Date: March 20, 2007

I, Christopher James Kemp,
hereby submit this work as part of the requirements for the degree of:

Master of Science

in:

Epidemiology and Biostatistics

It is entitled:

Plasma Levels of Brain-Derived Neurotrophic Factor in Obese Women
Assigned to a Very Low-Carbohydrate Diet or an Energy-Restricted Low-Fat Diet.

This work and its defense approved by:

Chair: Kim N. Dietrich, Ph.D.
        Paul Succop, Ph.D.
        Randy Seeley, Ph.D.
Plasma Levels of Brain-Derived Neurotrophic Factor in Obese Women Randomly Assigned to a Very Low-Carbohydrate Diet or an Energy-Restricted Low-Fat Diet.

A thesis submitted to the Division of Research and Advanced Studies of the University of Cincinnati in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in the Department of Environmental Health of the University of Cincinnati College of Medicine 2007

by

Christopher Kemp

B.S., The University of the West of England, 1996

Committee Chair: Kim N. Dietrich, Ph.D.
Abstract

Objectives: Studies have suggested a role for brain-derived neurotrophic factor (BDNF) in central control of food intake and energy balance. The objective of this study was to assess the effect on circulating BDNF of weight loss in an obese cohort of women.

Methods: A cohort of obese women was randomly assigned to: (1) an *ad libitum* low carbohydrate diet; or (2), a low fat diet with moderate caloric restriction. At baseline, three-months and six-months, height, weight, body fat, and circulating BDNF was recorded.

Results: Following weight loss, plasma BDNF showed a marginally significant increase. These data are contrary to the literature, which describes elevated serum BDNF levels in obese subjects compared to normal healthy-weight controls.

Conclusions: Weight loss is accompanied by an increase in plasma BDNF, regardless of the macronutrient composition of the diet employed. This effect was more pronounced in subjects assigned to a low carbohydrate diet.
Acknowledgments

I would like to thank Dr David D’Alessio and Dr Bonnie Brehm sincerely for allowing me access to serum samples generated for a previous study, and for providing generous counsel and guidance on my approach to this study. The instruction, financial support and patience of my supervisors Dr Stephen Benoit and Dr Debbie Clegg was instrumental in the completion of this thesis.

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Dr Kim Dietrich – Chair
Dr Paul Succop
Dr Randy Seeley

They each have donated significant time and energy to my further education, providing helpful advice on issues of study design, the interpretation of results, and the correct approach to statistical analysis.
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List of Abbreviations

AN – Anorexia nervosa
ARC – Arcuate nucleus
BDNF – Brain-derived neurotrophic factor
BMI – Body mass index
LC – Low carboydrate
LF – Low fat
POMC – Proopiomelanocortin
TBST – Tris buffered saline with Tween-20
VMN – Ventromedial hypothalamic nucleus
Introduction

In the past two decades, the prevalence of obesity in the United States has increased significantly (Hedley, 2004). An estimated 65 percent of adult Americans are overweight and 30 percent are obese, with a body mass index (BMI) of more than 30 kg/m². Obesity is associated with numerous medical conditions, including: adult-onset diabetes, coronary artery disease, heart failure, stroke, hypertension, depression, sleep apnea, and certain cancers (Pi-Sunyer, 1999). Furthermore, in the United States, obesity represents a profound social and economic burden, annually costing more than $100 billion to manage medically and causing the loss of an estimated 40 million workdays of productivity each year (Wellman, 2002). Obesity is the result of a long-term positive energy balance: if energy intake exceeds energy expenditure, the surplus energy is stored in the form of adipose tissue. Several important and well-delineated neural circuits play an important role in the regulation of energy homeostasis by controlling appetite and food intake. The central control of food intake is associated most frequently with the hypothalamus and regulated via the release of orexigenic and anorexigenic neuropeptides in response to physiologic and environmental stimuli (Broberger, 2005). Circulating factors such as insulin, which is released by the pancreas after eating, and leptin, which is released by adipose tissue, are able to cross the blood-brain barrier and bind to their hypothalamic receptors, initiating a cascade of cellular events that regulate food intake and appetite. Studies in human cohorts indicate that plasma leptin levels correlate significantly with BMI and other measures of adiposity (Kennedy, 1997, Jequier, 2002), and that a reduction in body weight is accompanied by a reduction in circulating levels of leptin (Havel, 1996).

