weight with changes in blood pressure (Greenfield et al., 2009; Sayk et al., 2010). More recently, a potent melanocortin receptor agonist, RM-493, has been administered as part of a Phase 1B proof-of-concept clinical trial in obese patients, including one cohort of patients with heterozygous loss-of-function mutations in *MC4R*, in whom there was promising weight loss after 4 weeks. If this compound moves forward, this may be one of the first examples of a personalized medicine approach for treating obesity in people with a genetically characterized subtype of obesity.

Processing and Trafficking of Melanocortin Peptides and Receptors

Melanocortin peptides are processed by enzymes including prohormone convertase 1 (PCSK1), which is involved in the cleavage of the precursor peptide POMC into ACTH, which is then further cleaved to generate α-MSH by carboxypeptidase E (Pritchard et al., 2002). Impaired *POMC* processing may contribute to the obesity seen in people with homozygous/compound heterozygous mutations in *PCSK1* who also have glucocorticoid deficiency, hypogonadotropic hypogonadism, and postprandial hypoglycaemia (as a result of impaired processing of proinsulin to insulin) (Jackson et al., 1997; O'Rahilly et al., 1995). Impaired processing of gut-derived peptides may contribute to the neonatal enteropathy seen in PCSK1 deficiency (Jackson et al., 2003; Martín et al., 2013). Intriguingly, common variants that affect the enzymatic activity of PCSK1 have been associated with obesity in multiple European, Asian, and Mexican populations, providing a clear example where both common and rare variants in the same gene can influence a spectrum of variation in body weight (Benzinou et al., 2008; Choquet et al., 2013; Rouskas et al., 2012).

Several human obesity disorders (e.g., Alström syndrome and Bardet-Beidl syndrome) disrupt genes involved in ciliary function (Ansley et al., 2003). The role of neuronal cilia in protein trafficking—in particular, of GPCRs involved in energy homeostasis as well as in leptin signaling (Ainsworth, 2007)—is beginning to

