RESEARCH ARTICLE | Obesity, Diabetes and Energy Homeostasis

Effect of food deprivation or short-term Western diet feeding on BDNF protein expression in the hypothalamic arcuate, paraventricular, and ventromedial nuclei

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Gilland KE, Fox EA. Effect of food deprivation or short-term Western diet feeding on BDNF protein expression in the hypothalamic arcuate, paraventricular, and ventromedial nuclei. Am J Physiol Regul Integr Comp Physiol 312: R611–R625, 2017. First published February 15, 2017; doi:10.1152/ajpregu.00256.2016.—Mutations in the brain-derived neurotrophic factor (BDNF) gene are associated with human obesity, and BDNF has potent inhibitory effects on eating and body weight. Little is known about the effects of energy balance manipulations on BDNF protein in the hypothalamus, though this brain region is critical for regulation of feeding and body weight and has high levels of BDNF. Here we investigated the effects of negative and positive energy status on BDNF protein levels in the arcuate (ARC), paraventricular, and ventromedial (VMH) hypothalamic nuclei and the ectorhinal cortex. To achieve this, mice were food deprived for 48 h or fed a Western diet (WD), a restricted amount of WD, or chow for 6 h, 48 h, 1 wk, or 3 wk. BDNF protein levels were estimated as the number of neurons in each brain region that exhibited BDNF-like immunoreactivity. Food deprivation decreased BDNF protein (and mRNA) expression in the ARC compared with fed mice (32%). In contrast, 1 wk of WD consumption increased BDNF protein expression in the VMH compared with chow or restricted WD feeding (40%) and, unexpectedly, increased BDNF protein in the ectorhinal cortex (20%). Furthermore, of the diet conditions and durations tested, only 1 wk of WD consumption was associated with both hyperphagia and excess weight, suggesting that effects of one or both contributed to the changes in BDNF levels. The decrease in ARC BDNF may support increased feeding in food-deprived mice, whereas the increase in the VMH may moderate overeating in WD-fed mice.

OBESITY HAS EXCEEDED EPIDEMIC levels and become pandemic, expanding globally and thus directly impacting a large proportion of the human population (5, 68). More indirectly, the care and treatment of obese and overweight people, many of whom also suffer from secondary disorders, including diabetes, cardiovascular disease, and some forms of cancer (25), have resulted in an enormous economic burden (7, 18, 49). The brain-derived neurotrophic factor (BDNF) locus is among the human chromosomal loci associated with susceptibility to obesity (66). Moreover, rare patients with Wilms’ tumor, aniridia, genitourinary anomalies, and mental retardation contiguous gene syndrome are obese and have chromosome deletions that include the BDNF locus (described by Kernie et al. [31]). Additionally, a variant of the BDNF receptor, tyrosine kinase B (trkB), has also been linked to human obesity (78).

A large body of evidence suggests that BDNF is involved in feeding behavior and body weight regulation, specifically as a potent anorexigenic factor that acts at least in part by contributing to satiety. For instance, BDNF is expressed in several brain regions important for controlling feeding behavior, including the ventromedial hypothalamic nucleus (VMH), the dorsomedial nucleus of the hypothalamus (DMH), the paraventricular nucleus of the hypothalamus (PVN), and the arcuate nucleus of the hypothalamus (ARC; 10).

Increases in central BDNF levels produced by injecting BDNF into the third ventricle (35, 53) or into specific hypothalamic and brain stem areas, including the dorsal vagal complex, VMH, and PVN, have all been associated with decreased food intake and body weight (1, 72, 74, 75). In contrast, decreases in BDNF levels produced by global heterozygous BDNF knockout (20, 31, 45), smooth muscle BDNF knockout combined with partial global BDNF knockout (19), forebrain-hypothalamic BDNF knockout (57), or virally targeted knockout of BDNF in the VMH (and adjacent DMH; 72) have all been associated with overeating and excess weight gain.

When BDNF binds to its high-affinity receptor, trkB, it results in autophosphorylation of trkB’s tyrosine residues, which activates several downstream signaling pathways (30). A mutation in humans that impairs this autophosphorylation and subsequent MAP kinase signaling resulted in hyperphagic obesity (78). This finding is consistent with the hypothesis that the effects of altered BDNF levels in the various hypothalamic regions on feeding behavior act through trkB receptor signaling.

An animal’s nutrition or energy status can influence levels of BDNF protein expression. For example, in the hippocampus, exercise and caloric restriction increase BDNF protein levels, whereas consumption of a Western diet (WD) or other forms of a high-energy diet (HED; a diet high in carbohydrate and fat) reduces BDNF protein expression (14, 24, 48, 73). Despite high levels of BDNF protein in the hypothalamus and the significance of hypothalamic regions such as the ARC, PVN, and VMH for regulation of food intake and body weight, little is known about the potential for nutrition or energy status to influence hypothalamic BDNF protein expression. Nevertheless, the potential for regulation of hypothalamic BDNF protein expression more generally is suggested by the observation of age-related changes in this BDNF (62). To date, and to our...
knowledge, the effect of nutrition or energy state on hypothalamic BDNF expression has only been examined at the mRNA level and mainly in the VMH (43, 71, 72, 77, 79). BDNF protein, however, not mRNA, is the functional molecule. Changes in protein and mRNA levels in general, and specifically for BDNF, do not always parallel one another and may even be opposite in direction (e.g., 52, 62). Therefore, it is surprising that measurement of hypothalamic BDNF protein levels has been largely absent from studies of feeding and body weight. Since our ultimate interest is in BDNF protein effects on food intake and body weight, it was essential and most relevant to determine how hypothalamic BDNF protein levels respond to energy status.

The first aim of the present study was to investigate the effects of 48 h of food deprivation on BDNF protein expression in the ARC, PVN, and VMH. Additionally, the cerebral cortex was examined as an initial assessment of the effects of food deprivation on BDNF protein expression outside the hypothalamus. On the basis of the outcomes of BDNF manipulations described above we hypothesized that food deprivation, because it causes increased food intake, will result in reduced BDNF protein levels in the VMH (reduced BDNF will reduce inhibition of feeding). A similar effect may occur in the PVN and the ARC. The PVN is also mainly inhibitory to feeding (34, 64, 70), although some neuropeptides instilled in the PVN have stimulated feeding (e.g., 33, 67). The ARC, however, is mixed: it contains subpopulations of neurons that are inhibitory or excitatory to feeding (61). The second aim was to examine the effects of short-term consumption of a WD on BDNF protein in these brain regions. This is crucial because changes in BDNF levels in the short term could contribute to early stages of hyperphagia and obesity. Understanding whether and how hypothalamic BDNF levels are altered by early stages of positive energy balance will be important for designing studies that test a causal role of these altered BDNF levels. Should BDNF play a causal role, this knowledge could be valuable for devising a treatment strategy that takes advantage of BDNF’s potent anorexigenic effect to help prevent or moderate obesity. A treatment that prevents obesity would be a significant advance given the tremendous difficulties most obese people have losing weight and maintaining weight loss (58).