Several studies have indicated a role for brain-derived neurotrophic factor (BDNF) in the central control of food intake and energy balance. An abundant growth factor widely expressed in the brain, previous reports implicate BDNF in the survival and maintenance of peripheral neurons (Ernfors, 1994), and in the synaptic plasticity that governs learning and memory in the
hippocampus (Binder, 2004). More recently, however, studies have shown that mice with a genetic whole-body reduction in BDNF levels exhibit hyperphagia and obesity (Kernie, 2000, Duan, 2003, Coppola, 2004, Fox, 2004). The specific central effects of BDNF on obesity were elucidated further with the generation of a conditional mutant mouse that displays a complete and postnatal silencing of the BDNF gene, which is limited to the brain (Rios, 2001). Significantly, these mice were more obese either than their intact wild-type littermates or the heterozygous BDNF-null mice that merely exhibit a non tissue-specific reduction in BDNF levels. By week 30 of age, mutant male and female mice with a brain-specific BDNF deletion were 80 percent and 150 percent heavier than their age-matched wild-type controls respectively. These data support a central role for BDNF in the regulation of appetite and satiety, reflected by the marked disregulation of food intake that occurs in its absence.

Figure 1. The BDNF signaling pathway.
Although the precise mechanism of BDNF action in the central nervous system is not known, some important known aspects of the BDNF signaling pathway are illustrated in Figure 1. Following food intake, leptin is released in the periphery by adipose tissue and crosses the blood-brain barrier, where it binds to proopiomelanocortin (POMC) releasing neurons in the arcuate nucleus (ARC) of the hypothalamus, one of several brain regions involved in the regulation of food intake and the control of energy balance. It is postulated that POMC then binds to BDNF releasing neurons in the ventromedial hypothalamic nucleus (VMN), initiating a cascade of cellular events that includes an increase in protein BDNF levels in the VMN, and an increased activity of TrkB, the BDNF receptor, and subsequently culminating in a marked decrease in food intake.

Conversely, BDNF gene expression in the VMN is reduced in mice by approximately 60% in response to prolonged food deprivation (Xu, 2003). Furthermore, acute and repetitive peripheral injections of rats with leptin, a circulating hormone that signals satiety, induced a concomitant increase in BDNF protein content in the brain (Bariohay, 2005). In the same animals, food deprivation for 48-hours caused a statistically significant reduction in central BDNF protein content; and subsequent refeeding of fasted animals increased BDNF protein levels, implying that BDNF is involved in the signaling of satiety and is likely a downstream effector of leptin. During a 14-day intraventricular administration of BDNF, Pelleymounter et al reported a severe, dose-dependent weight loss in male rats that persisted throughout the infusion period. Furthermore, weight loss was the result of an equally severe, and dose-dependent appetite suppression that was present for roughly half of the infusion period, leading the authors to conclude that appetite suppression during intraventricular administration of BDNF is centrally mediated (Pelleymonter, 1995). Genetic association studies carried out in human cohorts have identified specific polymorphisms in the BDNF gene that result in severe early-onset eating disorders (Friedel, 2005, Gray, 2006, Koizumi, 2004, Monteleone, 2006, Ribases, 2005). Additionally, mutations in the gene that codes for TrkB, the BDNF receptor, are associated with
severe early-onset obesity (Yeo, 2004).

In human studies, serum levels of BDNF are significantly decreased in women with anorexia nervosa and bulimia nervosa compared to healthy, normal-weight control subjects (Nakazato, 2003, Monteleone, 2005). Conversely, obese women display significantly elevated levels of serum BDNF (Monteleone, 2004) compared to normal-weight control subjects. Studies have shown that central fluctuations in BDNF concentration correlate closely with changes in serum BDNF concentration, providing evidence that serum BDNF measurements can be used as a surrogate for changes taking place in the brain (Karege, 2002). Most importantly, these data suggest an important role for BDNF in the long-term regulation of body weight: the significant increase in serum BDNF in obese subjects represents a mechanism designed to signal satiety and decrease food intake. Conversely, the reduction in circulating BDNF seen in anorexic subjects likely represents part of an adaptive mechanism geared toward increasing food intake and normalizing body weight. As yet, neither the effects of significant weight loss, nor the effects of different macronutrients on plasma levels of BDNF have been studied.

The significance of better understanding the factors that regulate food intake and the central control of appetite cannot be overestimated. In the United States, obesity is second only to cigarette smoking as a cause of preventable death, contributing to the deaths of an estimated 500,000 individuals annually. Previous studies indicate that BDNF plays an important role in the hypothalamic control of food intake, but this role has not been fully delineated. Better understanding factors with important mechanistic roles in regulating energy balance will allow the development of novel therapies for the treatment of obesity. The aims of this study are twofold: to investigate the effects of significant weight loss on plasma levels of BDNF in a cohort of obese women assigned to weigh-loss diets for a period of six-months; and to examine if the effects of weight loss on plasma BDNF levels differ depending on the macronutrient composition of the diet to which subjects are assigned. This study is designed to address two specific hypotheses. Firstly, it is hypothesized that a significant weight loss will result in a
significant reduction in plasma BDNF in a cohort of moderately obese women. Secondly, it is hypothesized that reductions in plasma BDNF levels following a period of significant weight loss are significantly greater in subjects assigned to a very low carbohydrate diet, compared to subjects assigned to a low fat diet.