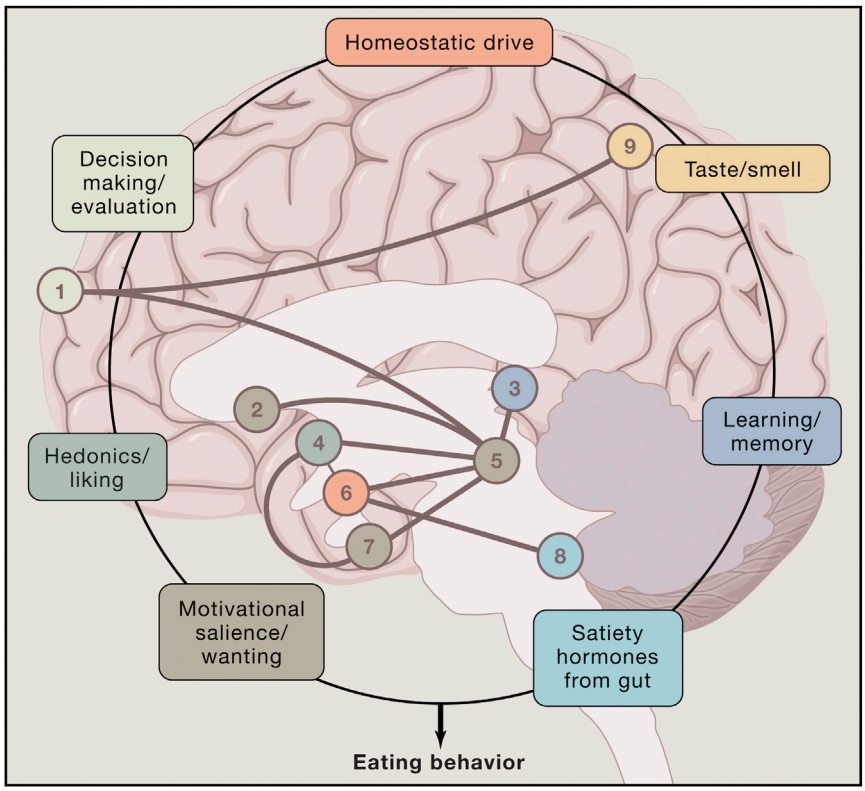

Figure 3. Neural Circuits Involved in Eating Behavior

Neural control of essential behaviors like eating requires the integration of multiple neural signals from different nodes in the brain. Dopaminergic circuits in regions such as the striatum (2), ventral tegmental area (5), and amygdala (7) encode motivational salience and wanting. Opioidergic circuits in regions such as the nucleus accumbens and the ventral pallidum (4) encode hedonic liking. These brain areas and others are integrated with the hypothalamus, cortical areas, and brainstem areas in the regulation of appetite and food intake. Brain regions: (1) prefrontal cortex, (2) dorsal striatum, (3) hippocampus, (4) nucleus accumbens/ventral pallidum, (5) ventral tegmental area, (6) hypothalamus, (7) amygdala, (8) nucleus of solitary tract, (9) gustatory/somatosensory cortex.

emerge. Furthermore, conditional postnatal knockout of proteins involved in intraflagellar transport in neurons and specifically when targeted to *pomc* neurons in mice results in hyperphagia and obesity (Davenport et al., 2007).

Additionally, there is currently a great deal of interest in identifying chaperones and accessory proteins that might modulate melanocortin signaling and melanocortin-dependent pathways. Mrap2, an accessory protein that interacts with Mc4r (and potentially other GPCRs) (Sebag et al., 2013) leads to obesity when disrupted in mice (Asai et al., 2013). Rare variants in *MRAP2* have been associated with severe obesity in humans, although the detailed molecular mechanisms underlying this association are not known (Asai et al., 2013).

Development and Maintenance of Neural Circuits Involved in Eating Behavior

Functional dissection of the neuronal circuits involved in the regulation of energy balance has until recently been limited to dissecting relatively simple linear relationships between neuronal populations that, in reality, are likely to be overlapping and interconnected. Peripheral signals such as leptin can modulate the development and maintenance of these neural circuits (Bouret et al., 2004) and their ability to adapt signaling by altering synaptic inputs (Pinto et al., 2004). While our current understanding of the dynamic and integrated nature of these neuronal networks is still at an early stage, optogenetic tools and other methodologies that permit the manipulation of gene expression in specific populations of neurons are paving the way for major advances in our understanding of the neural circuits connecting brain regions that contribute to the modulation of eating behavior (Betley et al., 2013; Wu et al., 2009) (Figure 3).

Several lines of evidence suggest that brain-derived neurotrophic factor (BDNF), a nerve growth factor that signals via the tyrosine kinase receptor tropomycin-related kinase B (TrkB), is important not only in energy balance, but also in anxiety and aggression. Haplo-insufficient mice and mice in which BDNF has been deleted postnatally are obese with hyperphagia and hyperactivity (Lyons et al., 1999; Xu et al., 2003); this unusual combination of phenotypes is also seen in individuals with genetic disruption of BDNF and TrkB (Gray et al., 2006; Yeo et al., 2004). While a Trkb agonist results in weight loss in mice (Tsao et al., 2008), central administration had no effect on food intake in primates (Perreault et al., 2013). Its potential utility in the treatment of a number of neurodegenerative diseases is still being explored (Yang et al., 2014).

Sim1 is a transcription factor involved in the development of the paraventricular and supraoptic nuclei of the hypothalamus and additionally may mediate signaling downstream of Mc4r (Michaud et al., 1998). *Sim1* haplo-insufficiency in mice and deletions, balanced translocations, and loss-of-function mutations in humans cause severe obesity (Bonnefond et al., 2013; Holder et al., 2000; Ramachandrappa et al., 2013). Oxytocin mRNA levels are reduced in mouse models of *Sim1* deficiency, and oxytocin administration reduces food intake in *Sim1*-haploinsufficient animals (Kublaoui et al., 2008). Impaired oxytocinergic signaling has also been implicated in the hyperphagia and obesity seen in Prader-Willi Syndrome (PWS) (Swaab et al., 1995), caused by lack of expression of a cluster of maternally imprinted snoRNAs on chromosome 15 (Sahoo et al., 2008). People with PWS and with *SIM1* mutations exhibit a spectrum of behavioral abnormalities that overlap with autism-like features and could be related to reduced oxytocinergic signaling (Ramachandrappa et al., 2013), although this has not been tested.