To capture potential changes in BDNF protein expression in the ARC, VMH, and PVN, four different durations of WD exposure were examined: 6 h, 48 h, 1 wk, and 3 wk. In parallel, the cerebral cortex was examined as an initial assessment of the effects of WD consumption on BDNF protein expression outside the hypothalamus. Again, on the basis of the effects of BDNF manipulations described above, because overeating and obesity should inhibit food intake, we hypothesized that one or more of the durations of short-term WD exposure tested will result in increased BDNF protein levels in the VMH and possibly in the ARC and PVN (increased BDNF will increase inhibition of feeding).

MATERIALS AND METHODS

Animals

Male C57BL/6 mice, 3–5 mo of age, were used. They were originally obtained from Harlen Industries (Indianapolis) and bred for several generations in our laboratory. Mice had ad libitum access to water and standard chow in pellet form (Laboratory Rodent Diet 5001; LabDiet, St. Louis, MO) and were bred and initially maintained at 22°C on a 14:10-h light-dark cycle with lights on at 0500 and off at 1900. At a minimum of 2 wk before beginning measurements to establish stable food intake (see Experiment 1: Effects of Food Deprivation on BDNF Protein Expression in ARC, VMH, and PVN), mice were switched to a 12:12-h light-dark cycle with lights on at 0200 and off at 1400. Optimal breeding conditions for our mice include a 14:10-h light-dark cycle. The reason for the shift from a 14:10-h to a 12:12-h cycle is that mouse feeding behavior has typically been studied using a 12:12-h or similar light cycle. Therefore, this shift in the light cycle made the conditions of our feeding experiments more comparable with those of other studies. The additional shift in the lights on phase was done so that perfusion of mice in the 6-h test diet duration groups could be done before 0000. The 2-wk duration of the adaptation phase for the shift in light cycle from 14:10 to 12:12 h was based on previous experiments in which we adjusted the light cycle and found that this duration of adaptation was sufficient time for mice to exhibit normal meal patterns, food intake, and body weight (data not shown; e.g., 8). Consistent with our past experience, mice in the present study appeared to have normal food intake and body weight (data not shown; e.g., 8). All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition) and American Association for Accreditation of Laboratory Animal Care guidelines and were approved by the Purdue University Animal Care and Use Committee.

Experiment 1: Effects of Food Deprivation on BDNF Protein Expression in ARC, VMH, and PVN

For all experimental runs, mice were housed individually in clear plastic cages. Control (FED) and food-deprived (DEP) mice were run through the entire experiment in pairs, one mouse from each group, to minimize the contribution of any variation between runs due to unintended differences in procedures or laboratory environment to any group differences observed. Chow pellet intake and body weight of 20 animals were measured just before 1400 daily, and stable food intake was determined. Stable food intake was defined as consumption of ±1 g each day, for 4 consecutive days, of the average intake for those 3 days (4). These mice were then divided into FED (n = 10) and DEP (n = 10) groups matched on food intake and body weight. Days 1–5 of the experiment were the 5 days of stable food intake followed by feeding or food deprivation on days 6 and 7. The FED group continued ad libitum feeding of standard Chow pellets for the final 48 h, whereas all food was removed from the DEP group at 1400 at the start of day 6. Forty-eight hours later at 1400 (end of day 7), mice were anesthetized and then fixed by cardiac perfusion.

Tissue processing. Mice were given a lethal dose of Brevital Sodium (sodium methohexitol; 100 mg/kg) and then perfused transcardially with saline at a rate of 4 ml/min for 10 min and then with chilled 4% paraformaldehyde dissolved in 0.1 M sodium phosphate-buffered saline (PBS) kept on ice for 30 min. Brains were removed and stored at 4°C in 4% paraformaldehyde in PBS overnight and then incubated in 30% sucrose PBS overnight at 4°C. Next, a block of brain containing the hypothalamus was excised, frozen in optimum cutting temperature compound (Tissue-Tek) using liquid nitrogen, and stored at −80°C until frozen sectioning.

Immunohistochemistry and Nissl staining. For the several potential approaches for assessing protein levels, we chose quantification of the cell numbers exhibiting BDNF-like immunoreactivity (BDNF-LIR) and of these cells’ staining intensity. Importantly, these approaches, compared with ELISA, for example, ensure independent assessment of protein levels in the VMH, PVN, and ARC as these nuclei lie in close apposition to one another.

First, a control brain was cross-sectioned at 30 μm through the VMH, PVN, and ARC and stained with 0.02% cresyl violet to aid location of sections used for counting BDNF-immunostained neurons.
and nucleus boundaries. Next, brains from WD- and chow-fed mice were cross-sectioned at 30 μm through the hypothalamus, which included ~50 coronal sections that contained the VMH, PVN, and ARC in their entirety. All sections were immunostained for BDNF except every ninth section, which was stained with cresyl violet and used to help determine the shape and location of nucleus borders in each brain. The BDNF-immunostaining protocol we employed was similar to that of Ewa et al. (16). Sections were stained using the free-floating technique with gentle agitation during the entire immunostaining procedure. Sections were first washed 3 × 15 min in PBS, incubated in goat block (8% normal goat serum, 0.5% Triton X-100, 2% bovine serum albumin, 0.08% sodium azide in PBS) at 4°C for 3 days. Next, sections were washed 3 × 15 min in PBS and incubated in secondary antibody (1:600; Cy3-conjugated goat anti-rabbit, 111-165-144; Jackson ImmunoResearch) in diluent for 2 h at 4°C in the dark. Sections were then washed 3 × 15 min in PBS and mounted on slides with glycerol, and coverslips were sealed with clear nail polish. Slides were stored at 4°C in the dark until confocal imaging, which was done within 2 wk of staining. Ewa et al. (16) tested the specificity of this primary antibody for BDNF using Western blots on hypothalamic and hippocampal tissues. Specific binding with bands at 18 kDa for mature BDNF protein and at 30 kDa for precursor BDNF was demonstrated. They also found no nonspecific staining when primary or secondary antibodies were omitted. We also tested the specificity of the primary antibody using the same immunostaining procedure described above except no primary antibody was added to the goat diluent. No nonspecific staining was observed.

Quantification of cells exhibiting BDNF-LIR. The ectorhinal cortex (hereinafter referred to as CORTEX) was selected to assess whether food deprivation (or in experiment 2, WD feeding) has global effects on BDNF protein expression (i.e., whether these manipulations have effects on BDNF protein outside the hypothalamus). The ectorhinal cortex was chosen for this purpose because it was present in sections used to confirm the BDNF protein expression findings in this experiment with commercial BDNF antibodies such as the one we employed resulted in an exceedingly small dynamic range in the staining intensity of our samples. Consequently, the magnitudes of group differences were <1%. Because meaningful comparisons of intensity could not be obtained, they are not discussed in RESULTS.

