Serum BDNF Levels in Response to Acute Exercise in Adults between 60-75 Years

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MAPSYK360, Master's Program in Psychology

Specialization: Behavioral Neuroscience

At

THE UNIVERSITY OF BERGEN

FACULTY OF PSYCHOLOGY

SPRING 2020

Word Count: 17537

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Abstract

Brain Derived Neurotrophic Factor (BDNF) has neuroplastic effects on the brain, and its emissions seem to be modulated by physical exercise both in humans and in animals. While having many interesting implications, the research surrounding this neurotrophic factor is incomplete. Especially little is known about how BDNF acts in older adult populations.

The aims of this study were to elucidate how BDNF acts in the ageing brain, and to investigate whether physical exercise could impact the emissions of this biomolecule by measuring peripheral levels of serum BDNF. Research on healthy adults in the second half of life is often underprioritized, and this study seeks to aid in the basic understanding of how BDNF may affect healthy populations in the age groups 60 and above.

26 individuals between the ages 62-75 years went through one aerobic exercise intervention, with blood samples drawn to measure serum BDNF levels right before and after the exercise session. Participants were also genetically tested for the genetic polymorphism called Val66Met which is suspected to influence BDNF emissions in both animals and humans.

Our analyses indicated that participants' baseline serum BDNF levels tended to rise with age. Results also showed that one acute aerobic exercise session generally led to increased levels of brain derived neurotrophic factor across our participants but that this response seemed to be influenced by each participant's genetic status.

Sammendrag

Brain Derived Neurotrophic Factor (BDNF) utøver en nevroplastisk effekt på hjernen, og dens utskillelse synes å være modulert av fysisk trening både hos mennesker og dyr. Selv om dette medfører flere spennende implikasjoner er forskningen rundt BDNF fremdeles ufullstendig. Man vet spesielt lite om hvordan BDNF opprører seg i eldre populasjoner.

Hensikten med denne studien var å belyse hvordan BDNF oppfører seg i den aldrende hjernen, samt å undersøke hvordan fysisk trening kan påvirke utskillelsen av dette biomolekylet ved å måle perifere nivåer av serum BDNF. Forskning på friske, eldre populasjoner blir ofte underprioritert, og denne studien søker å tilføre til den grunnleggende forståelsen for hvordan BDNF kan påvirke friske populasjoner i aldersgruppe 60 og oppover.

26 individer i alderen 62-75 år gjennomgikk en aerob treningsøkt, hvor blodprøver ble tatt for å måle serum BDNF-nivåer rett før og etter treningsøkten. Deltakere ble også genetisk testet for den genetiske polymorfismen kalt Val66Met, som antas å påvirke BDNF utskillelsen hos både dyr og mennesker.

Våre analyser viste at eldre deltakere hadde en tendens til å ha høyere serumnivåer av BDNF før treningsøkten. Resultatene viste også at en enkelt aerob treningsøkt generelt førte til økte nivåer med BDNF på tvers av deltakerne, men at denne responsen synes å være påvirket av deltakernes genetiske status.

Acknowledgements

First of all, I want to thank my main supervisors, Kristine Sirevåg and Silje Haukenes Stavestrand as well as my co-supervisor Jelena Mrdalj. You have all been amazing teachers throughout this year, and I have felt very lucky to have the three of you guiding me through the intricacies of writing a paper of this magnitude. Thank you for being so enthusiastic about walking me through both the basic and the more complicated aspects of this thesis. I have learned so much from all of you. Your passion for science and research has quite frankly been contagious, and it has motivated me to work hard so that I may one day inspire others as you have inspired me.

Second, I must thank my dear husband. Thank you for putting up with me for all these months. Though I know I must have been a frustrating room-mate, you have not shown it. You have met my frustrations and self-doubt with compassion and kindness, my sloppiness around the house with an unyielding will to pick up the slack and my irritability with humor and general silliness. I truly don't know what I ever did to deserve you.

To my close friends and family, thank you for checking up on me, cheering me on and for letting me be a weird dork when I most needed it. You have all been incredible. One last line goes out to my cat, for unceremoniously barging across my key-board or deciding to take a nap on my notes, giving me many great excuses for little breaks when I least knew I needed them.