Central administration of oxytocin in rodents is anorexigenic, and rodents that lack oxytocin or the oxytocin receptor become

obese (Olson et al., 1991). The exact sites of action of locally released oxytocin are unknown but likely involve areas with high oxytocin receptor expression, such as the VMH and amygdala. α-MSH, through its effects on MC4R, induces dendritic release of oxytocin, and this locally released oxytocin may be involved in the regulation of appetite (Sabatier et al., 2003). Modulation of central oxytocin signaling therefore forms another potential target in the treatment of obesity (Morton et al., 2014).

Neural Circuits Involved in Eating Behavior

The most consistent phenotype associated with genetic disruption of leptin-melanocortin signaling in humans is hyperphagia, an increased drive to eat (O'Rahilly and Farooqi, 2008). Additionally, detailed characterization of eating behavior in large numbers of twins suggests that eating behavior phenotypes such as satiety responsiveness, eating in the absence of hunger, reinforcing value of food, and the capacity to voluntarily inhibit eating are potentially heritable components of eating behavior (Carnell et al., 2008).This is not surprising, as one of the primary functions of the brain during periods of negative energy balance is to reprioritize behavioral outputs to obtain and consume food, thereby replenishing depleted energy stores. Ensuring sufficient energy stores is critical for survival of the species and, based on our understanding in other mammalian species, multiple processes that defend against starvation and fasting are hardwired.

In addition to this homeostatic regulation of eating behavior, which is driven by energy demands, hedonic food intake (i.e., beyond the need for energy repletion) in response to the rewarding properties of food (Kenny, 2011) is an important contributor to overeating. The palatability of a particular food source is assumed to be related to the flavor and taste of that food; high-fat diets are generally considered more palatable than low-fat diets and are preferentially overconsumed. Neural circuits involving the amygdala, the striatonigral pathway, orbito- and prefrontal cortex, and hippocampus have been implicated in transposing motivational aspects of stimuli into motor responses, as well as in hedonic evaluation of the stimulus and associative learning about the hedonic properties of food (Figure 3). Food reward has been considered to be encoded by distinct neural substrates, opioidergic brain pathways mediating liking (pleasure/palatability), whereas the wanting of food (incentive motivation) appears to be mediated by dopaminergic circuits (Berridge, 1996; Pecina et al., 2003). The overarching role of these responses is to shift attention and effort toward obtaining food reward.

Hormonal regulators of energy homeostasis can also act on brain reward circuits, most notably on the mesoaccumbens dopamine system, to increase or decrease the incentive value of food depending on energy requirements. This suggests that obtaining the pleasurable effects of food is a powerful motivating force that can override homeostatic satiety signals, and in agreement with this, meals that consist of palatable food are generally consumed with greater frequency and in greater portion size than those consisting of less palatable food. As a single meal of increased portion size can trigger increased food intake over several days, such hedonic overeating is likely to be an important contributor to weight gain and the development of obesity.

Human Brain Imaging Studies—Insights into Food Reward

Neural processes such as food reward can be challenging to measure in humans. Imaging studies using functional MRI (fMRI) permit the measurement of blood-oxygen-level dependent (BOLD) signals that reflect neural activity in specific regions involved in the response to food cues (Selvarajah et al., 2014). Pictures of food activate dopaminergic regions such as ventral striatum, and these effects are modulated by homeostatic state (Ziauddeen et al., 2012). In leptin-deficient humans, images of food (compared to non-food images) are associated with a marked increase in neuronal activation in the ventral striatum (Farooqi et al., 2007a). This response was normalized by 7 days of leptin treatment before significant weight loss had occurred, consistent with the view that activation in the ventral striatum does not directly encode the "liking" but, rather, the motivational salience, or "wanting," of food. Studies in obese volunteers in an energy-restricted, partially leptin-deficient state are consistent with the view that these responses are part of the physiological response to energy restriction (Rosenbaum et al., 2008) and are in keeping with findings in experimental studies in rodents (Fulton et al., 2006; Hommel et al., 2006).

Compared to obese controls, obese people with *MC4R* mutations have a preserved pattern of activation of the reward system to visual food cues, suggesting involvement of MC4R in the dopaminergic reward circuitry in humans (van der Klaauw et al., 2014). These findings are supported by evidence in rodents, which suggests that melanocortin signaling modulates food reward. Of note, fMRI studies in Prader-Willi Syndrome have also shown higher neural activity to food cues in reward areas compared to matched obese controls such as accumbens, amygdala, and ventromedial prefrontal cortex (Hinton et al., 2006).

