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The skinny on brain-derived neurotrophic factor: evidence from animal models to GWAS

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Abstract

Obesity and its sequelae constitute a major international healthcare problem. The obesity epidemic is due in part to higher calorie diets and reduced exercise over the past 30 years; however, increasing evidence has established genetic regulation of body weight as a major contributor to obesity. Brain-derived neurotrophic factor (BDNF) regulates development and plasticity of the central nervous system, and recent work has established a clear role for signaling through BDNF and its receptor TrkB in the control of body weight. Here we review research findings from animal models and human populations indicating that BDNF is a negative regulator of appetitive behavior and body weight.

Keywords

BDNF; TrkB; single nucleotide polymorphism; human obesity; hypothalamus

Introduction

In 2008 the World Health Organization (WHO) reported that 1.4 billion people worldwide were overweight and over half a billion people were obese. In addition, over 40 million children were reported to be overweight. The WHO report noted that the number of obese and overweight adults and children had doubled between 1980 and 2008. Most critically, severe morbidity has been associated with obesity, including (but not limited to) type 2 diabetes mellitus, cardiovascular disease, and almost all types of cancers (excluding esophageal, pancreatic, and prostatic cancers) [1]. The obesity epidemic has caused nations and healthcare systems to adopt policies and programs to help people decrease their intake of calories and increase their energy expenditure. The obesity epidemic has also triggered a new wave of research on the causal factors for weight gain and for the stability of that gained weight. The rapidly evolving field of obesity research is beginning to elucidate mechanisms of genetic and epigenetic regulation of body weight. Findings may have profound implications for the way we design weight loss treatment programs, with a new emphasis on individualized healthcare.

Regulation of energy balance involves the complex orchestration of a wide variety of peripheral and central mediators. The main organs involved in the control of energy balance are the pancreas, the adipose tissue, the gastrointestinal (GI) tract and the nervous system. Peripheral signals relay information about nutritional status to the central nervous system, which in turn controls metabolism and appetitive behavior. Some peripheral signals

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facilitate a negative energy balance and other peripheral signals facilitate a positive energy balance. A wide variety of factors provide information to the central nervous system that leads to a negative energy balance, including those that have an anorexigenic effect yielding loss of appetite and food rejection. Factors promoting negative energy balance include leptin produced in the adipose tissue [2], insulin produced in the pancreas [3], glucagon-like-polypeptide-1 produced in the ileum [4, 5], and peptide tyrosine tyrosine (PYY) produced in the terminal ileum and colon [6]. Additional factors leading to a negative energy balance include the peptide hormone cholecystokinin (CCK) produced in the duodenum [7] and various metabolites such as glucose, fatty acids, and amino acids [8–11]. In functional opposition, ghrelin produced in the stomach functions as an orexigenic signal increasing appetite, thus promoting a positive energy balance [12, 13].

All peripheral signals, whether negative or positive energy balance promoters, can be integrated in the hypothalamus and brainstem. Key hypothalamic nuclei regulating food intake include the arcuate nucleus (ARC), dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH), paraventricular nucleus (PVN), and the lateral hypothalamus/preformical area (LH/PFA) [14]. The main brainstem structure regulating energy homeostasis is the dorsal vagal complex (DVC). The DVC includes the area postrema (AP), the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (DMV) [15]. The hypothalamus and brainstem have many reciprocal connections, and both regions likely relay integrated messages forward to higher CNS structures or back to peripheral tissues to affect energy balance via alterations in metabolism and/or appetitive behavior.

Brain-derived neurotrophic factor (BDNF) belongs to a family of secreted proteins called neurotrophins that also includes nerve growth factor, neurotrophin 3, and neurotrophin 4/5. BDNF binds with high affinity to the tropomyosin receptor kinase B (TrkB) to promote signaling through three main pathways: mitogen-activated protein kinase (MAPK), phospholipase C-gamma (PLC- γ), and phosphatidylinositol-3 kinase (PI3-K). In addition, BDNF exerts some of its biological function through the p75^{NTR} receptor [16]. Research has demonstrated that BDNF is a key regulator of the development and synaptic plasticity of the central nervous system [17–19]. However, pharmacological and genetic research in the 1990s suggested that BDNF also plays a role in energy homeostasis [20–23]. Since then, studies have begun to unravel the complex and dynamic role that BDNF plays in the control of energy balance, revealing that its primary function is to promote a negative energy balance through a reduction in appetite [24–26]. This review provides a brief consideration of animal research followed by an outline of key evidence from research in humans for the role of BDNF in energy balance. More detailed information on the animal research can be found in recent excellent review articles [27–29].