Quantitative reverse transcriptase polymerase chain reaction. To confirm the BDNF protein expression findings in this experiment with a complementary method, BDNF mRNA levels were measured using quantitative PCR (qPCR). Additional groups of FED (n = 6) and DEP (n = 6) mice treated the same as described above were used to examine BDNF mRNA expression. BDNF mRNA was quantified from RNA extracted from the ARC, VMH, PVN, and CORTEX of FED and DEP. At the end of the 48-h food deprivation period for the DEP group, all mice were killed by cervical dislocation. Tissues were immediately dissected from 200-μm-thick coronal slices through the hypothalamus, using a tissue punch (23-gauge needle), and then RNA was extracted using the RNAqueous-Micro Total RNA Isolation Kit according to the manufacturer’s protocol (Thermo Fisher Scientific). Each RNA sample was incubated with DNase1 (Invitrogen) to remove genomic DNA. Then first-strand cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit in 30-μl PCR reactions according to the manufacturer’s instructions (Thermo Fisher Scientific).

To quantify BDNF mRNA levels from the hypothalamic and cortical samples, qPCR was performed on the mRNA samples. Amplification of 4-ng cDNA from the first-strand reaction was performed in triplicate using a previously established protocol (3, 11, 19, 72). On the basis of this protocol, primer sequences employed were as follows: BDNF forward, 5′ GAA AGT CCC GGT ATC ACA AG 3′; BDNF reverse, 5′ CCA GCC AAT TCT TTT TTT 3′; β-actin forward, 5′ GCC TGT ATT CCC TCC ATC G 3′; and β-actin reverse, 5′ CCA GTT GAG AAC AAT AGT AT T′. All primers were optimized such that the correlation coefficient was 0.99–1.0 and the PCR efficiency was 95–100%. Real-time PCR amplification was performed using an iCycler and PerfeCTa SYBR Green FastMix for iQ (Quanta Biosciences, Beverly, MA).

Data analysis. Values reported are means ± SE. Graphs were made using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA), and statistical analyses were performed using Statistica 6.0 (StatSoft; Tulsa, OK). In all statistical analyses, P < 0.05 was required for statistical significance. Body weight and food intake for FED and DEP groups were analyzed using an unpaired t-test. The independent variable was the diet condition (FED or DEP), and the dependent variable was the food intake in kilocalories or body weight in grams. For comparisons of stable food intake and body weight, respectively, of each group were collapsed across the 5 days of stable intake and then compared using the unpaired t-test. The same test was employed to assess differences between the FED and DEP groups in the numbers of BDNF-LIR cells and relative BDNF mRNA levels in the ARC, VMH, PVN, or CORTEX.
Fig. 1. Digital images of the brain regions used for BDNF-LIR quantification are shown here stained with cresyl violet (photomicrographs; A, C, and E) or immunohistochemistry using a BDNF antibody (confocal scans; B, D, and F). A and B: VMH and ARC are illustrated. C and D: PVN is shown. E and F: ectorhinal cortex (CORTEX) is illustrated on one side of the brain. The region of the CORTEX shown in the confocal image in F is the entire region used to count cells with BDNF-LIR as defined in MATERIALS AND METHODS. Scale bars = 100 μm.
the diet condition (FED or DEP), and the dependent variable was the number of BDNF-LIR cells within each brain region. Changes in BDNF mRNA levels between FED and DEP mice were determined by comparing changes in BDNF with β-actin, a constitutively expressed housekeeping gene. This was done using the Livak and Schmittgen method (44). Briefly, the difference in cycle threshold values between BDNF and β-actin in each tissue were calculated, and this difference was compared in FED and DEP mice as determined previously (3, 19). On the basis of studies that manipulated BDNF in the forebrain-hypothalamus, VMH, or PVN and suggested that BDNF inhibits feeding, we hypothesized that BDNF-LIR cell number and BDNF mRNA would decrease in the VMH and possibly in the ARC and PVN of the DEP compared with the FED group after 48-h food deprivation. To our knowledge, no studies have measured the effects of 24–72-h food deprivation on BDNF protein expression in this area of cortex. Chronic food restriction (e.g., 3–4 wk or longer) was found to have no effect on BDNF protein levels in the prefrontal cortex, but 3 mo of caloric restriction increased BDNF in an unspecifed region of cerebral cortex (14, 42, 51). This information is not sufficient to make a prediction about the effect of 48-h food deprivation on BDNF protein in the ectorhinal cortex. Therefore we predicted that there would be no change.

Experiment 2: Short-Term Effects of a WD on BDNF Protein Expression in ARC, VMH, and PVN

Diets. Two diets were used throughout this experiment: 1) a powdered diet modeling the “WD” (5TJN, catalog no. 1810850; TestDiet, St. Louis, MO), which contains ~39.9% fat, 44.1% carbohydrate (sucrose), and 16.3% protein and has an energy density of 4.49 kcal/g; and 2) a control diet, the standard chow maintenance diet, but in powdered form (Laboratory Rodent Diet 5001; LabDiet), which contains ~13.5% fat, 58% carbohydrates, and 28.5% protein and has an energy density of 3.34 kcal/g. To model a WD, a diet must contain high levels of fats and refined sugars and must produce dietary obesity: obesity due to increased food (calorie) intake (27). A WD is typically defined as a diet containing at least 55% fat, ~50% carbohydrates, and 15% protein (22). The diet selected for the current experiment was similar to diets used in many studies of the effects of nutrition on energy status on BDNF expression in the brain (e.g., 48). The control diet chosen for this experiment was a standard laboratory chow rather than a diet with ingredients matched to the WD because chow has been the control diet in the majority of the studies of WD effects on central BDNF expression. Moreover, our mice were maintained on this diet since birth. Therefore eliminating any changes in diet upon the start of the experiment reduced possible confounds.

Experimental design. Mice were housed individually and fed powdered standard chow in 2-oz spill-proof glass jars (Unifab, Kalama-zoo, MI; 65). Food intake and body weight were measured daily, and stable food intake was determined as defined in experiment 1. After food intake stabilized, all animals were fed only powdered WD overnight (15 h). This preexposure was done to prevent neophobia from occurring at the start of the test diet phase. Mice were then returned to powdered standard chow for 2 wk to minimize any potential effects of WD preexposure on BDNF expression.

Animals were assigned a duration of test diet exposure of either 6 h (n = 21), 48 h (n = 29), 1 wk (n = 30), or 3 wk (n = 27). The groups of animals assigned to each duration were matched on average food intake and body weight as measured during the period of stable food intake. Each duration group was further divided into WD, WD-food restricted (WD-FR), and CHOW test diet groups except for the 6-h group, which was divided into WD and CHOW test diet groups. The WD-FR group was included to assess the role of the nutrients composing the WD themselves independent of hyperphagia associated with ad libitum consumption of a WD in any changes in BDNF protein expression observed. To achieve this, these mice were fed daily an amount of the powdered WD equal to the average daily caloric consumption of chow by the CHOW group during the 5-day period of stable food intake. For each of the durations, the WD, WD-FR, and CHOW groups were matched using body weight and body weight during the period of stable intake. This should have minimized the possibility that differences in initial body weights confounded interpretation of group differences in the effect of the WD on BDNF expression. The original group sizes were as follows: 6-h WD (n = 10) and CHOW (n = 10); 48-h WD (n = 10), WD-FR (n = 10), and CHOW (n = 9); 1-wk WD (n = 10), WD-FR (n = 10), and CHOW (n = 10); and 3-wk WD (n = 9), WD-FR (n = 9), and CHOW (n = 9). The WD group received the powdered WD diet ad libitum during the test diet exposure. Fresh WD was given at a minimum of every 3 days. The WD-FR group was fed a predetermined amount of the powdered WD that contained the average number of calories consumed by the CHOW group during the 5-day period of stable food intake. The CHOW group received powdered chow ad libitum during the test diet exposure that was replaced every 3 days at a minimum.