The μ-opioid receptor system that subserves the neural substrates of "liking of food" is a key mediator in the hedonic valuation process of food intake. In addition, μ-opioid receptors were found to mediate the autoinhibition of β-endorphin on hypothalamic *pomc* neurons (Cowley et al., 2003). Antagonism of μ-opioid receptors thus likely results in alterations of hedonic valuation of food as well as potentially attenuates downregulation of *pomc* neuronal activity. Indeed, in humans, the μ-opioid receptor antagonist naloxone reduces the hedonic responses to, and consumption of, palatable foods. In clinical trials, the μ-opioid receptor antagonist GSK1521498 reduces the hedonic response to and motivation for high-fat foods (Ziauddeen et al., 2013). Recently, the combination of naltrexone, an opioid receptor antagonist with high affinity for the μ-opioid receptor, and bupropion, an atypical antidepressant that inhibits reuptake of dopamine and norepinephrine and increases activity of POMC neurons (Contrave) was approved for treatment of obesity by the FDA.

Taste and Food Preference

The orosensory properties of foods are perceived through a combination of taste, texture, and olfaction. The heritability of taste is well established in twin and family studies, with heritability estimates of 30%–50% for pleasantness, consumption, and cravings for sweet foods (Keskitalo et al., 2008). The central

sensing mechanisms for nutrients and quality of food have only recently become the subject of studies. Fat provides twice as many calories per gram as protein or carbohydrate. It is well established that palatable food that is rich in fat and refined sugars promotes larger meal sizes, less postprandial satiety, and greater caloric intake than diets that are high in carbohydrates but low in fat (Salbe et al., 2004). Traditionally, there have been contrasting perspectives on the mechanisms underlying food palatability. The homeostatic view of palatability suggests that palatability reflects the underlying biological need for nutrients, while the hedonic view of palatability suggests that certain foods engage reward processing and are therefore palatable. Studies in rodents have suggested that specific neural pathways, for example, involving the melanocortin-4 receptor (*Mc4r*), play a role in the preference for dietary fat and against dietary sucrose (Panaro and Cone, 2013). To date, very few studies have addressed the preference for specific nutrients in humans, although twin studies have found heritability estimates of 53%–62% for the intake of/preference for foods that are high fat/sucrose. There is considerable research being performed within the food industry focusing on the development of foods that offer some of the sensory properties of fat (fat mimetics) but do not have a high fat content. The potential to modify foods for health benefits is an area of considerable development; such work will need to take into consideration an understanding of the fundamental biology that underpins aspects of eating behavior.

Gut-Derived Satiety Signals

Peptides such as ghrelin, peptide YY (PYY), and glucagon-like peptide 1 (GLP-1) are secreted from gut entero-endocrine cells in response to meal ingestion and the presence of nutrients in the intestinal lumen (Batterham et al., 2002; Turton et al., 1996). Pioneering human infusion studies have demonstrated that a number of gut peptides modulate food intake when administered acutely in humans (Tan and Bloom, 2013), suggesting that modulating satiety signals could be a useful therapeutic strategy in obesity (Finan et al., 2015). The synthetic GLP-I receptor agonist liraglutide has recently been approved for the treatment of obesity alone by the FDA. Several other gut peptide analogs, as well as gut hormone receptor agonists, are currently being studied in clinical trials (Tan and Bloom, 2013).

Satiation, the sensation of fullness that results in meal termination and satiety, the persistence of fullness that determines the timing to the next meal, are heritable traits that influence weight gain (Carnell et al., 2008). Although common obesity seems to be associated with low circulating PYY levels (Batterham et al., 2006), rare genetic variants in PYY or its receptors have not been associated with obesity. Fasting ghrelin levels have been found to be increased in children (Haqq et al., 2003) and adults with PWS (Cummings et al., 2002), potentially contributing to the hyperphagia and impaired satiety associated with this syndrome, although the potential mechanisms involved are not known.

Additionally, there is a growing literature on changes in the composition of the gut microbiome in response to acute/short-term changes in the diet, chronic states such as obesity and bariatric surgery (Turnbaugh et al., 2006), and the impact of specific organisms on nutrient absorption and on metabolic parameters in mice and humans (Cox et al., 2014).

