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# Brain derived neurotrophic factor response during endurance cycle training: Impact of carbohydrate and protein supplementation

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# Brain Derived Neurotrophic Factor Response During Endurance Cycle Training: Impact of Carbohydrate and Protein Supplementation

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## Table of Contents

## List of Tables

## List of Figures

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## Abstract

**Purpose:** Brain Derived Neurotrophic Factor (BDNF) is part of the superfamily of neurotrophins including: nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4). Recent evidence suggests that aerobic activity can increase BDNF levels both during and following exercise. Likewise, dietary habits have been shown to alter BDNF levels. However, nothing is known about the effect of BDNF levels during aerobic activity under specific dietary interventions. The purpose of this study was to examine the effects of specific macronutrient supplementation on BDNF levels during aerobic exercise. **Methods:** We had the opportunity to determine BDNF levels in blood samples obtained from two separate projects whereby subjects completed 2 hours of cycling. Specifically, 14 endurance-trained cyclists ($VO_{2\,max}$: 62 ± 7 ml/kg/min) from two previous studies: Study A focused on protein (PRO) and placebo (PLA) supplementation (6 subjects: 3 males and 3 females) and Study B focused on carbohydrate (CHO) supplementation (8 subjects: 6 males and 2 females) during constant load cycling. Both studies had nearly identical exercise protocols of constant load cycling for 120 minutes at 50% (CHO) and 55% (PLA/PRO) of peak power at $VO_{2\,max}$. In both studies blood samples were obtained prior to exercise and immediately following the 120-minute ride, allowing us to make preliminary comparisons between studies. Upon the start and every 15 minutes during steady state cycling, subjects were given a PLA, PRO, or CHO drink. Serum BDNF concentration both before and after the two-hour fixed intensity trial were analyzed with standard ELISA (enzyme-linked immunosorbent assay) methodology. The following comparisons were made: PRO vs. PLA (Study A) and CHO (Study B) vs. PLA (Study A). **Results:** BDNF levels 'almost

certainly' increased (24 ± 10%) following 120 minutes of cycling with PLA. Similarly, BDNF levels were 'very likely' elevated (32 ± 32%) following 120 minutes of cycling with PRO. All 6 subjects experienced an increase in BDNF levels under the PLA condition, while 4 out of 6 subjects increased BDNF levels with PRO. Conversely, the change in BDNF levels was 'unclear' (2 ± 17%) following 120 minutes of cycling with CHO. There was an 'unclear' difference between the PLA vs. PRO trials, while the PLA vs. CHO trials conveyed a 'likely' difference in post-exercise BDNF response. **Conclusion:** BDNF levels were elevated in PLA and PRO treatments, while the CHO treatment had unclear effects. PLA data confirms elevated BDNF response due to aerobic exercise, while PRO data suggests a similar response. BDNF response seemed to be blunted by CHO treatment. The results from this project justify follow-up investigations to determine the precise relationship between CHO supplementation and the BDNF response to aerobic exercise.

## Chapter One

## Introduction

Brain-derived neurotrophic factor (BDNF) was first discovered during the purification of pig brain matter by Barde and colleagues in 1982 (Barde et al, 1982). BDNF is one of four known neurotrophin growth factors, the others being nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) (Bath and Lee, 2006). The primary functions of BDNF are to promote neuron survival, plasticity, and maturation (Numakawa et al, 2010). BDNF functions to promote the survival of damaged neurons in those who, for example, have Parkinson's disease or who have suffered a blow to the head. Otherwise, healthy neurons are able to survive a human lifetime (Barde et al, 1993). Neuroplasticity is a prevalent function of BDNF where neurons are able to adapt to developmental and environmental influences as well as from disease and injury (World Health Organization, 1983). BDNF is also able promote the maturation of neurons and the maintenance of mature neurons (Numakawa et al, 2010). Since BDNF is active in the hippocampus, cortex, and cerebellum, it has a strong effect on higher order processes, such as learning and memory, especially later in life (Bath and Lee, 2006; Huang & Reichardt, 2001). Ostensibly, an abundance of BDNF would be physiologically advantageous.