BDNF and the central control of energy balance: evidence from animal studies

BDNF and its receptor TrkB are expressed in many brain regions involved in energy homeostasis. In the hypothalamus, BDNF is expressed in the VMH, DMH, PVN, and LH, with highest expression demonstrated in the VMH, but little to none in the ARC [30]. TrkB, however, has been shown to be expressed in all hypothalamic nuclei [31]. In addition, BDNF and TrkB are expressed in the dorsal vagal complex (DVC) of the brainstem and in higher cortical circuitry involved in appetitive behavior [31, 32]. The first evidence that implicated BDNF in energy homeostasis was published in the early 1990s and showed that intracerebroventricular infusion of recombinant BDNF induced weight loss [20, 21]. These initial animal studies suggested that BDNF was a negative regulator of body weight.

Mice lacking BDNF expression die perinatally due to the crucial role of BDNF in development [33, 34]. *Bdnf* heterozygous mice (*Bdnf*^{+/-}) express reduced amounts of total BDNF but nonetheless they do survive into adulthood [22, 23]. Investigations of *Bdnf*^{+/-} mice further established the role of BDNF in energy balance. These mice were found to be hyperphagic and have significantly higher body weights than WT controls by two months of age [22, 23]. Importantly, this was not due to an irreversible developmental effect, as infusion of BDNF into the third ventricle of adult mice was able to rescue the observed phenotype [23]. In further support of BDNF's roles in energy balance, conditional *Bdnf* deletion in the postnatal brain using the Cre-loxP recombination technology led to hyperphagia and an 80% (male) and 150% (female) increase in body weight [35]. Interestingly, several studies found that *Bdnf* mutant mice did not develop obesity when they were limited to have the same amount of daily food intake as wild-type mice [24–26]. Collectively, these genetic studies indicate that central BDNF primarily regulates appetite to control body weight.

Evidence has further suggested that BDNF's anorexigenic effect occurs through its high affinity receptor TrkB. Mice expressing TrkB at approximately a quarter of the normal level exhibited hyperphagia and developed massive obesity [30]. Moreover, central administration of TrkB agonists was found to ameliorate obesity and associated metabolic conditions in mice and non-human primates [36, 37]. However, it is puzzling that peripheral administration of TrkB agonists was found to increase appetite and body weight gain in non-human primates [37].

Food deprivation was found to drastically and selectively reduce levels of *Bdnf* mRNA in the mouse VMH [30]. This remarkable observation has been independently confirmed [25, 38], indicating that the VMH is likely a brain area that synthesizes BDNF to suppress appetite. In support of this view, research has shown that deletion of the *Bdnf* gene in the adult VMH and DMH results in overeating and increased weight gain in mice [25] and that overexpressing BDNF in the VMH and its nearby regions prevents the development of obesity in *Bdnf* mutant mice [26]. It is important to note that the extra weight gain caused by *Bdnf* deletion in the adult VMH and DMH is much smaller than that induced by early disruption of the BDNF function throughout the brain [25, 26, 35], indicating that BDNF produced in other brain regions also plays a role in the control of energy balance.

One such brain region could be the PVH, as mouse obesity induced with a high fat diet was reversed with administration of BDNF directly into the PVN [39]. Outside the hypothalamus, DVC in the brainstem expresses BDNF and likely plays a role in BDNF's control of energy balance. Within the DVC, BDNF and TrkB are expressed in the nucleus of the solitary tract (NTS), while TrkB is also expressed in the area postrema (AP) [31, 32]. Levels of BDNF in the DVC were found to decrease with fasting and increase with re-feeding, and infusion of BDNF into the DVC decreased food intake and body weight of rats [40]. BDNF also acts in neural circuits outside the hypothalamus and brainstem to regulate food intake, as evidenced by work showing that BDNF negatively regulates consumption of palatable, high fat chow through signaling in the mesolimbic dopamine system in mice [41].