Methods

Participants

A cohort of fifty-three moderately obese female subjects was recruited from the general population by placing advertisements in *The Cincinnati Enquirer*. All participants gave written, informed consent to principal investigator, Bonnie J. Brehm, PhD, for participation in the study, which was approved by the University of Cincinnati College of Medicine Institutional Review Board (IRB number: 06-11-09-01, *The Effects of Weight Loss and Macronutrient on Brain-Derived Neurotrophic Factor in Obese Women*, approved November 26, 2006). Following recruitment, the cohort was randomized, using a random number table, to receive either: (1) an *ad libitum* very low carbohydrate (LC) diet, with carbohydrates representing only 10% of total kilocalories consumed; or (2), a low fat diet (LF) with a moderate caloric restriction to approximately 1200kcal/d (5024kJ/d), and recommended distributions of fat and carbohydrate to 30% of kcal and 55% of kcal, respectively. All subjects had to satisfy the following inclusion criteria: an age of at least 18-years; a moderate level of obesity, characterized as a body mass index (BMI) of 30-35; and a stable body weight, requiring subjects to have undergone no changes in weight exceeding 10% of total body weight in the preceding six-months. The exclusion criteria were as follows: untreated hypertension, the presence of cardiovascular disease, diabetes, hypothyroidism, substance abuse, pregnancy, or lactation. Medical histories were compiled for each subject, an electrocardiogram was given, and measurements were taken of height, weight,
blood pressure, and fasting blood glucose levels. Subjects attended weekly counseling sessions with a registered dietitian during the first three-months of the six-month study period, either in small groups or on a one-on-one basis. Dietitians reviewed subjects’ three-day food records weekly, along with a weekly assessment of body weight, blood pressure, and urinary ketone levels. At baseline, three-month and six-month timepoints, height, weight, body fat (via DEXA scan), and blood pressure of each subject was measured and recorded. Additionally, blood samples were taken at each of the three timepoints in order to assess circulating levels of BDNF, lipids, glucose, insulin, β-hydroxybutyrate, ghrelin, leptin, and inflammatory markers.

**Sample Collection**

Following a 12-hour overnight fast by subjects, 10-milliliters of blood were drawn by venipuncture directly into heparinized vacutainers. Samples were centrifuged to collect plasma, which was stored at -80°C until required.

**BDNF Assay**

Plasma levels of BDNF were measured using the BDNF E<sub>max</sub> Immunoassay System kit (Promega, Madison, WI, USA), according to manufacturer's directions. In this colorimetric format, the amount of BDNF in a test sample is proportional to the color generated by an enzyme-linked oxidation-reduction reaction. Using a BDNF standard supplied at a concentration of 1µg/ml BDNF, a linear standard curve is generated, which includes BDNF concentrations between 7.8-500pg/ml.

Briefly, flat-bottomed 96-well culture plates were coated with anti-BDNF monoclonal antibody, sealed and incubated overnight at 4°C. Following overnight incubation and a vigorous wash using Tris-buffered saline with Tween-20 (TBST), 200µl of Block & Sample 1X buffer was
added to each well using a repeat pipettor. The plate was incubated for one hour at room temperature without shaking. Human plasma samples were centrifuged at 4000rpm for 10-minutes to remove particulate matter, and subsequently screened in triplicate at a single concentration, by loading wells with 50µl of each test sample, diluted 1:1 with Block & Sample 1X buffer. Plates were incubated for two-hours at room temperature on a shaking platform (400+/-100rpm). Following incubation, wells were washed five times with TBST and incubated with anti-human BDNF polyclonal antibody for two-hours at room temperature with shaking, after which plates were washed and incubated with anti-IgY antibody conjugated with horseradish peroxidase for one-hour with shaking.

Following TBST washes, plates were incubated with peroxidase substrate and tetramethylbenzidine solution for 10-minutes, until a yellow color developed. The reaction was halted by adding 100µl of 1N hydrochloric acid to wells and the absorbance of each well at 450nm was measured with an automated microplate reader. Using the absorbance of the serially-diluted BDNF standards, the concentration of BDNF in each test sample was calculated with GraphPad Prism 4, a statistical software package. Initially, all test samples were diluted 1:1 with Block & Sample 1X buffer, in order to generate BDNF concentrations within the range of the BDNF linear standard curve. In those instances that BDNF concentrations of test samples exceeded the range of the standard curve, samples were diluted 1:9 with Block & Sample 1X buffer and the assay was repeated.

**Data Analysis**

All data were de-identified by the co-Principal Investigator and subjects were assigned unique identification numbers. Personal information collected during recruitment was maintained securely in a central location for the duration of the study period and only the principal investigator had access to it. Prior to analysis, plasma BDNF concentration measurements
underwent a logarithmic transformation to normalize their distribution. Plasma BDNF concentrations were analyzed with the statistical software package SAS (version 8.2 SAS Institute, Inc., Cary, NC), utilizing a mixed model 2-factor repeat measures analysis of variance model, with plasma concentrations of BDNF as the outcome (dependent variable) and diet and time-point of study as the two independent variables (factors). Interactions between age of subject and diet, and between diet and time-point of study also were analyzed via this model. For all analyses, statistical significance was set at $P < 0.5$.