Tissue preparation, immunohistochemistry, quantification, and data analysis were performed as described for experiment 1 unless stated otherwise. The brain regions examined for BDNF expression were also the same as in experiment 1, including the ARC, PVN, VMH, and CORTEX. The CORTEX was included to gauge the effects of WD feeding on BDNF protein levels outside the hypothalamus, similar to experiment 1.

Tissue processing. All animals were killed and perfused with fixative at 1400, immediately before onset of the dark phase of the light cycle and within ±1 h of the end of their test diet exposure duration. Animals were processed in groups of three that included one WD, one WD-FR, and one CHOW mouse from either the 6-h, 48-h, 1-wk, or 3-wk group. Data from mice with numbers of BDNF-LIR cells that were >2 SD from the mean of their group were also excluded from analysis (6-h group n = 2, 1 WD and 1 CHOW; 48-h group n = 3, 1 WD, 1 WD-FR, and 1 CHOW; 1-wk group n = 5, 1 WD, 2 WD-FR, and 2 CHOW; and 3-wk group n = 1, 1 WD). Data from mice with numbers of BDNF-LIR cells that were >2 SD from the mean of their group were also excluded from analysis (6-h group n = 2, 1 WD and 1 CHOW; 48-h group n = 2, 1 WD and 1 WD-FR; and 1-wk group n = 3, 1 WD, 1 WD-FR, and 1 CHOW). The final group sizes were as follows: 6-h WD (n = 8) and CHOW (n = 8); 48-h WD (n = 8), WD-FR (n = 8), and CHOW (n = 8); 1-wk WD (n = 8), WD-FR (n = 7), and CHOW (n = 7); and 3-wk WD (n = 8), WD-FR (n = 9), and CHOW (n = 9).

Data analysis. For the 6-h test diet exposure groups, differences in body weight and food intake were analyzed using a Student’s unpaired t-test, whereas, for the 48-h groups, one-way ANOVA with Bonferroni’s multiple-comparisons post hoc test was employed. The independent variable was the diet condition (WD, WD-FR, and CHOW), and the dependent variable was either food intake in kcalories or body weight in grams. Repeated-measures ANOVA across days was used to analyze group differences in food intake and body weight in the 1- and 3-wk test diet exposure groups. When the overall repeated-measures ANOVA was significant, pairwise group comparisons were made between test diet groups using repeated-measures ANOVA across days. Pairwise group comparisons of the numbers of cells exhibiting BDNF-LIR were made according to a priori hypotheses, using a Student’s unpaired t-test. The independent variable was the time exposed to each diet condition, and the dependent variable was the number of BDNF-LIR neurons within each brain region. On the basis of studies that manipulated BDNF in the forebrain-hypothalamus, VMH, or PVN and suggested that BDNF inhibits feeding, we hypothesized that BDNF-LIR cell number would increase in the VMH and possibly in the ARC and PVN of WD mice compared with the WD-FR and CHOW group at all test diet durations. As there was no evidence for short-term effects of a WD on CORTEX BDNF protein levels, we predicted that there would be no effect.
RESULTS

Experiment 1: Effect of Food Deprivation on BDNF Protein Expression in ARC, VMH, and PVN

Food intake and body weight. ANOVA demonstrated that there were no group differences in age, and Bartlett’s test showed that there were no group differences in variances in age (data not shown). During the period of stable food intake, the FED and DEP groups exhibited similar average daily food intake ($t = 0.44, P = 0.33; \text{Fig. 2A}$) and body weight ($t = 0.64, P = 0.27; \text{Fig. 2B}$). Over the course of the 48-h deprivation period, food intake for FED animals was greater than for DEP mice (Fig. 2A; no statistical test was done as no variance in DEP group). Body weight of DEP mice was significantly reduced after 24 h (13%; 21.88 ± 0.45 g) and 48 h (21%; 20.67 ± 0.52 g) compared with their initial weight (24.22 ± 0.43 g; $t = 4.17, 6.47$, respectively, both $P < 0.01; \text{Fig. 2B}$). Body weight for the FED group after 48 h was 24.94 ± 0.45 g, which was similar to their weight at 0 h (24.85 ± 0.54 g; $t = 0.13, P = 0.45$) and significantly greater than the weight of DEP mice at 48 h (20.67 ± 0.52 g; $t = 6.66, P < 0.01; \text{Fig. 2B}$).

**BDNF protein expression.** The number of cells that exhibited BDNF-LIR in each brain region examined is shown in Fig. 2C. The number of BDNF-LIR cells in the ARC was significantly decreased in DEP compared with FED mice (32%, $P < 0.05$). The number of BDNF-LIR cells in the VMH was significantly decreased in DEP compared with FED mice (31%, $t = 1.12, P = 0.12$). The number of BDNF-LIR cells in the PVN was similar to their weight at 0 h (24.85 ± 0.54 g; $t = 0.44, P = 0.64$) and significantly greater than the weight of DEP mice at 48 h (20.67 ± 0.52 g; $t = 6.66, P < 0.01; \text{Fig. 2B}$).

The effects of 6 h, 48 h, 1 wk, or 3 wk of WD consumption on BDNF protein expression in the ARC, VMH, and PVN were examined. Expression in the CORTEX was also investigated to gauge the potential for an effect of diet on brain regions outside the hypothalamus. The $t$ and $P$ values for comparisons of BDNF-LIR cell numbers are listed in Table 1. ANOVA demonstrated that there were no group differences in age, and Bartlett’s test showed that there were no group differences in variances in age (data not shown).

**Six-hour test diet exposure. Food intake and body weight.** During the period of stable food intake, WD and CHOW animals consumed similar amounts of food ($t = 0.06, P = 0.90$). There were no differences in BDNF-LIR cell numbers between FED and DEP groups in the PVN ($P = 0.12$) and CORTEX ($P = 0.27$). **BDNF mRNA expression.** To confirm the effect of 48-h food deprivation on BDNF protein expression in the ARC, we compared BDNF mRNA expression in the ARC as well as in the PVN, VMH, and CORTEX in the FED and DEP groups (Fig. 2D). Levels of BDNF mRNA normalized to $\beta$-actin mRNA in the brain regions of the FED group were set at 100%. Similar to BDNF protein expression, qPCR showed a significant decrease in BDNF mRNA levels only in the ARC (62%, $t = 2.57, P < 0.05$), a nonsignificant decreasing trend in the VMH (31%, $t = 1.12, P = 0.12$), no change in the PVN ($t = 0.49, P = 0.32$), and a nonsignificant increasing trend in the CORTEX ($t = 1.624, P = 0.07$).