One strategy to enhance BDNF levels is physical activity/exercise. Both acute strength and endurance training result in transient increases in BDNF during exercise (Correia et al, 2010; Gold et al, 2003, Seifert et al, 2010). Chronic resistance training yields the same results. Five weeks of strength training can robustly increase serum BDNF levels, although BDNF returned to resting levels within 30 minutes of exercise

completion in both pre and post-training intervention (Yarrow et al, 2010). Endurance exercises, such as running and cycling, have conveyed similar results. Five weeks of endurance training can elevate basal plasma BDNF levels (3-fold higher) (Zoladz, 2008). Additionally, chronic aerobic exercise can elicit high BDNF levels under resting conditions (Zoladz et al, 2008). Exercise intensity also appears to be associated with the magnitude of BDNF production. As intensity increases so does the rate at which BDNF levels increase (Griffin et al, 2011). During aerobic exercise BDNF is synthesized and secreted by sympathetic and sensory target organs (Huang & Reichardt, 2001). BDNF then travels in the blood to the brain, primarily to the hippocampus and cerebral cortex, and to working muscle. BDNF has also been proven to be produced in skeletal muscle cells, where it only acts locally and is not released into the blood stream (Wrann et al, 2010; Matthews et al, 2009). Elevated BDNF levels in the brain can lead to an increased hippocampus size, which has been linked to neurological health by a study that examined the positive correlation between brain size and endurance exercise capacity (Raichlen & Gordon, 2011).

In addition to exercise, nutrition can affect BDNF levels. Both high fat and high carbohydrate diets have been linked to decreased BDNF levels (Molteni et al, 2004; Maioli et al, 2012). On the other hand, caloric restriction and reduced meal frequency can increase BDNF and improve brain function (Mattson & Wan, 2005). Certain foods and diets can also positively impact BDNF. For example, blueberries have been shown to improve brain function, in aged rats, by increasing BDNF levels (Williams et al, 2008). Also, a Mediterranean diet, with an emphasis on nuts, has been linked to increased BDNF levels in those with prevalent depression (Sanchez-Villegas et al, 2011). Though

the impact of daily nutritional habits on BDNF levels have been examined, the potential effects of nutrition during exercise on the BDNF response has not been examined.

The purpose of this investigation was to explore the effects of macronutrient supplementation during exercise on BDNF. Specifically, we had the opportunity to assay blood samples obtained before and after 2 hours of cycling exercise performed with carbohydrate (CHO) feedings, protein (PRO) feedings, and water (PLA) (Schroer et al, 2014; 'unpublished observations'). A possible link between nutrient intake during exercise and increased BDNF levels could have implications for maximizing BDNF production in order to promote higher levels of brain function as well as neuron survival in those suffering from disease and injury.

# Chapter Two

## Methods

*Study Design*

BDNF levels were determined in blood samples collected from two previous studies that examined the effects of PRO (Schroer et al, 2014) and CHO supplementation (Luden et al, 2014) on cycling performance. Both studies implemented virtually identical exercise trials with subjects that possessed a $VO_{2max}$ ≥ 50 ml/kg/min or 4.0 L/min. Additionally, subjects in the CHO study completed ≥ 7 hours of cycle training each week for ≥ 2 months preceding participation in the study, while no specific training duration requirements were implemented in the PRO study. Performance trials commenced with 120 minutes of constant load cycling, during which subjects received either placebo or treatment beverages that contained macronutrients (CHO or PRO).

In both studies blood samples were obtained prior to exercise and immediately following the 120-minute ride, allowing us to make preliminary comparisons between studies. As seen in Table 3.1, Study A included 6 college-aged (3 males and 3 females) endurance-trained cyclists who completed 4 exercise trials (one being a familiarization trial where only water was consumed) that each consisted of 120 minutes of constant load cycling at 55% of peak power at $VO_{2max.}$ Treatments were artificially sweetened placebo (Splenda) and 45 g/L whey protein hydrolysate (purchased from American Casein Company). Each solution also contained 470 mg/L sodium chloride, 200-mg/L potassium chloride, and 4.1 g/L artificial vanilla flavoring (Bakers Imitation Vanilla). Treatment beverages were provided immediately prior to the beginning of exercise and every 15 minutes throughout the constant load cycling (Schroer et al, 2014).