My Contribution to the Dataset in this Thesis

As I had finished the first year of my master program, I was offered the opportunity to receive a student scholarship and work for the research project *Physical Exercise Augmented Cognitive Behaviour Therapy for Older Adults with Generalised Anxiety Disorder* (PEXACOG) for one year at the outpatient clinic Solli DPS, while being on leave from my studies. During this time, I was in charge of recruiting and coordinating testing for the control group in this clinical trial.

This entailed everything from reaching out to the group we were seeking, preliminary screening of each potential participant by phone of, inviting potential candidates to an in-depth interview as well as assisting in this interview. I also coordinated any contact between the participants in the control group and the rest of the research group. Additionally, I was in charge of the administrative work pertaining to the testing of each participant included in the control group. During my year at Solli DPS I was also allowed to complete certain physiological tests like administrating buccal samples and measuring heart rate variability in the participants in the control group.

Being a part of a large clinical study like PEXACOG was an immense teaching experience, and I was able to observe up close the workings of a large research study. It was during this year I first became familiar with Brain Derived Neurotrophic Factor (BDNF) and its possible implications for ageing populations. As my year ended, I was allowed to formulate a set of hypotheses surrounding BDNF in relation to the control sample of the PEXACOG project, for my master thesis. My main supervisors Silje Haukenes Stavestrand and Kristine Sirevåg guided me through this process, and the foundation for this thesis was born.

Table of Contents

Abbreviations

ANOVA	analysis of variance
BDNF	brain derived neurotrophic factor
BMI	body mass index
CNS	central nervous system
CSF	cerebrospinal fluid
HR	heart rate
HPA	hypothalamic pituitary adrenal
HRmax	maximum heart rate
mRNA	messenger ribonucleic acid
ml	milliliters
M.I.N.I.	Mini International Neuropsychiatric Interview
MMS-E	Mini Mental State Examination
ng	nanograms
SNP	single nucleotide polymorphism
TrkB	receptor kinase b
VO2max	maximal oxygen uptake

Introduction

This paper will investigate the relationship between one single session of physical exercise and peripheral levels of serum Brain Derived Neurotrophic Factor (BDNF) in adults aged 62-75 years, while accounting for genetic variations between individuals. The current scientific discussion surrounding this interaction is spread across a diverse array of research fields, and a unified understanding has yet to be accomplished. The following text will attempt to give the reader a comprehensive overview of the current status of research done on BDNF through several levels.

Firstly, a brief examination of BDNF on a molecular level will be presented, followed by a short overview of the effects BDNF is thought to have on the human brain. The three main methods of collecting and measuring BDNF will be described before discussing the impact physical exercise has on levels of BDNF in both humans and animals. The Neurotrophic Hypothesis for Depression will be discussed to give an insight into how BDNF is theorized to impact mental health. This will be followed by a detailed overview of research done on older populations in both humans and animals, before venturing into a discussion of the possible impact genetic variations may have on regulation of BDNF.

Neurotrophic Factors

Neurotrophic factors are described as either peptides or small proteins that are found across the entire central nervous system. They signal through tyrosine kinase receptors which are a class of cell surface receptors (Maisonpierre et al., 1991). These receptors play an essential role in regulating most cellular processes by causing either excitatory or inhibitory signaling-cascades (Maisonpierre et al., 1991). Neurotrophic factors generally interact with this intracellular signaling mechanism in order to promote neural proliferation and survival (Szuhany, Bugatti & Otto, 2014). In short, this means that Neurotrophic Factors have the

potential to exert neuroplastic effects on certain areas of the brain. BDNF are proteins belonging to a subgroup of the Neurotrophic Factors.

Neuroplasticity

Neuroplasticity describes the modifications that can occur in neural structures and circuits caused by individual experiences or environmental stimuli. These changes can often lead to developmental advantages (Herholz & Zatorre, 2012). Neuroplasticity can happen either through neurogenesis or through apoptosis. Neurogenesis is the process of creating new neurons as well as connections between these. Apoptosis is when connections between neurons that are deemed superfluous are severed, through a form of programmed cell death (Herholz & Zatorre, 2012). Children, whose brains are still under development, exhibit the greatest capacity for neuroplasticity, but it can occur throughout the entire adult life-span either spontaneously or through targeted therapeutic interventions (Marcotte et al., 2012). Stegemöller (2014) describes neuroplasticity as something that can occur on vastly different levels, ranging from one single neuron creating a new connection to reorganizing entire regions of the brain to develop one or more responses to an entirely new set of stimuli.