Experiment 2: Short-Term Effects of a WD on BDNF Protein Expression in ARC, VMH, and PVN

The effects of 6 h, 48 h, 1 wk, or 3 wk of WD consumption on BDNF protein expression in the ARC, VMH, and PVN were examined. Expression in the CORTEX was also investigated to gauge the potential for an effect of diet on brain regions outside the hypothalamus. The $t$ and $P$ values for comparisons of BDNF-LIR cell numbers are listed in Table 1. ANOVA demonstrated that there were no group differences in age, and Bartlett’s test showed that there were no group differences in variances in age (data not shown).
Fasting and Western Diet Effects on Hypothalamic BDNF

Table 1. Values of t and P for group comparisons of number of BDNF-LIR cells in experiment 2

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<td>t Value</td>
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<td>0.33</td>
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<td>PVN</td>
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<tr>
<td>CORTEX</td>
<td>0.46</td>
<td>0.32</td>
<td>1.22</td>
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<tr>
<td>Three-week group</td>
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<tr>
<td>ARC</td>
<td>0.78</td>
<td>0.23</td>
<td>0.92</td>
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<tr>
<td>VMH</td>
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<tr>
<td>CORTEX</td>
<td>0.46</td>
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In the CORTEX there were trends toward increases in BDNF-LIR cells in WD (18.5%) and WD-FR (17%) compared with CHOW mice, but they were not significant.

One-week test diet exposure. Food intake and body weight. During the period of stable food intake, WD, WD-FR, and CHOW animals consumed similar amounts of food ($F_{2,19} = 0.08, P = 0.93$; data not shown) and exhibited similar body weights ($F_{2,19} = 1.19, P = 0.33$; data not shown). Moreover, these groups did not differ in body weight ($F_{2,19} = 0.55, P = 0.59$) or food intake ($F_{2,19} = 0.27, P = 0.77$) the day before test diet exposure (data not shown). Repeated-measures ANOVA of group food intakes over days during test diet exposure showed significant main effects of diet and days ($F_{2,19} = 10.67, F_{7,133} = 2.80, both P < 0.01$) and a significant interaction (days × diet; $F_{14,133} = 3.55, P < 0.01$; Fig. 5A). Pairwise comparisons between these same group food intakes using repeated-measures ANOVA over days showed significant increases between WD and WD-FR and WD and CHOW groups (23 and 31% increases, respectively, based on averages...
over the 7 days; $F_{7,91} = 3.22$ and 4.86, respectively, both $P < 0.01$ but no difference between WD-FR and CHOW mice ($F_{7,84} = 1.27, P = 0.27$). Repeated-measures ANOVA of group body weights over days during test diet exposure demonstrated a significant main effect of days ($F_{7,133} = 16.80, P < 0.01$), but not of diet ($F_{2,19} = 2.28, P = 0.71$), and a significant interaction (days $\times$ diet) ($F_{14,133} = 8.12, P < 0.01$; Fig. 5B). Pairwise comparisons of these same group body weights using repeated-measures ANOVA over days showed significant increases in WD compared with WD-FR and CHOW mice (both *$P < 0.05$).

Fig. 4. Food intake (A), body weight (B), and number of BDNF-LIR cells counted (C) are plotted for the 48-h test diet exposure groups. A: food intake was increased by 34 and 29% in the WD mice compared with the WD-FR and CHOW groups (both $P < 0.01$), respectively, after 48 h of test diet consumption. *$P < 0.01$. B: in contrast, there were no differences in body weight between the WD, WD-FR, and CHOW groups after 48 h of test diet exposure ($P = 0.12$). C: there were no differences in the number of BDNF-LIR cells in any of the brain areas examined. There were trends toward an increase in the VMH (WD vs. CHOW, 33% $P = 0.07$) and CORTEX (WD vs. CHOW, 18.5%, $P = 0.09$; WD-FR vs. CHOW, 17%, $P = 0.05$), but they were not significant. Note that BDNF-LIR cell counts for a given nucleus varied across test diet durations. In particular, BDNF-LIR cell numbers in a given nucleus in WD, WD-FR, and CHOW groups changed in parallel from one test diet duration to another.

Fig. 5. Food intake (A), body weight (B), and number of BDNF-LIR cells counted (C) are plotted for the 1-wk test diet exposure groups. In A and B, day 0 represents the day before test diet exposure. A: ANOVA with repeated measures over days showed significant main effects (diet and days) and interaction (days $\times$ diet) (all $P < 0.01$) for the food intake of the WD, WD-FR, and CHOW groups. Repeated-measures pairwise comparisons showed differences in food intake between WD and WD-FR groups and WD and CHOW groups (23 and 31% increases, respectively; both $P < 0.01$), but no difference between WD-FR and CHOW mice ($P = 0.27$). B: ANOVA with repeated measures over days demonstrated a significant main effect of days ($P < 0.01$), but not diet ($P = 0.72$), and a significant interaction (days $\times$ diet) ($P < 0.01$) for the body weights of the WD, WD-FR, and CHOW groups. Repeated-measures pairwise comparisons of the body weights detected differences between WD and WD-FR groups and WD and CHOW groups (23 and 31% increases, respectively; both $P < 0.01$), but no difference between WD-FR and CHOW mice ($P = 0.27$). C: number of cells that exhibited BDNF-LIR was increased in WD compared with CHOW mice in the VMH and CORTEX (40 and 20%, respectively) and a trend toward an increase in WD compared with WD-FR mice in the CORTEX was not significant (13% increase, $P = 0.13$). There were no differences in cell numbers showing BDNF-LIR between the WD, WD-FR, and CHOW groups in the ARC or PVN. *$P < 0.05$. In A and B the same lowercase letters placed to the right of each line in each graph indicate no significant difference between groups, whereas different letters indicate a significant difference between groups.
11% based on averages over the 7 days; \( F_{7.91} = 6.31 \) and 2.49, respectively, both \( P < 0.01 \)), whereas WD-FR and CHOW mice were not different (\( F_{7.84} = 1.96, \ P = 0.07 \)).

**BDNF Protein Expression.** No group differences in the mean number of BDNF-LIR cell numbers were observed in the ARC (Fig. 5C). The increasing trend in the mean number of BDNF-LIR cells in the VMH of WD compared with CHOW mice observed after 48-h test diet exposure was further increased to 40% and became significant after 1 wk of exposure (Fig. 5C). No group differences in the mean number of BDNF-LIR cell numbers were observed in the PVN (Fig. 5C). In the CORTEX, similar to the VMH, the increasing trend in the mean number of BDNF-LIR cells of WD compared with CHOW mice observed after 48-h test diet exposure was further increased to 20% and became significant after 1 wk of exposure (Fig. 5C). In contrast, the increasing trend of 13% in the WD compared with the WD-FR group was not significant (Fig. 5C).