As seen in Table 3.1, Study B included 8 college-aged (6 males and 2 females) endurance-trained cyclists who completed a large-scale study designed to examine the impact of PRO supplementation on physiological responses to 10 days of heavy cycling and 10 days of recovery. Similar to study A, subjects performed constant load trials consisting of 120 min at 50% of peak power. The exercise that marked the beginning of the heavy training phase was used for this project, where subjects consumed 45 g of CHO per hour (Gatorade with 6% carbohydrate) (Luden et al, 2014). This study provided no placebo trial for comparison. Therefore, the following comparisons were made: PRO vs. PLA (Study A) and CHO (Study B) vs. PLA (Study A).

*Serum BDNF*

Study A collected 3 ml blood samples from an antecubital vein before exercise and following the two-hour constant load trial. After 30 minutes of clotting, samples were centrifuged at 3,000 rpm for 10 minutes then stored at -80$^{o}$C for later analysis (Schroer et al, 2014). Study B collected 10 ml blood samples from an antecubital vein before exercise and following the two-hour constant load trial. After 30 minutes of coagulation, samples were centrifuged at 10,000 rpm and later stored at -80$^{o}$C for later analysis (Luden et al, 2014). Serum BDNF concentration both before and after the two-hour constant load trial were analyzed with standard ELISA (enzyme-linked immunosorbent assay) methodology.

*Statistical Analysis*

Data were log transformed to diminish the effects of nonuniformity. Magnitude-based inferences about the data were derived using methods described by Hopkins and colleagues (Hopkins et al., 2009). The ‘smallest worthwhile change’ in BDNF was

calculated and used as the threshold value for a substantial treatment effect (i.e. change in BDNF with CHO vs. change in BDNF with PLA) (Hopkins, 2004). The smallest worthwhile change in BDNF was defined as 0.2 x the between subject variability in the change in BDNF with exercise under placebo conditions (artificially sweetened non-caloric beverage).

Published spreadsheets (Hopkins, 2006a and b) were then used to determine the likelihood of the true treatment effect (of the population) reaching the substantial change threshold (above); these percent likelihoods were classified as: <1% almost certainly no chance, 1-5% = very unlikely, 5-25% = unlikely, 25-75% = possible, 75-95% = likely, 95-99% = very likely, and >99% = almost certain. Mechanistic inference criteria was used to classify the effects of treatment on the BDNF response. Specifically, if the percent chance of the effect reaching the substantial change threshold was <25% and the effect was clear, it was classified as 'trivial'. The effect was classified as unclear if 90% confidence intervals included values that exceeded the substantial change threshold for both a positive and negative effect; effects were classified as unclear (>5% chance of reaching the substantial threshold for both a positive and negative effect)

## Chapter Three

## Results

Subject demographics are displayed in Table 3.1. BDNF levels before and after exercise are displayed in Table 3.2 and Figure 3.1. Data reported parenthetically are the mean effect ± 90% Confidence Interval. BDNF levels were ‘almost certainly’ increased (24 ± 10%) immediately following 2 hours of cycling with PLA. Similarly, BDNF levels were ‘very likely’ higher (32 ± 32%) following 2 hours of cycling with PRO. All 6 subjects experienced an increase in BDNF levels under the PLA condition, while 4 out of 6 subjects increased BDNF levels with PRO. Conversely, the change in BDNF levels was ‘unclear’ (2 ± 17%) following 2 hours of cycling with CHO. There was an ‘unclear’ difference between the PLA vs. PRO trials, while the PLA vs. CHO trials conveyed a ‘likely’ difference in post-exercise BDNF response.