BDNF and Neuroplasticity

BDNF exerts crucial neuroplastic effects in both the fully formed adult brain as well as throughout each of the developmental stages of the brain during infancy and childhood (Lista & Sorrentino, 2010). These neuroplastic effects are executed by BDNF-proteins binding to tropomyosin receptor kinase B (trkB), which are a receptor subgroup of the previously mentioned tyrosine kinase receptors (Erickson, Miller & Roecklein, 2012). BDNF expression is regulated by activity-dependent transcriptional and translational mechanisms, as well as certain epigenetic processes. Several factors influencing BDNF regulation have been identified; physical exercise, neuronal activity, stress, antidepressants and specific hormones

like estrogen (Miranda, Morici, Zanoni, & Bekinschtein, 2019). BDNF has been found across several neural structures such as the hippocampus, the hypothalamus, the cerebellum and throughout the cerebral cortex, being able to produce long-term potentiation in these areas (Montag et al., 2014). Long term potentiation is a form of neuroplasticity, and it is generally considered to be a neural correlate of learning and memory. This is represented through increased synaptic activity (Baudry et al., 2015). The highest levels of BDNF expression have been located in the hippocampus and it seems like this structure is more vulnerable to changes in BDNF levels than any other area of the brain. The hippocampus is deeply involved in the formation of new memories and it acts as somewhat of a temporary storage space, where a declarative memory can be held before it is permanently stored in our long-term memory (Montag et al., 2014). BDNF levels in the hippocampus may also influence the executive functions of the brain (Sakata et al., 2013). Executive functions are the cognitive processes that enable us to organize and plan our activities, shift and direct our attention, engage in problem solving, and act according to long-term goals (Miyake et al., 2000). Overall, our executive functions allow us to have a flexible mind that can adapt to novel situations and stimuli.

The hippocampal susceptibility to BDNF-related fluctuations was demonstrated when Ma, Wang, Wu, Wei, and Lee (1997) performed an experiment that blocked the endogenous release of BDNF in the hippocampi of mice. The intervention resulted in a significant decrease in long-term potentiation. They also found that injecting a BDNF-depleted hippocampus with exogenous BDNF led to a significant increase in long-term potentiation in this neural structure. More recent findings support that BDNF plays a crucial role in long-term potentiation through what is called synaptic consolidation. This is a crucial step in long-term potentiation and is associated with the formation and maintenance of memories (Miranda et al., 2019).

Measuring BDNF

In research, collecting BDNF from subjects can generally be done using one of three methods; taking blood samples, collecting cerebrospinal fluid or harvesting brain tissue (Klein et al., 2011; Laske et al., 2007; Michalski & Fahnestock, 2003).

The most widely used method of collecting measuring BDNF in human studies is by collecting blood samples (Håkansson et al., 2017; Saucedo Marquez, Vanaudenaerde, Troosters, & Wenderoth, 2015). Even though some BDNF is produced peripherally, outside of the central nervous system, the assumption is still that the majority of BDNF in the blood stream originates from the brain (Klein et al., 2011). This is because BDNF has been found to be able to cross the blood brain barrier and enter the peripheral blood stream, which allows for measuring BDNF in either the plasma or the serum of the blood (Saucedo Marquez et al., 2015).

Plasma is the fluid in which the cellular components that make up the blood are suspended. Serum is defined as plasma where clotting components such as fibrinogen are lacking due to a clotting process already having occurred (Widmaier, Raff, & Strang, 2011). BDNF-proteins are stored in the platelets of the blood which are “set free” through coagulation processes. As the blood clots it allows for BDNF to enter the serum of the blood. BDNF concentrations in serum are about 200-times higher than the concentrations of BDNF found in the plasma of the blood (Saucedo Marquez et al., 2015). Rasmussen and colleagues (2009) and Klein and colleagues (2011) reported that the brain-blood correlation of BDNF is close to 75% when comparing hippocampal BDNF-expression to the levels found in the blood and plasma in rats and in pigs.

Some studies have also collected cerebrospinal fluid (CSF) from human subjects suffering from neurodegenerative diseases like Alzheimer’s disease or dementia (Laske et al.,

2007). Even though collecting BDNF through CSF is thought to be a reliable way to measure BDNF-emissions in humans, this method is more rarely used due to its invasive nature (Fernandes, Berk, Turck, Steiner, & Gonçalves, 2013). The fact that BDNF levels in cerebrospinal fluids have been found to be highly correlated to the levels found in the serum and plasma of the blood (Fernandes et al., 2013) likely contributes to measuring BDNF through blood samples being the preferred method.