**Three-week test diet exposure. Food intake and body weight.** During the period of stable food intake, WD, WD-FR, and CHOW animals consumed similar amounts of food (\( F_{2.23} = 3.34, \ P = 0.05 \); data not shown) and exhibited similar body weights (\( F_{2.23} = 0.34, \ P = 0.71 \); data not shown). Furthermore, these groups did not differ in body weight (\( F_{2.23} = 0.09, \ P = 0.91 \)) or food intake (\( F_{2.23} = 2.63, \ P = 0.09 \)) on the day before test diet exposure (data not shown). Repeated-measures ANOVA of group food intakes over days during test diet exposure revealed significant main effects of days and diet (\( F_{21,483} = 5.02, F_{2.23} = 5.74, \ P < 0.01 \)), and a significant interaction (days \( \times \) diet; \( F_{42,483} = 2.75, \ P < 0.01 \); Fig. 6A). Pairwise-repeated-measures ANOVA of these same group food intakes over days showed a significant decrease in WD-FR compared with CHOW mice (18% decrease based on averages over 3 wk; \( F_{21,336} = 2.95, \ P < 0.01 \)). In contrast, an increasing trend in the WD compared with the WD-FR group failed to reach significance (20% increase based on averages over 3 wk; \( F_{21,315} = 1.56, \ P = 0.06 \)). Some WD-FR mice did not consistently consume all of the food they were offered each day. The amount of food they were offered matched the average daily caloric intake of the CHOW group during the 5-day period of stable chow intake. This amount of food did not fully account for the slight increase in caloric consumption of the CHOW group with age from the 5 days of stable intake to the 3-wk test diet phase (an average of 1.5 kcal/day). Nevertheless, on average, the WD-FR mice ate even less (13.21 kcal/day) than they consumed during the stable intake phase (13.89 kcal/day) and CHOW mice ate during this phase (14.62 kcal/day). This suggests that their decreased intake relative to the other groups was mainly due to their failure to consistently consume all the restricted amount of food they were offered. Repeated-measures ANOVA of group body weights over days during test diet exposure showed a significant main effect for days (\( F_{21,483} = 33.65, \ P < 0.01 \)), but not diet (\( F_{2.23} = 1.04, \ P = 0.37 \); Fig. 6B), and a significant interaction (days \( \times \) diet; \( F_{42,483} = 5.65, \ P < 0.01 \)). Pairwise comparisons of these same group body weights using repeated-measures ANOVA over days demonstrated increases in the WD and WD-FR compared with CHOW mice (9 and 7% increases; \( F_{21,315} = 9.99 \) and \( F_{21,336} = 10.96, \) respectively, both \( P < 0.01 \)), whereas the WD and WD-FR mice were similar (\( F_{21,315} = 0.49, \ P = 0.97 \)).
BDNF PROTEIN EXPRESSION. No significant differences in the numbers of cells exhibiting BDNF-LIR were observed between the WD, WD-FR, and CHOW groups in the ARC, VMH, and PVN, although there were trends toward a decrease in the WD group compared with the WD-FR and CHOW groups in the PVN of 17 and 14.5%, respectively (Fig. 6C). In the CORTEX, however, there was a significant increase in the number of BDNF-LIR cells in the WD-FR compared with the CHOW group (12.5%). None of the other comparisons in the CORTEX were significant (Fig. 6C).

DISCUSSION

The goal of the first experiment was to determine the effect of food deprivation on BDNF protein in the ARC, VMH, and PVN. The CORTEX was also examined to make an initial assessment of whether food deprivation altered BDNF levels outside the hypothalamus. BDNF protein levels were estimated by quantifying the number of neurons exhibiting BDNF-LIR in each of these brain areas. Forty-eight hours of food deprivation produced a decrease in BDNF protein expression in the ARC. A decreasing trend also occurred in the VMH, but it was not significant. The aim of the second experiment was to determine the effect of short-term (6 h, 48 h, 1 wk, or 3 wk) WD consumption on BDNF protein in the ARC, VMH, and PVN, measured as in experiment 1. Caloric intake was increased in WD compared with WD-FR and CHOW mice over the course of the 6-h, 48-h, and 1-wk, but not the 3-wk, duration of test diet consumption. In contrast, body weight increased significantly in WD compared with WD-FR and CHOW mice only after 1 wk of test diet consumption and in WD and WD-FR compared with CHOW mice only after 3 wk. WD consumption resulted in an increasing trend in BDNF protein expression in the VMH after 48 h that became significant after 1 wk and returned to control levels by 3 wk. Within the hypothalamus, this effect appeared to be specific to the VMH as none of the durations of WD diet consumption tested altered BDNF expression in the ARC or PVN. BDNF protein in the CORTEX, however, was also increased after 1 wk of WD exposure and 3 wk of restricted WD consumption.

Effect of Food Deprivation on BDNF Protein Expression in ARC, VMH, and PVN

In most areas of the brain that have been examined, BDNF protein expression has been observed to increase in response to chronic dietary restriction, appearing to have a protective effect against some insults to the brain (e.g., hippocampus, cerebral cortex, and striatum; 14). To our knowledge, the effect of food deprivation on BDNF protein expression in the hypothalamus has not been examined. In the present study, there was a decrease in BDNF-LIR cell numbers in the ARC in response to 48-h food deprivation. Similarly, BDNF mRNA in the ARC was reduced by this duration of food deprivation. There were trends toward decreases in the VMH for both protein and mRNA, but they did not reach significance. In contrast, three previous studies have found significant reductions of BDNF mRNA in the VMH (71, 72, 77). The failure for this decrease to reach significance in the present study could have been due to methodological differences between studies. Two of the previous studies used in situ hybridization to detect BDNF mRNA (72, 77), and the third measured expression of only two of several different BDNF transcripts (71). Therefore, if some BDNF transcripts that were not measured did not decrease, or increased, and all transcripts were combined for measurement, it is possible that the significant decreases would have washed out and been a nonsignificant trend as observed in the present study.

The degree to which this reduction of BDNF expression in the ARC contributes to satiety will depend on which ARC neurons are regulated by this BDNF and whether they are excited or inhibited. The ARC contains both proopiomelanocortin (POMC) neurons that inhibit feeding and agouti-related protein (AGRP) neurons that promote feeding. A small percentage of POMC and AGRP neurons express trkB receptors (39). Consequently, a large number of neurons of unknown neurochemical identity in the ARC express trkB receptors and could therefore be regulated by BDNF. Ultimately, the present findings raise the possibility that reduced BDNF levels in the ARC following food deprivation may alter the sensitivity or activity of the AGRP, POMC, or unidentified ARC neurons that express trkB receptors to contribute to food deprivation-induced eating.