## Chapter Four

## Discussion

The primary objective of this project was to provide insight into the possibility that nutritional intake during aerobic exercise could influence the BDNF response to exercise. As anticipated, BDNF levels increased under PLA conditions. PRO intake during exercise did not appear to influence this response. Interestingly, BDNF did not increase with CHO intake in a separate group of subjects. However, the lack of a BDNF response with CHO may have been influenced by factors unrelated to the CHO supplementation. Specifically, the subject group that received the CHO treatment exhibited higher baseline BDNF values than the PLA/PRO group and a higher training status.

The PLA group experienced an 'almost certain' increase in BDNF following 2 hours of fixed intensity cycling with both PLA and PRO. This result is consistent with a growing body of literature that indicates BDNF concentrations increase as a result of acute endurance exercise (Gold et al, 2003). Studies show that acute endurance exercise can increase BDNF mRNA and its tyrosine kinase receptor, TrkB (Kim et al, 2005). Specifically, during exercise an action potential is sent down a motor nerve to release the neurotransmitter, glutamate. Once glutamate binds to post-synaptic receptors, AMPA and NMDA, the post-synaptic nerve depolarization opens up voltage-gated calcium channels. The influx of calcium stimulates exocytosis of storage vesicles containing neurotrophins (i.e. BDNF). Once BDNF is released it binds to its TrkB receptor and stimulates calcium secretion from intracellular stores causing a continuous BDNF release from a single nerve cell (Lessman et al, 2003). With enhanced

transcription due to increased mRNA of BDNF and TrkB this process is able to occur at a faster rate, therefore increasing BDNF levels. Furthermore, this process seems to be more prevalent 24 hours after exercise, meaning increased BDNF levels immediately following exercise are not due to enhanced transcription (Yarrow et al, 2010). Instead, the immediate increases in BDNF may be facilitated by platelet activation (Matthews et al, 2009). Platelets are known to store and release BDNF (Fujimora et al, 2002). In a study conducted by Matthews et al, the pattern of increased platelet count after a 2-hour fixed intensity cycle bout nearly matched that of serum BDNF. Furthermore, plasma levels of P-selectin, a marker of platelet activation, were also increased immediately following exercise (Matthews et al, 2009). Additionally, with PRO supplementation during the 2-hour fixed intensity cycle, there was a 'very likely' increase in BDNF levels. Compared to the results of the PLA group, there was an 'unclear' difference between the two treatments. To our knowledge, these are the first data gathered on the impact of PRO during exercise on BDNF levels and PRO supplementation did not seem to effect BDNF levels in a positive or negative way.

We also had the opportunity to assess the BDNF response to a virtually identical exercise session in a separate group of subjects that received CHO supplementation. Interestingly, in contrast to the clear increase in BDNF with PLA and PRO in the aforementioned subjects, there was an 'unclear' change in BDNF levels following exercise, suggesting that CHO availability may mediate the BDNF response to exercise. Although BDNF is generally thought to be produced by- and concentrated in- the brain (Barde et al, 1982), BDNF can also be produced in skeletal muscle cells. BDNF produced by skeletal muscle cells is not released into circulation and only acts locally

(Matthews et al, 2009). In this study, Matthews et al treated the extensor digitorum longus and tibialis cranialis muscles of a rat with BDNF via electroporation. Introducing BDNF vectors into these muscle cells caused increased phosphorylation of AMP-activated protein kinase (AMPK) and acetyl coenzyme A carboxylase ß (ACCß) as well as enhanced fat oxidation in vitro and ex vivo. The increased fat oxidation due to BDNF was AMPK-dependent, as the BDNF response was abrogated in cells treated with AMPK inhibitors. AMPK is activated via muscle contraction; meaning during the fixed intensity cycle AMPK was activated. Ultimately, this study identified BDNF as a contraction-inducible protein in skeletal muscle that is capable of increasing fat oxidation via activation of AMPK (Matthews et al, 2009). Furthermore, phosphorylation of AMPK has been found to decrease in rats exposed to CHO supplementation post-meal vs. rats exposed to water (placebo) post-meal (Wilson et al, 2011). This possible relationship between CHO and AMPK as well as the AMPK and BDNF relationship, may partially explain effects of CHO supplementation on BDNF levels. Another possible explanation for the effects of CHO supplementation on BDNF levels is the inverse relationship represented between BDNF and plasma glucose levels (Krabbe et al, 2007). Mean plasma glucose levels at 120 minutes in this study were 72, 76, and 87 mg/dL for PRO, PLA, and CHO, respectively. Glucose levels were much higher in the CHO group, which, according to Krabbe et al, would suggest decreased BDNF levels. Out of 8 subjects, 5 experienced decreased BDNF levels following the 120-minute trial.