**Results**

Forty-two of the 53 subjects enrolled (79%) completed the six-month study. The age range of the subjects was 29.01- to 58.55-years-of-age (with a mean age of 43.7-years-of-age +/- 7.6 years). Before randomization to diets, the mean BMI of all subjects was 33.6 +/- 0.3kg/m$^2$. At initiation of the diets, there were no statistically significant differences between subjects assigned to the LF or LC diet groups, with respect to age, height, body weight, or BMI (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Low-Fat Group</th>
<th>Low-Carb Group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(yr)</td>
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</tr>
<tr>
<td>Height(m)</td>
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<td>1.66</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight(kg)</td>
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<td>91.16</td>
<td>0.61</td>
</tr>
<tr>
<td>BMI</td>
<td>34.04</td>
<td>33.12</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*Table 1.* Descriptive characteristics before diet initiation of subjects who completed the 6-month study period.
At the onset of the study, subjects assigned to the restricted low-fat (n=20) and the ad libitum low-carbohydrate (n=22) diets consumed a similar amount of calories: 1707 +/- 104 kcal per day and 1608 +/- 123 kcal per day, respectively. During the study, all subjects showed a daily decrease in caloric intake of approximately 450 calories compared to baseline, including those in the ad libitum low-carbohydrate group. Despite the similarities in caloric intake in both groups, the relative percentages of total calories represented by carbohydrates, fats, and proteins differed markedly between groups; by the three-month time-point, caloric intake in the LC group was distributed as follows: 15% carbohydrate, 28% protein, and 57% fat. At the same timepoint, calories in the LF group were distributed as 54% carbohydrate, 18% protein and 28% fat. By both the three-month and six-month time-points, subjects in the LC group had lost a significantly greater amount of weight than those in the LF group (p< 0.001 at three-months and six-months, as shown in Figure 2.). Specifically, at three-months, subjects assigned to the LC diet had lost an average of 7.6 +/- 0.7kg and, by six-months, a total of 8.5 +/- 1.0kg. Subjects assigned to the LF diet group had lost 4.2 +/-
0.8kg at three-months, and 3.9 +/- 1.0kg at six-months. Changes in body weight, BMI, plasma leptin and plasma BDNF levels at three-months and six-months compared to baseline for each of the two diets are displayed in Table 2. A more complete display of the data over time for each subject, organized with respect to diet, is collected in Table 3 (See Appendix B). Specifically, plasma leptin levels did not differ between diets but exhibited a significant time effect. Plasma leptin levels were reduced at three-months in both study groups compared to baseline levels (P<0.0001). By the six-month time-point, plasma leptin levels had begun to return to baseline levels and the reduction observed at the three-month time-point with both diets was no longer present in subjects on either diet by the termination of the study period. Repeat measures analysis of variance of logarithmically-transformed BDNF plasma concentrations indicated a marginally significant negative effect of weight loss on plasma BDNF concentration: plasma BDNF concentrations increased as a subject’s body weight decreased (P = 0.0525). Additionally, the concomitant increases in plasma BDNF levels that accompanied reductions in body weight were more pronounced in subjects assigned to the LC diet group than those randomized to the LF group, although the extent of the difference between the two diets was not statistically significant (Figure 3.). Specifically, the negative effect of weight loss on plasma BDNF concentration was -0.054 in subjects assigned to the LC diet and -0.032 in subjects randomized to the LF diet, but the
difference in the effects of the two diets was not significantly different \((P = 0.4820,\) as shown in Appendix A: SAS output). The average effects of the two diets are best summarized via the least square means of plasma BDNF in subjects assigned either to the LF or LC diet, which are 398.74 pg/ml, and 321.38pg/ml respectively \((p = 0.5303)\).

In previously published studies using this cohort (Brehm, 2003), investigators reported significant differences in plasma triglyceride concentrations between the two diet groups immediately following randomization, but before initiation of the diets \((P < 0.05)\). Subsequently, plasma triglyceride concentrations did not differ either at the three-month or six-month time points, and the authors concluded that this difference reflected a difference between the two diet groups at baseline.