Measuring BDNF-expression directly in cerebral tissue is done by looking at the magnitude of messenger ribonucleic acid (mRNA) molecules that carry the "blueprints" necessary to produce BDNF to the ribosomes of the cell. This is done through a process called polymerase chain reaction (PCR). PCR is therefore an indirect way of measuring protein activity, because it is not the protein itself that is being measured, but the mRNA molecules that signal a demand for more production of the given protein ("Gene expression quantitation by real-time PCR. (Report)," 2013). Even though some studies have allowed researchers to harvest human brain tissue post-mortem to analyze BDNF levels directly in the brain (Chen, Dowlatshahi, Macqueen, Wang, & Young, 2001; Karege, Vaudan, Schwald, Perroud, & La Harpe, 2005; Michalski & Fahnestock, 2003), these studies are rare. Harvesting of brain tissue is therefore generally associated with animal studies.

Some studies (Brooks et al., 2014; Kirk et al., 2011) have also used neuroimaging techniques as an additional tool to examine whether the BDNF levels in the blood is reflected in the implicated neural structures, such as the hippocampus. Seeing as the hippocampus is particularly sensitive to fluctuations in BDNF levels, several studies have looked at this structure in relation to a rise or fall in baseline BDNF-production (Ma et al., 1997; Rasmussen et al., 2009). Findings in studies looking at the connection between BDNF levels and hippocampal volume have been somewhat mixed. Some studies (Erickson et al., 2012; Kirk et

al., 2011) have found a correlation between rising BDNF levels and increased hippocampal volume and vice versa, while others have found no such significant correlation (Driscoll et al., 2012; Gruber et al., 2012).

The concrete biological processes can be more easily investigated in animals, as animal studies allows for a direct harvesting of the cerebral tissue in question, and more invasive experiments are therefore possible in animal compared to in human studies. Even though this presents a clear advantage, animal studies are not completely unproblematic. Examining behavioral and psychiatric phenomena in animals presents a different set of challenges. Per today it is impossible to ensure that the human experience and cognitive range can be accurately transferred onto an animal model. Measuring depressive and anxious traits in animals is not necessarily transferable to human subjects, as behavioral and neurological phenomena likely differ greatly between species. Based on this information, it seems like the clearest understanding of BDNF and BDNF production will come from looking at human and animal studies in tandem. Research done on animals can increase our understanding of how BDNF acts in humans, and further validate and improve the methods used when measuring BDNF in human subjects.

Physical Exercise and BDNF-Mediated Neuroplasticity

Regular or acute physical exercise has consistently been proven to have a positive impact on brain health through increased neuroplasticity and improved cognition (Ratey & Loehr, 2011). The areas of cognitive improvement that have been most reliable across studies have been improved memory functions and improved executive functions (Hötting, Schickert, Kaiser, Röder, & Schmidt-Kassow, 2016). The fact that physical exercise and increased levels of BDNF promote similar cognitive benefits has led to the assumption that the two are somehow interacting with each other (Alomari, Khabour, Alzoubi, & Alzubi, 2013). This becomes increasingly plausible when presented with the fact that BDNF is the neurotrophic

factor that is most susceptible to change through physical exercise (Kramer, Erickson & Colcombe, 2006). Both animal and human studies have shown that even one single bout of physical exercise leads to a temporary increase in BDNF emissions (Saucedo Marquez et al., 2015; Håkansson et al., 2017). These, and similar findings, have become the foundation for an idea that physical exercise could lead to BDNF-mediated neuroplasticity and possible neuroprotective effects (Ratey & Loehr, 2011).

Physical Exercise and BDNF: Animal Studies

Vaynman, Ying & Gomez-Pinilla (2004) have found that those mice who voluntarily ran in their wheels overall exhibited higher BDNF levels in their hippocampi compared to sedentary controls Vaynman and colleagues, (2004) explored the connection between physical exercise, higher BDNF levels and performance on recall and learning in the Morris Water Maze test. The Morris Water Maze is used to measure spatial and long-term memory in the hippocampus of rats or mice through a spatial learning task (Morris, Garrud, Rawlins, & Keefe, 1982; R. Morris, 2008). Rats in the test group were injected with trkB-IgG, which renders trkB-receptors unable to bind to BDNF-molecules, thus blocking the effects of increased BDNF-expression. They were then divided into two groups; sedentary and active. The active group had access to a running wheel, but were never forced to run, as the researchers wanted to mimic voluntary exercise in humans as closely as possible. Both groups had a corresponding control group of rats who received a placebo injection. Otherwise they were given the exact same treatment as either the sedentary or active test groups. The rats in the active control group performed significantly better on the Morris Water Maze compared to sedentary controls. The most interesting finding in this study, though, was the difference between the two active groups; rats who had received a trkB-IgG injection saw a complete absence of the exercise-mediated improvements the control group experienced (Vaynman et

al., 2004). This study supports the claim that the cognitive benefits from physical exercise are mediated by a release of BDNF in the brain.