While a link between CHO availability and BDNF is an intriguing prospective, it is important to note that there are a number of confounding factors that make if it difficult for us to definitely conclude that the CHO treatment directly impacted the BDNF

response. A small difference in training status was present between the two groups with an average $VO_{2max}$: 59.6 ± 4.2 mL/kg/min for the PLA/PRO group and $VO_{2max}$: 63.2 ± 8.2 mL/kg/min for the CHO group, which may have effected basal BDNF levels. The average baseline BDNF levels for the CHO group were about 42 ng/mL while baseline BDNF levels were about 22 and 20 ng/mL for PLA and PRO group, respectively. A more plausible reason may be the exercise in days leading up to fixed intensity cycle. Exercise in PRO/PLA group was minimal compared to the intensified training of the CHO group, which provides a possible explanation for decreased baseline BDNF levels in the PLA/PRO group. Chronic endurance training is thought to increase basal BDNF levels, which may offer a potential link in the between-treatment differences seen with the PLA and CHO treatments (Zoladz et al, 2008). Higher training status and exercise leading up to 120-minute fixed-intensity trial are both associated with increased baseline BDNF levels, which may explain the increased baseline BDNF levels in the CHO group (Zoladz et al, 2008). BDNF levels typically stay elevated for at least 24 hours post-exercise in chronic aerobic exercise, which would result in increased basal BDNF levels in CHO group, who continued “normal training” consisting of ≥ 7 hours of cycling a week for ≥ 2 months (including at least one ride ≥ 3 hours every 14 days) leading up to the 120-minute fixed-intensity trial (Matthews et al, 2009; Schroer et al, 2014). Compared to the PRO/PLA group, who were instructed to avoid any heavy exercise 48 hours leading up to the exercise trial, BDNF response was likely elevated on the day of the fixed-intensity trial, making it difficult to cross-reference to the PLA/PRO group. These increased baseline BDNF levels provide another possible explanation as to why BDNF response was ‘unclear’ in the CHO trials.

It is also worth noting that the BDNF response in the CHO group was associated with marked variability between subjects. Some subject's experienced considerable decreases in BDNF while others experienced increases in BDNF. This suggests that other variables (i.e. training status, stress levels, sleep, recovery, genetics, etc.) could have influenced the BDNF change from pre to post-exercise as well. A major limitation of the current study is that we utilized data from two separate studies albeit with a nearly identical exercise protocol. Unfortunately, the two subject groups appeared to differ in training status therefore adding uncertainty to the results. Regardless, the current findings extend previous literature indicating that endurance exercise can increase BDNF levels (Seifert et al, 2010). Furthermore, PRO supplementation during the acute endurance exercise produces very similar results to that of the PLA. The most provocative finding was that CHO supplementation had an unclear effect on BDNF levels. These results should be reproduced using a within-subject design before definitive conclusions can be made. Interesting aspects of this study worth revisiting include the effect CHO supplementation has on this group and how BDNF response with CHO or PRO supplementation differs across varied training status'. The previously mentioned confounding variables notwithstanding, the mechanistic relationship between CHO supplementation and BDNF levels is unclear. The interrelationship between carbohydrate availability, substrate source utilization, and BDNF levels warrant further investigation, as BDNF seems to be a candidate novel biomarker of metabolic stress. Perhaps more importantly, though well beyond the scope of this project, and developing a better understanding of the biological significance of BDNF, in both clinical and healthy populations.

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