<table>
<thead>
<tr>
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<th>Low Fat Diet Group (n=20)</th>
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<tr>
<td></td>
<td>0</td>
<td>3-mths</td>
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<tr>
<td><strong>Body wt (kg)</strong></td>
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<td>88.11(1.60)</td>
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<td><strong>BMI</strong></td>
<td>34.04(0.41)</td>
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<tr>
<td><strong>Leptin (ng/ml)</strong></td>
<td>30.08(1.88)</td>
<td>25.35(1.82)</td>
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<tr>
<td><strong>BDNF (pg/ml)</strong></td>
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</table>

Table 2. Body weight, BMI, plasma leptin and plasma BDNF concentrations at baseline, three-months and six-months, displayed with standard error of the mean for both diet groups.
Discussion

There is a mounting body of research that supports a role for BDNF in the pathophysiology of eating disorders. Numerous reports have described the hyperphagia and severe obesity that result from functional mutations in the BDNF gene (Rios, 2001, Gray, 2006). Several previous studies have demonstrated altered levels of circulating BDNF levels in individuals who exhibit chronic disregulation of body adiposity and BMI; specifically, compared to normal healthy-weight controls, serum BDNF is elevated in obese individuals and reduced in anorexic individuals to a statistically significant degree (Monteleone, 2004). However, the mechanisms that drive these changes in circulating BDNF have not yet been elucidated. The parallels between BDNF and leptin are several: both can be detected in the periphery, are elevated in obese cohorts, and bind to their receptors in the hypothalamus, where they signal satiety and bring about a reduction in food intake (Ref). In obese subjects assigned to weight loss diets, a significant reduction in body weight has been shown to significantly reduce circulating levels of leptin (Ref).

A primary hypothesis of this study, that a reduction in body weight would dismantle the negative feedback loop elevating BDNF in obese subjects and subsequently reduce plasma BDNF levels, was rejected. Moreover, in this cohort, a period of weight loss was accompanied instead by a marginally statistically significant increase in plasma BDNF levels in these subjects and not a reduction. Previously, Nakazato et al did not observe any changes in serum BDNF in subjects with anorexia nervosa (AN) following an increase in body weight, leading the authors to conclude that reduced serum BDNF levels in AN patients are not due to changes in body weight (Nakazato, 2006). In a study of 140 adults, Lommatzsch et al reported a statistically significant negative correlation between body weight and plasma BDNF consistent with the increase in plasma BDNF seen in this cohort following a reduction in body weight (Lommatzsch, 2005). Specifically, by dividing a cohort of healthy adults into three groups, based on body weight (group A: <70kg; group B: 70-90kg; group C: >90kg) Lommatzsch showed significantly lower
plasma BDNF levels in the heavier subjects of group C compared to those in group A ($P < 0.01$). These data suggest a very different relationship between body weight and BDNF concentration in plasma and serum respectively.

The mechanisms that control the storage and release of BDNF remain unclear, but the literature suggests BDNF is produced primarily in the brain and spinal cord, and then stored and later transported to the periphery via platelets (Yamamoto, 1990). Studies have shown that serum contains 50-fold higher levels of BDNF than plasma (Radka, 1996). As such, the difference between serum and plasma BDNF levels represents the amount of BDNF stored in platelets (Lommatzsch, 2005). Importantly, platelets do not produce BDNF but instead acquire their BDNF stores from circulating plasma via a process of internalization that has not yet been fully elucidated. Following agonistic stimulation, BDNF is released from platelets in the periphery; but strong evidence indicates that an activated platelet releases only half of its stored BDNF, the remainder of which represents a significant non-releasable pool of BDNF that plays no physiological role (Fujimura, 2002).

Davi et al demonstrated increased and persistent levels of platelet activation in a cohort of obese women (Davi, 2002); and Coban et al showed a statistically significant increase in mean platelet volume in an obese cohort, compared to non-obese controls matched for age and gender (Coban, 2005). In a comparison of serum BDNF in children and adolescents, El-Gharbawy et al hypothesized a positive relationship between serum BDNF concentrations and body adiposity consistent with the published literature. Conversely, serum BDNF concentrations were significantly lower in overweight subjects ($P = 0.03$) following adjustment for sex, race pubertal status, age, and platelet count. Subsequent multiple regression analysis found no significant contribution to serum BDNF levels from sex, race, or adiposity. Platelet count ($P < 0.001$) and age ($P = 0.006$) were significant predictors of serum BDNF concentration, leading the authors to conclude that serum BDNF is little more than a reflection of platelet BDNF stores and is unrelated to obesity (El-Gharbawy, 2006). These data have far reaching implications for those
attempting to link elevated serum BDNF concentrations to body adiposity. More precisely, increases in serum BDNF concentrations in obese subjects are little more than a residual artifact of the well-documented changes in platelet function that accompany obesity. The increase in plasma BDNF that occurred with a reduction in body weight in this cohort mirrors the relationship between serum BDNF and adiposity, once the data are adjusted for platelet count. Together these findings suggest that plasma provides a more accurate representation of BDNF production in the central nervous system and that the effects of weight loss are opposite to those reported elsewhere in the literature.