Targeting Energy Expenditure

A number of large family-based population studies, most notably the Quebec family study, have addressed the contribution of genetic versus environmental factors to energy expenditure, including physical activity (Pérusse et al., 1989). For example, the heritability of exercise participation is entirely accounted for by common familial environment, while for physical activity level, the heritability is ~20%. As such, promotion of increased levels of physical activity is a useful strategy for weight loss and, in particular, for weight maintenance.

In contrast, basal metabolic rate (BMR) and respiratory quotient (ratio of carbohydrate versus fat oxidation; a marker of substrate utilization) are highly heritable (47% and 36%, respectively) (Bouchard and Tremblay, 1990). Very few genes have been shown to modulate BMR in humans, although the reduced basal metabolic rate reported in obese people harboring loss-of-function mutations in the cellular scaffolding protein *KSR2* (kinase suppressor of Ras2) suggests that genetic variation in energy expenditure phenotypes may contribute to weight gain in some individuals (Pearce et al., 2013). In this study, almost all of the *KSR2* variants identified in obese individuals impaired glucose oxidation and fatty acid oxidation in cells, suggesting a defect in substrate utilization, which was rescued by the addition of metformin. Further work will be needed to see whether these observations can be replicated in experimental clinical studies and to investigate the cellular mechanisms underlying these effects which, in part, may be mediated by the interaction of KSR2 with the cellular fuel sensor, AMP-kinase (Brommage et al., 2008; Costanzo-Garvey et al., 2009).

The development of compounds that might increase energy expenditure is being explored as a possible therapeutic strategy. One potential route is to activate brown adipose tissue, thereby generating heat through uncoupling protein 1 (UCP1) (Lowell and Spiegelman, 2000). UCP1-positive cells in white adipose tissue depots in rodents (often called beige/brite cells) can be stimulated to dissipate energy by thermogenesis and pharmacological stimulation of these processes, potentially through circulating myokines that drive brown-fat-like development (Wu et al., 1999), has attracted the interest of a number of pharmaceutical companies. Although UCP1-positive cells that show similarity to murine beige adipocytes have been found in human fat depots (Wu et al., 1999), the translation of these findings in rodents to therapies that can be administered safely in humans presents some challenges. For example, what influences the exact amount of brown fat and/or beige fat available in adult humans, and can this be increased? To what extent do sex steroids (or other gender-specific factors) influence the activity/quantity of brown/beige fat, as women seem to have more than men (Cypess et al., 2009)? How much extra energy would be expended through the stimulation/overstimulation of such processes, and would this be clinically relevant? Would an increase in energy expenditure lead to a compensatory increase in food intake, and how might such an effect be managed?

Building an Integrated View of the Pathways that Regulate Energy Homeostasis

Given the complexity of neurobiological processes underlying body weight homeostasis, it is likely that future drugs will need

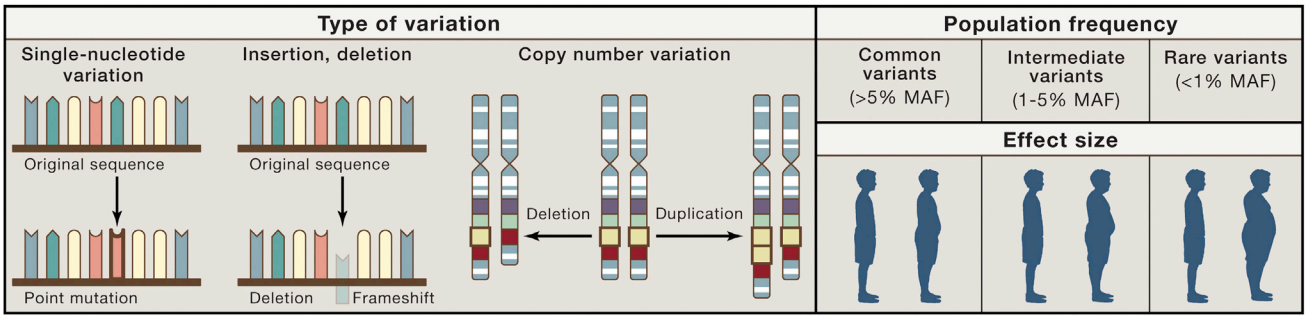

Figure 4. Types of Genetic Variation Contributing to Body Weight Regulation

Genetic effects on body weight are mediated by different types of variants, their frequency in the population, and the effect of the variant on the phenotype. Variants include single-nucleotide variations in which only one nucleotide is changed, copy number variations in which a stretch of DNA is repeated or deleted (often containing many genes), or small insertions and deletions of a few base pairs. Common variants are found at a minor allele frequency (MAF) of more than 5% in a population, whereas intermediate (1%–5%) and rare variants (< 1%) are found at lower frequencies. Generally, the effect size of common obesity-associated variants on body weight is modest. Several rare variants have been associated with severe obesity.