Peripheral fat stores release the peptide hormone leptin to convey satiety signals to central neuronal populations. BDNF appears to be a downstream mediator of leptin in the hypothalamus. The *Bdnf* gene produces two populations of mRNA with either a short or a long 3' untranslated region (3' UTR) due to two alternative polyadenylation sites [42]. It has been shown that short 3' UTR *Bdnf* mRNA is restricted to cell bodies whereas long 3' UTR *Bdnf* mRNA also localizes to neuronal dendrites for local translation [43]. Mice lacking long 3' UTR *Bdnf* mRNA developed severe hyperphagic obesity, which was completely rescued by the viral expression of long 3' UTR *Bdnf* mRNA, but not short 3' UTR *Bdnf*

mRNA, in the hypothalamus. Furthermore, leptin stimulated local synthesis of BDNF in dendrites of hypothalamic neurons and, without the long isoform of *Bdnf* mRNA, mice were resistant to leptin [26]. These findings indicate that leptin suppresses appetite in part by stimulating local BDNF synthesis in neuronal dendrites in the hypothalamus.

BDNF also closely interacts with the melanocortin system to regulate energy balance, in both the VMH and brainstem. One group of neurons in the ARC expresses proopiomelanocortin (POMC), one of whose cleavage products, alpha melanocyte stimulating hormone (α -MSH), activates melanocortin-4 receptor (MC4R) to promote anorexia and weight loss [14]. It has been shown that activation of melanocortin-4 receptor (MC4R) stimulated expression of BDNF in the VMH, and hyperphagia and excessive weight gain were attenuated with BDNF infusion into the brain of mice with decreased MC4R activity [30]. Similarly, the hyperphagia induced by MC4R antagonist infused into the fourth ventricle was reversed with simultaneous administration of BDNF [44]. Furthermore, BDNF delivered directly to the medial NTS reduced food intake, which was reversed by pre-treatment with a TrkB antagonist [45], while acute blockade of TrkB activation abolished the anorexigenic effect of MC4R agonists in the brainstem [44].

In summary, animal studies have accumulated strong evidence to implicate BDNF as a crucial player in the control of energy balance. However, the molecular mechanism and neural circuit through which BDNF regulates energy balance remain unclear. For example, pharmacological studies indicate that TrkB signaling is essential for the anorexigenic effect of MC4R agonists in the DVC [44, 45]; however, it is unknown whether BDNF is synthesized in neurons inside the DVC or reaches the DVC through anterograde or retrograde transport, whether BDNF acts on TrkB in DVC neurons or at axonal terminals residing in the DVC, and which effector of the TrkB signaling cascades is essential for the anorexigenic activity of MC4R.

BDNF and human obesity: From case studies to GWAS

There is already substantial work linking BDNF to energy balance in human populations. Rare genetic mutations have shown the most dramatic BDNF-linked phenotypes, but recent genome wide association studies (GWAS) have identified more prevalent polymorphisms with more subtle phenotypes.

The first report to link BDNF signaling to human obesity described an 8 year-old boy, heterozygous for a de novo missense mutation in the TrkB receptor, who developed severe hyperphagic obesity [46]. The missense mutation resulted in a Y722C substitution, which impaired activation of the TrkB receptor and its downstream signaling pathways [46]. A mutation in the *Bdnf* gene also causes obesity in children. An 8-year-old girl had a de novo chromosomal inversion, rendering functional loss of one *Bdnf* allele, and she developed hyperphagia and severe obesity [47]. As expected from loss of one functional *Bdnf* allele, the subject's serum BDNF levels were severely reduced compared to age and weight matched subjects [47].