The study addressed a second hypothesis: that changes to plasma BDNF levels during a period of significant weight loss differ with regard to the macronutrient composition of the diet ingested. Despite not reaching statistical significance, the effects of the two diets on plasma BDNF concentrations did differ and these differences are worthy of consideration. The marginally significant increases in plasma BDNF that accompanied weight loss were more pronounced in subjects assigned to an ad libitum low carbohydrate diet than those randomized to receive the more restrictive low fat diet. This effect is likely a reflection of the larger amount of weight loss in subjects assigned to the low carbohydrate diet group. In a previously published study by Archer et al, rats were randomized to one of two test diets with markedly different macronutrient compositions. Despite dramatically different long-term effects on weight gain, body adiposity and energy intake, hypothalamic BDNF gene expression in response to the two diets was not different (Archer, 2005). Whereas, rats fed diets rich in saturated fat and refined sugar show dramatic long-term reductions in BDNF gene expression and protein levels in brain regions involved with memory and learning (Molteni, 2002).
Conclusion

This study assessed the effects of weight loss on circulating levels of BDNF, a neurotransmitter that signals satiety in the hypothalamus, thereby regulating food intake and energy balance in the central nervous system. In a moderately obese cohort of women, a significant reduction in body weight was accompanied by a marginally significant increase in plasma BDNF levels. The primary hypothesis, that a reduction in body weight would dismantle the negative feedback loop elevating BDNF in obese subjects, and subsequently normalize plasma BDNF levels, was rejected. Instead, plasma BDNF was elevated in subjects following a period of weight loss. These data are novel and provide valuable information on the effects of weight loss on circulating BDNF; additionally, these data are contrary to those of previously published studies, which report the opposite findings in serum. Perhaps most importantly, these findings suggest that measuring BDNF levels in serum does not provide an accurate means of assessing circulating levels of BDNF in the periphery. In fact, the widely reported changes in serum BDNF observed in obese cohorts and AN patients are little more than a reflection of the alterations to platelet count and function that accompany these conditions.

Changes in plasma BDNF levels differed slightly in subjects assigned to an *ad libitum* low carbohydrate diet, compared to those randomized to a restrictive low fat diet; however, the differences were not statistically significant. Subjects in the low carbohydrate diet group lost significantly more weight than those in the low fat diet group at all time points; additionally, previously published studies have reported no significant effect on hypothalamic BDNF gene expression of diets with different macronutrient compositions. Taken together, these data suggest that it is the reduction in body weight and not dietary macronutrient composition that is responsible for changes in plasma BDNF levels in this cohort. Furthermore, as the evidence suggests, the greater the reduction in body weight, the larger the increase in plasma BDNF.
Bibliography


9. Duan W, Guo Z, Jiang H, Ware M, Mattson MP. Reversal of Behavioral and Metabolic Abnormalities, and Insulin Resistance Syndrome, by Dietary Restriction in Mice


Appendix A

The SAS System

full model for ln(bdnf)  13
12:24 Thursday, December 14, 2006

The Mixed Procedure

Model Information

Data Set                     WORK.RES
Dependent Variable           lnbdnf
Covariance Structure         Autoregressive
Subject Effect               Subject
Estimation Method            REML
Residual Variance Method     Profile
Fixed Effects SE Method      Model-Based
Degrees of Freedom Method    Between-Within

Class Level Information

Class    Levels    Values
Subject  39        1 2 3 4 5 6 7 8 9 10 11 12 13
          14 15 16 17 18 19 20 21 22 23
          24 25 26 27 28 29 30 31 32 33
          34 35 37 39 41 42
DIET     2         1 2
Dimensions

Covariance Parameters     2
Columns in X             17
Columns in Z             0
Subjects                  39
Max Obs Per Subject      2

Number of Observations

Number of Observations Read       84
Number of Observations Used       64
Number of Observations Not Used    20

Iteration History

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The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

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Fit Statistics

-2 Res Log Likelihood: 186.7
AIC (smaller is better): 190.7
AICC (smaller is better): 190.9
BIC (smaller is better): 194.0

Null Model Likelihood Ratio Test

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<tr>
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<td>0.6329</td>
</tr>
</tbody>
</table>

full model for ln(bdnf) 15

The Mixed Procedure

Least Squares Means

| Effect | DIET | Estimate | Error | DF | t Value | Pr > |t| |
|--------|------|----------|-------|----|---------|------|---|
| DIET   | 1    | 6.0031   | 0.2053| 30 | 29.24   | <.0001|
| DIET   | 2    | 5.6323   | 0.2282| 30 | 24.68   | <.0001|

model for ln(bdnf) with insignificant 2-way interactions removed 16

The Mixed Procedure
Model Information

Data Set                     WORK.RES
Dependent Variable           lnbdnf
Covariance Structure         Autoregressive
Subject Effect               Subject
Estimation Method            REML
Residual Variance Method     Profile
Fixed Effects SE Method      Model-Based
Degrees of Freedom Method    Between-Within

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
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<tbody>
<tr>
<td>Subject</td>
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<td>1 2 3 4 5 6 7 8 9 10 11 12 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 15 16 17 18 19 20 21 22 23</td>
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<tr>
<td></td>
<td></td>
<td>24 25 26 27 28 29 30 31 32 33</td>
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<tr>
<td></td>
<td></td>
<td>34 35 37 39 41 42</td>
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<td>1 2</td>
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</table>