Effect of a WD on BDNF Protein Expression in ARC, VMH, and PVN

Mice in the WD group showed an increase in BDNF-LIR cell number in the VMH compared with the WD-FR and CHOW groups after 1 wk of consuming their test diets. Food intake was increased in WD animals after 48 h and 1 wk of test diet exposure, whereas their body weight did not increase until 1 wk of exposure. This could imply that either the duration of overconsumption of the WD, the consequent increase in body weight, or the combination of both contributed to the increase in VMH BDNF protein expression after 1 wk of WD consumption. These group comparisons also identify some factors that are unlikely to have contributed to the increased VMH BDNF. In particular, the 1-wk WD-FR group consumed the same WD diet for the same duration as the 1-wk WD mice but was not permitted to overeat and did not exhibit any change in VMH BDNF protein levels. This observation suggests that the 1-wk duration of WD consumption on its own could not have produced the increase in BDNF protein expression in the VMH. It further suggests that the high-carbohydrate, high-fat macronutrient content of the WD on its own was not likely to have caused the increase in VMH BDNF. Thus it appears that overconsumption of the WD for 1 wk was necessary to increase VMH BDNF protein.

The increase in VMH BDNF protein expression in the present study appeared to be selective within the hypothalamus to the VMH after 1 wk of WD exposure, as no changes in BDNF protein levels were observed in the ARC or PVN after any of the durations of WD consumption tested. It remains possible, however, that changes in BDNF expression within these nuclei could have been missed if they were associated with a subset of PVN or ARC neurons defined spatially, cytoarchitecturally, or neurochemically. Counts of these neurons would have been diluted by counts of the remaining subsets of neurons that did not exhibit a change.

The most parsimonious hypothesis to explain the increase in BDNF expression in the VMH after 1 wk of WD consumption...
is that it was a compensatory response activated by the effects of the initial hyperphagia or excess weight gain to inhibit food intake and prevent or moderate excess weight gain. It remains to be demonstrated, however, whether this increase in BDNF is successful at inhibiting food intake and, if so, to what degree. Little is known about how diet influences expression of BDNF or other molecules involved in neural function and communication. It was recently reported that 48 h of hyperglycemia and hyperinsulinemia led to increased BDNF expression in the posterior VMH (50). Therefore, if 1 wk of WD consumption caused similar metabolic changes, these could have contributed to the increased BDNF protein expression we observed in the VMH. It is also possible that the increase in VMH BDNF was mediated by leptin. As animals develop dietary obesity, circulating leptin levels typically increase (e.g., 43). Intravenous injection of leptin has been shown to increase BDNF protein in VMH neurons and in axons within the VMH and DMH that were closely opposed to neurons expressing trkB (32). Moreover, C57BL/6 mice consuming a WD for 1 wk still respond to leptin by reducing food intake to a similar degree as controls, suggesting that leptin resistance had not yet developed (40).

Thus, if an increase in circulating leptin occurred by 1 wk of WD consumption in the present study, it could have led to an increase in VMH BDNF levels. BDNF protein expression returned to control levels between 1 and 3 wk of WD feeding. Understanding the mechanism underlying this decrease could be valuable for identifying a means of maintaining increased VMH BDNF, which might moderate hyperphagia and weight gain. One process that could account for this decrease in VMH protein is the buildup of free radicals that lead to oxidative stress (OS). OS occurs in the hypothalamus of obese rodents, and it decreases BDNF expression by reducing binding of a key transcription factor, cAMP response element-binding protein (CREB), to the BDNF promoters (17, 29, 41, 56, 69, 80). Another process that could contribute to the decrease in VMH BDNF protein that occurred between 1 and 3 wk of WD consumption is hypothalamic insulin resistance (9, 13), which could lead to reduced glucose uptake by glucose-sensitive VMH neurons and therefore result in decreased activity of these neurons (63). The probability that such an effect could lead to reduced VMH BDNF levels is high given that a large proportion of VMH BDNF neurons have insulin receptors (38) and that BDNF expression is activity dependent, possibly involving epigenetic modification of the BDNF promoter (47). Finally, if leptin was involved in the increase in VMH BDNF protein, then leptin resistance could have contributed to the decrease of BDNF expression to control levels after 3 wk of WD exposure. In rodents, leptin resistance appears to develop after a few weeks of HED or WD consumption (59, 60) and therefore could have prevented leptin from maintaining an increase in VMH BDNF expression.

A potential alternative explanation for the increase in numbers of BDNF-LIR cells in the VMH after 1 wk of WD consumption is that neurogenesis supplied additional BDNF neurons to the VMH. The available evidence, however, suggests that it is unlikely that neurogenesis occurred in the VMH in the present study. First, hypothalamic neurogenesis associated with consumption of a HED has only been observed in females: it was not detected in males (36), and we only studied males. Second, only a small number of new neurons were generated in the hypothalamus of females in this study. Moreover, the new neurons only occurred in the median eminence, not in the VMH. Third, if neurogenesis accounted for the increased BDNF-LIR cell numbers present after 1 wk of WD consumption, then the cells generated should still be present after 3 wk of WD feeding. We found no increase, however, in BDNF-LIR cells in the VMH in the mice that consumed the WD for this duration. Finally, in a preliminary analysis we found no evidence for neurogenesis in the VMH. To examine whether total VMH neuron number increased, as would be expected if WD-induced neurogenesis had occurred, we performed total counts of cresyl violet-stained neurons in the ARC, VMH, PVN, and CORTEX for mice that consumed the WD for 1 wk, using the sections from the BDNF immunostaining that had been set aside for cresyl violet staining (see MATERIALS AND METHODS). No significant differences in the total number of cresyl violet-stained neurons were found between the WD, WD-FR, and CHOW 1-wk test diet groups for the ARC, VMH, PVN, or CORTEX (data not shown). These preliminary results are consistent with the interpretation that the increase in number of VMH cells exhibiting BDNF-LIR after 1 wk of WD consumption was due mainly to increased expression of BDNF from undetectable to detectable levels in some cells.

Effect of a WD on BDNF Expression in CORTEX (Ectorhinal Cortex)

The ectorhinal cortex was investigated as an initial assessment of whether food deprivation (experiment 1) or WD feeding (experiment 2) has global effects on BDNF protein expression (i.e., whether these manipulations have effects on BDNF protein outside the hypothalamus). In a large number of previous studies, 16–21 wk of HED (in some studies these diets fit the criteria of a WD, and in others they did not) feeding have typically reduced BDNF protein expression in the prefrontal cortex or an unspecified cortical region (e.g., 6). Instances of decreased BDNF protein expression in the prefrontal cortex, typically ~35% in magnitude, were associated with impairment of certain types of memory such as recognition (e.g., 6).