to be directed at highly specific targets and may consist of combinations of compounds that target different mechanisms, as illustrated by recent studies demonstrating the efficacy of dual melanocortin-4 receptor and GLP-1 receptor agonism (Clemmensen et al., 2015). The central and peripheral regulation of food intake, energy expenditure, physical activity, fat absorption, and oxidation are all being explored as potential mechanisms that can be targeted in rodent studies. In parallel, genetic approaches into human eating behavior and obesity may inform the focus of experimental approaches in rodents and might generate new potential drug targets in which the potential relevance to humans may be established at an earlier stage than has previously been the case.

Common Genetic Variants and Genome-wide Association Studies

Genetic influences are likely to operate across the weight spectrum but may be more penetrant when studying childhood-onset obesity and at both extremes of the BMI distribution—thinness and severe obesity. Genetic variance depends on the nature and amount of mutational variance in a population, the segregation and frequency of the alleles that influence a trait in a particular population, the effect sizes of the variants (which may be additive or non-additive), the mode of gene action, and the degree of genetic control of phenotypic variance of the trait in question (Figure 4).

Genome-wide association studies (GWAS) seek to identify the common variants (minor allele frequency [MAF] of more than 5%) that contribute to the heritability of common diseases. High-throughput arrays have facilitated the genotyping of thousands of common variants (directly or by imputation) in large population-based cohorts on whom BMI data is available. The first GWAS-derived loci to be reported were intronic variants in *FTO* (fat mass and obesity associated) and a variant ~200 kb downstream of *MC4R* (Dina et al., 2007; Frayling et al., 2007; Loos et al., 2008). To date, more than 80 genetic loci associated with BMI and body fat distribution (often measured by waist-to-hip ratio) have been identified by GWAS approaches, and many of these have been replicated in different populations and ethnicities (Locke et al., 2015). GWAS in childhood-onset obesity and in severely obese children and adults have shown that there is some overlap between the common variants that contribute to early-onset and adult-onset weight gain, but also that both of these approaches can identify novel variants (Bradfield et al., 2012; Wheeler et al., 2013). Cumulatively, the common variants identified in GWAS are characterized by modest effect sizes (per allele odds ratios between 1.1 and 1.5), and the proportion of variability of BMI explained by GWAS-identified loci to date remains relatively modest (< 5%). Nevertheless, variants that explain a small proportion of phenotypic variance may provide substantial biological or therapeutic insights, although the road to establishing causal variants and their functional relevance is often a challenging one.

GWAS-associated loci are often identified by the name of the nearest gene; this may or may not be the gene in which variation contributes to variation in BMI. Some of the GWAS loci encompass genes previously appreciated to play a role in energy homeostasis (e.g., *LEPR*, *SH2B1*, *MC4R*, *BDNF*), and in some cases, specific variants have been associated with changes in expression based on eQTL data (Wheeler et al., 2013). Other loci contain genes that seem to be plausible biological candidates or can suggest genes for which there was no previous evidence (Locke et al., 2015). Many of the signals identified to date map to non-coding regions of the genome that may potentially be involved in gene regulation.

The strongest association signal for BMI has consistently been found with variants in the first intron of FTO, which have been associated with increased BMI and eating behavior in a number of studies (Cecil et al., 2008; Wardle et al., 2008a). Deletion or overexpression of *fto* and other genes in this region (*IRX3*, *RPGRIP1L*) in rodents (Church et al., 2010; Fischer et al., 2009; Gerken et al., 2007; Stratigopoulos et al., 2008) (Smemo et al., 2014) can impact energy homeostasis. Despite these obvious challenges, these studies have demonstrated progress toward identifying new biology based on GWAS (Tung et al., 2014).