Similarly, Ambrogini and colleagues, (2013) found that rats who were physically active through running, experienced higher rates of hippocampal long-term potentiation than controls did. The Dentate Gyrus of the hippocampus in these rats showed increased levels of BDNF mediated neurogenesis (Ambrogini et al., 2013). This could indicate that the improved synaptic plasticity that has been observed in the brain after one or more exercise sessions might be mediated by BDNF (Ambrogini et al., 2013).

Physical Exercise and BDNF: Studies in Humans

Studies on humans have also been able to support the claim that there is a distinct connection between physical exercise and BDNF levels. A 2010 literature-review (Knaepen, Goekint, Heyman & Meeusen, 2010) looked at studies which measured peripheral BDNF levels in either serum or plasma after an exercise intervention. They found that 69% of the studies done on healthy subjects showed an increase in peripheral BDNF levels after exercise. One important note is that these changes were temporary, and their magnitude seemed to be affected by the intensity of the exercise-protocol used in each study. Protocols of higher intensity usually yielded higher peripheral BDNF levels. This trend seemed to only hold up when comparing healthy groups to each other, as subjects with disabilities or chronic diseases seemed to need less intense exercise to achieve increased BDNF levels in the bloodstream. This review observed that one single exercise-session generally led to an increase in peripheral BDNF ranging from 11.7% to 410%. Even though these results seem promising, not all the studies included in the review were able to find significant changes in BDNF levels following physical exercise (Schiffer, Schulte, Hollmann, Bloch, & Strüder, 2009; Schulz et al., 2004). One study (White & Castellano, 2008) even found decreasing BDNF levels

following physical exercise in both the clinical and the control group. The authors of the review attributed these vastly different results to two factors; (1) a wide variety of exercise protocols have been utilized across studies and (2) the difference between the time blood was drawn from subjects after the exercise session varied greatly between studies. The peripheral increase in BDNF levels in the blood is volatile, and there seems to be only a small window of time where these levels can be measured before they return to baseline levels (Knaepen et al., 2010). This claim is supported by a study by Saucedo Marquez and colleagues (2015) conducted on young, healthy men (28 +/-5 years). They concluded that 20 minutes of aerobic physical exercise was the minimal requirement needed to produce BDNF-emissions observable in the peripheral blood-flow. In addition to this they recommended that blood-samples should be collected within 10 minutes after finishing an exercise session, this is because physical exercise generally leads to increased vascularity which in turn leads the BDNF in the blood to more rapidly passing through the blood stream (Saucedo Marquez et al., 2015). There also seems to be a connection between being physically fit and having lower resting BDNF levels (Chan, Tong, & Yip, 2008; Nofuji et al., 2008) which seems to be a consequence of the increased blood circulation in these individuals, though it is important to note that this effect seems to be strongest in individuals who are close to an athletic level of fitness (Zoladz et al., 2008).

Within this context it is important to note that aerobic exercise, as contrary to anaerobic exercise, has been found to be more effective in improving brain health through rising BDNF levels (Araya, Orellana, Godoy, Soto, & Fiedler, 2013; Griffin et al., 2011; Seifert et al., 2010). Examples of aerobic exercise are jogging, running, swimming and cycling. Aerobic exercise is the capacity to engage in an activity over time, due to the muscles ability to convert oxygen to cells through metabolism. Aerobic exercise has been found to increase BDNF levels both in the central nervous system and in the peripheral blood stream

(Griffin et al., 2011). Aerobic capacity can be operationalized by the maximal oxygen uptake (VO2max). VO2max is the maximum amount of oxygen an individual can utilize during exercise and can be measured through physical tests. Throughout this paper, unless otherwise specified, the term physical exercise will refer to aerobic exercise.