A few rare syndromes are associated with obesity, and the obesity symptom in some of these cases is a result of reduced BDNF expression. Wilm's tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome is a rare disorder (1 case per 500,000 to 1,000,000 persons) that is caused by heterogeneous contiguous deletions of the *WT1* and *PAX6* genes located in the 11p13 chromosomal region. The *Bdnf* locus is at 11p14.1, and a subset of patients with WAGR syndrome also have deletions that include the *Bdnf* gene, resulting in BDNF haploinsufficiency. Importantly, the subset of WAGR patients with BDNF haploinsufficiency had decreased serum BDNF levels and developed childhood-onset obesity [48]. Smith-Magenis syndrome (SMS) is caused by haploinsufficiency of the

retinoic acid induced 1 (*RAI1*) gene, and patients have cognitive and behavioral deficits and early-onset obesity [49, 50]. *RAI1* heterozygous mice (*RAI1*^{+/-}) are hyperphagic, have increased adipose deposition, and have decreased *Bdnf* expression in the hypothalamus. *In vitro* assays revealed that *RAI1* regulates *Bdnf* transcription through an intronic enhancer element, indicating that obesity in SMS patients may be due to decreased *RAI1*-induced expression of BDNF in the hypothalamus [51]. Taken together, these examples of syndromes associated with altered BDNF expression provide evidence that decreased BDNF expression in humans is related to obesity.

As discussed above, serum BDNF levels are decreased in obese subjects with one nonfunctional *Bdnf* allele [47, 48]; however, the relationship between serum BDNF levels and obesity in the general population is unclear. BDNF in peripheral blood is found in serum, plasma, and platelets. In humans, increased age and weight were correlated with decreased plasma levels of BDNF; however, these parameters did not correlate with BDNF levels in platelets [52, 53]. Furthermore, it appears that levels of serum BDNF are decreased even in non-obese subjects that have low insulin sensitivity, indicating that decreases in serum BDNF occur in altered metabolic states [54]. More work is needed to determine the role of peripherally circulating BDNF in energy homeostasis.

Starting in 2009, a series of GWAS have linked BDNF to human obesity. Due to the large sample size of these studies, they were able to detect more genetic variations with subtler effects than previous research in humans. Four large-scale GWAS using over 30,000 to nearly 250,000 subjects found 7 single nucleotide polymorphisms (SNPs) in or near the *Bdnf* gene (rs4074134, rs4923461, rs925946, rs10501087, rs6265, rs10767664, and rs2030323) that were associated with increased body mass index (BMI) [55–58]. Some of these SNPs have been found to be associated with obesity in specific populations in smaller GWAS. In an adult Japanese population (n=2,865), 3 of these 7 SNPs (rs4074134, rs4923461, and rs10501087) were significantly correlated with BMIs greater than 30 kg/m² [59], while in a pediatric population (n=6,078) only one of these SNPs (rs6265) was found to significantly correlate with childhood-onset obesity [60]. Similarly, in a pediatric study looking at Chinese children (n=3,503), investigators found a significant association between rs6265 and obesity [61]. Finally, a recently published longitudinal study found that rs925946 was significantly associated with greater childhood weight gain, faster linear growth, and adult obesity [62].

The aforementioned human data clearly shows an association between BDNF and obesity in both adult and pediatric populations across many ethnicities. Severe phenotypes in rare cases where the function of one *Bdnf* or *NTRK2* (encoding TrkB) allele is abolished or greatly impaired indicate a crucial role of the BDNF-TrkB pathway in the control of human body weight. Moreover, the association of several common *Bdnf* SNPs with increased BMI revealed by GWAS suggests that *Bdnf* gene variants have a significant contribution to the current obesity epidemic.

Potential mechanisms underlying the role of *Bdnf* SNPs in obesity

The human *Bdnf* gene has at least eight exons, the first seven of which contain 5 untranslated regions (5 UTRs), with exon 8 containing the entire coding sequence as well as the 3' UTR sequence (Figure 1). Transcription can be initiated at any of the first seven exons, which are spliced to the common coding exon 8, generating mRNA species with different 5 UTRs that code for the same protein. The intron sequence upstream of each of the first seven exons likely contains cis-regulatory elements that control transcription initiation at each of these exons. Among the 7 SNPs associated with increased BMI, two (rs2030323 and rs10767664) localize to the intron (intron 3) upstream of exon 4, one

(rs6265) in the coding region, and four (rs10501087, rs925946, rs4923461 and rs4074134) downstream of the *Bdnf* 3' UTR (Figure 1).