Is there yet more common variation to find? Newly developed statistical methods that assess the contribution of common genetic variation across the genome (Zhu et al., 2015) support the growing consensus that there is a long tail of common

variation. As such, meta-analyses of even larger population-based data sets are currently underway. The available evidence suggests that BMI is highly polygenic (high number of contributing genes) (Gusev et al., 2014). One of the challenges of such studies is how to capture the full spectrum of genetic variation (Figure 4), including complex multi-allelic CNVs, which show lower linkage disequilibrium with surrounding SNPs and are consequently less detectable by conventional SNP-based genome-wide association studies. For example, in a large family-based association study of Swedish families ascertained through the identification of siblings who were discordant for obesity, integrating data from CNV analysis with transcriptomic data from adipose tissue revealed an association with copies of *AMY1* with obesity (Falchi et al., 2014).

Finding New Rare Highly Penetrant Variants

Rare variants, which outnumber common variants in the human genome, may explain a proportion of the heritability of obesity and may be more readily identified at the extremes of the phenotypic distribution. The earliest studies were performed in children with clinically identifiable syndromes often associated with developmental delay or dysmorphic features as well as obesity. Rare CNVs that often disrupt a number of genes have recently been implicated in highly penetrant forms of obesity (Bochukova et al., 2010; Walters et al., 2010). Candidate gene studies based on the molecules known to cause severe obesity in experimental animals have shown that these genes also contribute to childhood-onset human obesity, often in the absence of developmental delay. The functional and physiological characterization of these mutations and of the mutation carriers has illustrated a high degree of convergence of the mechanisms that regulate energy balance across mammalian species.

Exome sequencing of cohorts with severe childhood-onset and adult-onset obesity, as well as those at the extremes of the BMI distribution in population-based cohorts, is well underway and may lead to the identification of new genes whose functions will need to be explored in cells, model organisms, and humans. Whole-genome sequencing provides the ''most complete'' view of genomic variation but poses challenges in terms of proving causality, but these are beginning to be addressed. Recent studies have now shown that human inducible pluripotent stem cell (iPSC)-derived neurons may facilitate a mechanistic understanding of how specific genes disrupt cellular and neuronal mechanisms that may be involved in the pathogenesis of obesity (Wang et al., 2015).

Therapeutics Opportunities in Obesity

Lifestyle modification remains the first step in weight management. While intervention programs that focus on supporting people to change their diet and/or levels of physical activity can be effective in inducing weight loss in the short to medium term in some people, they lose efficacy in the long term. As such, in addition to the focus on prevention of obesity, treatment of obese patients, preferably at a stage before complications have emerged, is an important priority (Gray et al., 2012). However, current therapeutic options in obesity are very limited; the only currently approved anti-obesity drug for long-term use in the US and Europe is Orlistat, which reduces intestinal lipid absorption by inhibiting pancreatic lipase and often has limiting adverse effects that preclude its long term use.

Previously available anti-obesity drugs targeted cannabinoid signaling (rimonabant), noradrenergic (phentermine) and serotoninergic signaling (fenfluramine, dexfenfluramine), and reuptake (sibutramine). These compounds were moderately effective but, as with many centrally acting agents, at the expense of many off-target effects, reflecting lack of specificity of the neural targets. Lorcaserin, a selective 5HT2cR agonist with limited activity at the other serotonin receptors, has been approved for use in the US (Smith et al., 2010), although concerns about potential cardiac valvulopathy and cancer risk have prevented European approval of the drug to date. The combination of the anticonvulsant topiramate and phentermine, which increases central noradrenaline levels (Qsymia), is also approved in some countries.

Finally, development of personalized medicine by selecting the optimal pharmacological intervention for particular people through genetics or other molecular/cellular analyses is an exciting and evolving area. Synthetic-biology-inspired therapeutic systems that integrate sensor and effector devices into cells have been developed to monitor disease-relevant metabolites, process on/off level control, and coordinate adjusted therapeutic responses. These systems have the potential to restore metabolite homeostasis in a seamless, automatic, and self-sufficient manner, which is particularly attractive for future gene- and cell-based therapies. As an example, a closed-loop synthetic intracellular lipid-sensing receptor (LSR)-pramlintide circuit represents a potential prototype for such a cell-based therapy. The LSR sensor captures a wide range of lipids within their physiologic concentration range, becomes dose-dependently activated by peak fatty acid levels, and is turned off at physiological concentrations (Rössger et al., 2013). Such emerging methodologies offer fresh perspectives for drug delivery and potentially personalized medicine in the future.

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