Dimensions

Covariance Parameters       2
Columns in X                9
Columns in Z                0
Subjects                    39
Max Obs Per Subject         2

Number of Observations

25
Number of Observations Read              84
Number of Observations Used              64
Number of Observations Not Used          20

Iteration History

<table>
<thead>
<tr>
<th>Iteration</th>
<th>Evaluations</th>
<th>-2 Res Log Like</th>
<th>Criterion</th>
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<tbody>
<tr>
<td>0</td>
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<td>0.00001728</td>
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<tr>
<td>2</td>
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model for ln(bdnf) with insignificant 2-way interactions removed

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The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

<table>
<thead>
<tr>
<th>Cov Parm</th>
<th>Subject</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR(1)</td>
<td>Subject</td>
<td>0.2442</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>0.8419</td>
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</table>

Fit Statistics

<table>
<thead>
<tr>
<th>-2 Res Log Likelihood</th>
<th>182.6</th>
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</thead>
<tbody>
<tr>
<td>AIC (smaller is better)</td>
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<tr>
<td>AICC (smaller is better)</td>
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</table>
BIC (smaller is better) 190.0

Null Model Likelihood Ratio Test

<table>
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<tr>
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<th>Pr &gt; ChiSq</th>
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<tbody>
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Type 3 Tests of Fixed Effects

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<th>Den</th>
<th>Effect</th>
<th>DF</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>DIET</td>
<td>1</td>
<td>32</td>
<td>0.90</td>
<td>0.3510</td>
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<td>1.23</td>
<td>0.2764</td>
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<tr>
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<td>32</td>
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<td>0.6908</td>
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<td>32</td>
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<tr>
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Least Squares Means

<table>
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<tr>
<td>Effect</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>DIET 1</td>
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<tr>
<td>DIET 2</td>
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model for \( \ln(\text{bdnf}) \) with insignificant 2-way interactions and covariates removed

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The Mixed Procedure

Model Information

Data Set                     WORK.THESIS
Dependent Variable           lnbdnf
Covariance Structure         Autoregressive
Subject Effect               Subject
Estimation Method            REML
Residual Variance Method     Profile
Fixed Effects SE Method      Model-Based
Degrees of Freedom Method    Between-Within

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>42</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42</td>
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<td>1 2</td>
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</table>

Dimensions

<table>
<thead>
<tr>
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</thead>
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<tr>
<td>Covariance Parameters</td>
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<tr>
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<tr>
<td>Subjects</td>
<td>42</td>
</tr>
<tr>
<td>Max Obs Per Subject</td>
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</tbody>
</table>
Number of Observations

Number of Observations Read 84
Number of Observations Used 70
Number of Observations Not Used 14

Iteration History

<table>
<thead>
<tr>
<th>Iteration</th>
<th>Evaluations</th>
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<th>Criterion</th>
</tr>
</thead>
<tbody>
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<td>0</td>
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model for ln(bdnf) with insignificant 2-way interactions and covariates removed

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The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

<table>
<thead>
<tr>
<th>Cov Parm</th>
<th>Subject</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR(1)</td>
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<tr>
<td>Residual</td>
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<td>0.7568</td>
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Fit Statistics

-2 Res Log Likelihood 184.0
AIC (smaller is better) 188.0
AICC (smaller is better)        188.2  
BIC (smaller is better)         191.5

Null Model Likelihood Ratio Test

<table>
<thead>
<tr>
<th>DF</th>
<th>Chi-Square</th>
<th>Pr &gt; ChiSq</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1.83</td>
<td>0.1758</td>
</tr>
</tbody>
</table>

Solution for Fixed Effects

| Effect   |   DIET | Estimate | Error | DF  | t Value | Pr > |t| |
|----------|--------|----------|-------|-----|---------|------|---|
| Intercept|        | 1.6703   | 3.7061| 38  | 0.45    | 0.6548|
| DIET 1   |        | -1.6847  | 2.6602| 38  | -0.63   | 0.5303|
| DIET 2   |        | 0        | .     | .   | .       | .     |
| HT       |        | 5.2561   | 2.8369| 38  | 1.85    | 0.0717|
| wt       |        | -0.05372 | 0.02649| 27  | -2.03   | 0.0525|
| wt*DIET 1|        | 0.02216  | 0.03109| 27  | 0.71    | 0.4820|
| wt*DIET 2|        | 0        | .     | .   | .       | .     |

Type 3 Tests of Fixed Effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num</th>
<th>Den</th>
</tr>
</thead>
<tbody>
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<td>DF</td>
<td>DF</td>
</tr>
<tr>
<td>DIET</td>
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<td>38</td>
</tr>
<tr>
<td>HT</td>
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</tr>
<tr>
<td>wt*DIET</td>
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</tr>
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</table>
model for ln(bdnf) with insignificant 2-way interactions and covariates removed