In the present study, after much shorter durations of WD exposure, increased rather than decreased BDNF protein expression occurred in the CORTEX. These increases occurred after 1 wk for the WD group (20%) and 3 wk for WD-FR mice (12.5%). Because these increases were modest, their physiological relevance should be interpreted with caution. Moreover, the augmented BDNF expression in the CORTEX of the 3-wk WD-FR group was confounded by a significant decrease in caloric intake compared with the 3-wk CHOW mice. Thus it is not possible to distinguish the contributions of WD consumption and reduced caloric intake to the increase in BDNF protein expression. The increase in BDNF protein in the ectorhinal cortex after 1 wk of WD feeding suggests that WD consumption can affect BDNF protein expression outside the hypothalamus. Similar to the argument presented above regarding increased VMH BDNF protein after 1 wk of WD feeding, the hyperphagia or excess body weight gain associated with this duration of WD consumption probably contributed to the increased CORTEX BDNF protein in the WD group. The increase in CORTEX BDNF observed in the WD-FR group...
after 3 wk of WD consumption raises the possibility that such a long exposure to the nutrient composition of the WD may produce a modest increase in CORTEX BDNF protein even without overeating. Interestingly, similar to the contrasting effects of WD vs. restricted WD feeding on VMH vs. CORTEX BDNF protein levels in the present study, altered BDNF mRNA expression was observed in the VMH and hippocampus of mice after long-term (14 wk) ad libitum consumption of a WD but occurred only in the hippocampus of mice pair fed the WD (79).

The scarcity of information on the ectorhinal cortex makes it difficult to envision what the potential significance of diet-induced increases in its BDNF levels might be. The ectorhinal cortex appears to regulate autonomic function, especially involving the sympathetic nervous system (76). It receives input from the posterior basomedial amygdala, which is involved in assigning emotional significance to sensory stimuli, especially those associated with stress, anxiety, and fear (23, 26, 46). The ectorhinal cortex projects to the amygdala transition area and extended amygdala (54). These areas project to brain stem autonomic regions to regulate the adrenal gland and peripheral sympathetic ganglia, including the stellate and celiac ganglia, which innervate the heart and gastrointestinal tract, respectively (54, 76). Thus the ectorhinal cortex may be involved in activation of sympathetic responses that contribute to emotion, anxiety, and fear, and increased BDNF levels might modulate this activation.

In conclusion, the effects of manipulations that produce negative (food deprivation) and positive (WD feeding) energy balance on BDNF protein expression in the hypothalamic ARC, VMH, and PVN nuclei, key brain regions in the regulation of feeding and body weight, were examined in normal mice. Considering the widespread expression of BDNF, we also examined the ectorhinal cortex to begin to assess the potential for these energy balance manipulations to have global effects on BDNF protein. BDNF protein expression was estimated as the number of cells exhibiting BDNF-LIR in each brain region examined. Compared with ad libitum CHOW intake, 48-h food deprivation caused a decrease in BDNF protein expression in the ARC. In contrast, WD feeding increased BDNF protein expression in the VMH and CORTEX after 1 wk, but not after 6 h, 48 h, or 3 wk and not in the ARC or PVN. Additionally, 3 wk of restricted WD food intake (WD-FR) produced a small, selective increase in BDNF protein expression in the CORTEX. These results suggest that opposite manipulations of energy balance such as food deprivation versus WD consumption can have opposite effects on hypothalamic BDNF protein expression. These effects occurred, however, in different hypothalamic nuclei (ARC vs. VMH) and over different time courses (48 h vs. 1 wk). These changes in BDNF levels are most probably part of the compensatory homeostatic responses activated by the deviations from energy balance that result from food deprivation and WD feeding. For example, one of the goals of responses to food deprivation would be to increase food intake, an effect that occurs in response to decreased BDNF. In contrast, one of the goals of responses to WD feeding would be to decrease eating, an effect that occurs in response to increased BDNF. The degree to which these altered BDNF levels contribute to changes in food intake remains to be established. The possible role of the effects of the WD and WD-FR manipulations on BDNF protein expression in the CORTEX is not clear but may involve modulation of cortical regulation of autonomic, probably sympathetic, responses that accompany some emotional behaviors.

**Perspectives and Significance**

Long-term (4–6 mo) consumption of WDs has consistently reduced BDNF protein levels in the various brain areas that have been examined, including the hippocampus and prefrontal cortex (6, 48, 73). Given BDNF’s potent anorexigenic activity, if a WD also reduced BDNF in the VMH, this would essentially decrease the baseline level of inhibition of feeding and thus could contribute to the WD-induced hyperphagia (1, 35, 53, 72, 74, 75). Instead, we found that short-term (1 wk) consumption of a WD increased VMH BDNF protein. This raises the possibility that the increase in VMH BDNF was part of a homeostatic response to counter the WD-induced overeating and excess weight gain. Consistent with this possibility, global BDNF heterozygous knockout mice that could not be distinguished from wild types on the basis of food intake, body weight, meal size, or meal frequency when fed a balanced diet exhibited an exaggerated early hyperphagia compared with wild types when fed a WD (20). This could imply that the knockout mice were not able to sufficiently increase BDNF levels to oppose the increase in food intake stimulated by the WD. In the present study, exposure to the WD for 3 wk erased the increase in VMH BDNF that occurred after 1 wk. Accordingly, prolonged WD feeding appears to neutralize this increase in VMH BDNF protein expression. If the increase in BDNF did comprise part of a compensatory response to oppose hyperphagia, by removing a potentially inhibitory influence on feeding, this neutralization may have contributed to the development of dietary obesity.

Similar to this pattern of reduced expression of VMH BDNF protein between 1 and 3 wk of WD consumption, dietary obesity is associated with altered expression of other central and peripheral feeding regulatory molecules or their receptors (15, 28). The direction of these changes in expression often suggested that they would reduce the effectiveness of anorexigens and increase the effectiveness of orexigens. In some instances, this has been borne out. For example, in dietary obese animals, inhibition of feeding by CCK is less effective than in Chow-fed controls (12). These changes would favor maintenance of obesity because they reduce inhibitory influences on feeding or increase excitatory influences.

Importantly, if changes in the effectiveness of feeding signals occur before obesity develops, they are more likely to play a causal role in the obesity than effects that occur after an animal has become obese. Most of the research on the plasticity of feeding signals in dietary obesity has been done in animals that are already obese, and consequently, little is known about the timing of this plasticity relative to different stages of dietary obesity [e.g., 15, but see Bhagat et al. (2)]. If the increase in VMH BDNF that occurs after 1 wk of WD feeding is involved in a response that limits hyperphagia and obesity but subsequently falters, it may be valuable to figure out why this reversal occurs and whether it is possible to prevent it or, even better, how to bolster the increase. Such knowledge could contribute to development of a strategy for preventing or moderating dietary obesity. Thus the finding of...
early BDNF responses to WD exposure illustrates the importance of determining the timing and direction of changes to a feeding signal in relation to the timeline of dietary obesity. In particular, determination of whether any change in a signaling pathway in dietary obesity occurs before an animal becomes obese identifies candidates that should be tested for a causal role. This strategy may be valuable to aid identification of the signaling pathways that deserve the most attention.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.E.G. and E.A.F. designed research; K.E.G. performed experiments; K.E.G. and E.A.F. analyzed data; K.E.G. and E.A.F. drafted manuscript; K.E.G. and E.A.F. edited and revised manuscript; K.E.G. and E.A.F. approved final version of manuscript.

REFERENCES