Neuronal activity stimulates transcriptional initiation at the promoter for exon 4 by inducing Ca^{2+} influx through the L-type voltage-gated calcium channel and the NMDA receptor [63]. This promoter is located within intron 3, and several cis-regulatory elements that respond to Ca^{2+} influx have been identified in the 200 bp immediately upstream of exon 4 [64]. The two SNPs within intron 3, rs2030323 and rs10767664, locate 5476 bp and 2922 bp upstream of the exon 4, respectively (Figure 1). It is very likely that these two SNPs are within additional cis-regulatory elements for this promoter and their minor alleles impair the interaction between these regulatory elements and their binding proteins, thus reducing activity-induced BDNF expression.

SNP rs6265 is the most extensively studied *Bdnf* gene variant. It is in the sequence encoding the pro-domain of the BDNF precursor, producing a valine to methionine substitution at codon 66 (Val66Met). It has been shown that mice homozygous for the Val66Met substitution have significantly higher body weight than normal mice [65], further supporting a role for rs6265 in human obesity. In humans, *Bdnf*^{Met} has been found to be associated with an array of psychiatric disorders, neurodegenerative disorders, and cognitive deficits in addition to increased BMI [65–70]. Cellular and biochemical analyses have revealed that the methionine substitution results in decreased BDNF localization to synapses and decreased activity-dependent BDNF secretion in hippocampal neurons [70, 71]. It is possible that these same deficits occur in the hypothalamus or brainstem. Obesity in humans harboring two copies of the methionine allele could therefore be due to deficits in activity-dependent release of BDNF in the hypothalamus or brainstem.

Alternative utilization of two polyadenylation sites at exon 8 produces short 3' UTR *Bdnf* mRNA and long 3' UTR *Bdnf* mRNA (Figure 1). Local translation of long 3' UTR *Bdnf* mRNA and release of the translational product in dendrites are activity-dependent processes in hippocampal neurons [43, 72, 73]. The same processes likely occur in hypothalamic neurons, as leptin stimulates local translation of long 3' UTR *Bdnf* mRNA in the dendrites of hypothalamic neurons through neuronal activity [26]. The minor alleles of the four SNPs downstream of exon 8 may interfere with production of long 3' UTR *Bdnf* mRNA and thus reduces activity-dependent local synthesis of BDNF in the dendrites of hypothalamic neurons. This would likely increase human BMI, as mice lacking long 3' UTR *Bdnf* mRNA become severely obese [26].

From the discussion above, it becomes clear that neuronal activity may be the key to understanding the effect of the seven *Bdnf* SNPs on BMI. The minor alleles of these SNPs may impair activity-dependent BDNF production at levels of transcription, translation, and secretion, and lead to increased susceptibility to obesity.

Perspective

Both animal and human studies have established a conclusive role for BDNF in the control of body weight; however, we still do not have a clear picture as to how BDNF regulates energy balance. The slow progress in this research field may be related to the complex action of BDNF in the nervous system. In contrast to some anorexigenic factors (e.g. - MSH), which are synthesized in a small number of cells, BDNF is expressed in many brain regions and can act locally through an autocrine or paracrine mode or remotely through anterograde or retrograde transport. Recent GWAS have repetitively linked *Bdnf* gene variants with human BMI. These exciting findings will attract new investigators to this research field and speed up progress. It is imperative to identify the neuronal circuitry through which BDNF acts to regulate energy balance by employing neuronal tracers and

mouse mutants with deletion of the *Bdnf* or *NTRK2* gene in selective neuronal populations. It also is important to understand how the SNPs identified in the GWAS affect BDNF production by using iPS cells derived from humans harboring these SNPs. These studies will increase our understanding of the molecular and neural mechanisms underlying the role of BDNF in the control of body weight. On the basis of the results from animal and GWAS, we speculate that anorexigenic factors such as leptin and melanocortin increase BDNF production at multiple levels through neuronal activity, and that BDNF in turn controls energy balance by regulating synaptic function.

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Figure 1. Human *Bdnf* gene organization and location of SNPs

This diagram of the human *Bdnf* gene is based on the annotation for locus AF411339 in GeneBank. The gene consists of 8 exons and two alternative polyadenylation (pA) sites in exon 8 (arrows above exon 8). The position (bp) for each BMI-associated SNP is according to Build 36.