The Mixed Procedure

Least Squares Means

| Effect | DIET | Estimate | Error  | DF  | t Value | Pr > |t| |
|--------|------|----------|--------|-----|---------|-------|
| DIET 1 | 5.9883 | 0.1751 | 38    | 34.21 | <.0001 |
| DIET 2 | 5.7726 | 0.1683 | 38    | 34.30 | <.0001 |

The UNIVARIATE Procedure

Variable: Resid (Residual)

Moments

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Sum Weights</th>
<th>Sum Observations</th>
<th>Variance</th>
<th>Std Error Mean</th>
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</thead>
<tbody>
<tr>
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<td>-0.6673939</td>
<td>-0.706382</td>
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<tr>
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<td>0.706382</td>
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Basic Statistical Measures

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<th>Variability</th>
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<tbody>
<tr>
<td>Mean</td>
<td>Std Deviation</td>
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<tr>
<td>-0.00953</td>
<td>0.84047</td>
</tr>
<tr>
<td>Median</td>
<td>Variance</td>
</tr>
<tr>
<td>0.13270</td>
<td>0.70638</td>
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</tbody>
</table>
Mode       .      Range               4.60859
         Interquartile Range   1.00829

Tests for Location: $\mu_0=0$

<table>
<thead>
<tr>
<th>Test</th>
<th>Statistic</th>
<th>$p$ Value</th>
</tr>
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<tbody>
<tr>
<td>Student's $t$</td>
<td>$t = -0.09491$</td>
<td>0.9247</td>
</tr>
<tr>
<td>Sign</td>
<td>$M = 1$</td>
<td>0.9050</td>
</tr>
<tr>
<td>Signed Rank</td>
<td>$S = 50.5$</td>
<td>0.7699</td>
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</table>

Tests for Normality

<table>
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<th>Test</th>
<th>Statistic</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shapiro-Wilk</td>
<td>$W = 0.95645$</td>
<td>0.0160</td>
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<tr>
<td>Kolmogorov-Smirnov</td>
<td>$D = 0.09717$</td>
<td>0.0981</td>
</tr>
<tr>
<td>Cramer-von Mises</td>
<td>$W-Sq = 0.147909$</td>
<td>0.0245</td>
</tr>
<tr>
<td>Anderson-Darling</td>
<td>$A-Sq = 0.927144$</td>
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Quantiles (Definition 5)

<table>
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<tr>
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<tr>
<td>99%</td>
<td>1.934475</td>
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<tr>
<td>95%</td>
<td>1.068368</td>
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<td>90%</td>
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<td>75% Q3</td>
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<td>50% Median</td>
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The UNIVARIATE Procedure
Variable:  Resid  (Residual)

Quantiles (Definition 5)

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Extreme Observations

<table>
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<th>Value</th>
<th>Obs</th>
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</table>
Missing Values

-----Percent Of-----

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<th>Count</th>
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<tbody>
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</table>

model for ln(bdnf) with insignificant 2-way interactions and covariates removed

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The UNIVARIATE Procedure
Variable: Resid (Residual)

Stem Leaf # Boxplot
18 3 1 |
16 6 1 |
14 |
12 |
10 74 2 |
8 22467 5 |
6 001134 6 |
4 00444603589 11 +------+
2 26823455 8 |
0 33 2 *------*
-0 664444110 9 | + | |
-2 907 3 |
-4 865441 6 +--------
-6 86418331 8 |
-8 6 1 |
-10 8 1 |
-12 9 1 |
-14 488 3 |
model for ln(bdnf) with insignificant 2-way interactions and covariates removed

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The UNIVARIATE Procedure
Variable: Resid (Residual)

Normal Probability Plot

-2.7  -1  0  +1  +2

1.9
## Appendix B

<table>
<thead>
<tr>
<th>LAB #</th>
<th>DIET</th>
<th>Age (yrs)</th>
<th>Height (m)</th>
<th>Body weight (kg)</th>
<th>Body Mass Index</th>
<th>Plasma BDNF (pg/ml)</th>
<th>Plasma Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
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<td>58.55</td>
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<td>85.2</td>
<td>83.6</td>
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<td>14</td>
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**Notes:**
- **LAB #** represents the laboratory number.
- **DIET** indicates the diet group.
- **Age (yrs)** is the age in years.
- **Height (m)** is the height in meters.
- **Body weight (kg)** is the body weight in kilograms.
- **Body Mass Index** is calculated by dividing the body weight by the square of the height.
- **Plasma BDNF (pg/ml)** is the blood plasma brain-derived neurotrophic factor in picograms per milliliter.
- **Plasma Leptin (ng/ml)** is the blood plasma leptin in nanograms per milliliter.
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**Table 3.** Descriptive statistics for each subject at baseline, three-month and six-month time points, organized by diet (Diet 1 = Low fat diet with caloric restriction; Diet 2 = *ad libitum* low carbohydrate diet).