The Neurotrophic Hypothesis for Depression

The hippocampus is the neural structure that has been most closely researched in relation to BDNF. Seeing as the hippocampal structure is closely involved in regulating the negative feedback-loop of the Hypothalamic Pituitary Adrenal (HPA) axis, as well as it is tied to the amygdala and the prefrontal cortex, it is also associated with emotional and cognitive regulation (Duman & Monteggia, 2006). In fact, several studies have confirmed that stress can decrease hippocampal expression of BDNF (Nibuya, Takahashi, Russell, & Duman, 1999; Smith, Makino, Kvetnansky, & Post, 1995). Removal of the adrenal glands in rats have led to increased levels of hippocampal BDNF, as well as neuroprotective effects during stress. This is because the adrenal glands produce corticosterone, which is the stress hormone that corresponds to cortisol in humans. One study injected corticosterone directly into the rodent hippocampus and saw that this intervention led to hippocampal atrophy and decreased BDNF levels (Smith et al., 1995). Chronic stress has therefore been viewed as a possible contributing factor to low BDNF levels in both humans and animals.

An abated hippocampal volume and a dysfunctional HPA axis are factors often associated with depression. Additionally, post-mortem analyses of brain tissue in severely depressed and/or suicidal patients have shown lower BDNF-expression than those found in control samples (Chen et al., 2001; Karege et al., 2005). Putting this together with the fact that the subjects who had been on antidepressants at the time of death exhibited higher BDNF levels than those who had not, these results became the foundation for what is called the

"Neurotrophic Hypothesis of Depression" (Duman & Monteggia, 2006). Brain imaging studies (Sawyer, Corsentino, Sachs-Ericsson, & Steffens, 2012; Sheline, Gado, & Kraemer, 2003) have since supported this theory. Patients with major depression disorder have reliably been found to have smaller hippocampi compared to non-depressed subjects. Once started on antidepressants, severely depressed patients have been observed to regain their hippocampal volume to a size comparable to healthy controls. These increasing levels of BDNF-production are attributed to the treatment by anti-depressants. This effect has been found across all different types of treatment of depression, but particularly positive effects have been observed through treatments using monoamine-oxydase inhibitors or by electroconvulsive therapies (Duman & Monteggia, 2006). Evidence also points toward a decrease of serum BDNF levels in severely depressed patients, and that this effect also can be reversed by administering anti-depressants to the patient (Chen et al., 2001; Karege et al., 2005). Though this is very promising research more studies are needed to confirm whether BDNF-serum levels can successfully be used as a biomarker for depression (Duman & Monteggia, 2006). Physical exercise has been known to decrease depressive symptoms in humans, and physical exercise can in some cases be used as a stand-alone treatment for depression (Kvam, Kleppe, Nordhus, & Hovland, 2016). This theory ties neatly into the current discussion about the relationship between BDNF and physical exercise (Ten Have, de Graaf, & Monshouwer, 2011).

Though the neurotrophic hypothesis has an almost intuitive appeal, there are still some important points that need to be addressed before the validity of this hypothesis can be confirmed. Not all studies have replicated the finding that antidepressants lead to an up-regulation of BDNF-levels, and some studies done on mice have failed to establish the role BDNF is given in this theory of depression (Chourbaji et al., 2004; Macqueen et al., 2001).

BDNF and Ageing

Cognitive decline is an inevitability that comes along with growing older. As we age, neural structures will atrophy, and our cognitive functioning will decline (Alkadhi, 2018; Lipnicki et al., 2013). One of the brain functions that seems most vulnerable to the ageing process are the executive functions (Turner & Spreng, 2012). Working memory, which describes the capability of temporarily holding information to be processed at the "forefront" of consciousness falls under the executive functioning umbrella (Kramer et al., 2006). Certain memory processes are also prone to age-related decline, such as prospective memory and declarative memory. Both prospective and declarative memory are connected to the hippocampus (Kennedy et al., 2015). Additionally, the hippocampus is one of the brain structures most prone to age-related atrophy. Studies who have examined healthy older adult samples found an average decrease of 1-2% per year (Kirk et al., 2011). This hippocampal atrophy is considered to be one of the factors that cause age-related cognitive decline. This becomes increasingly interesting when presented with the fact that BDNF levels have been shown to decline with age as well (Kirk et al., 2011). The dentate gyrus of the anterior hippocampus has consistently been found to be the neural structure most vulnerable to plasticity-deficits and to lower BDNF levels caused by ageing (Miranda et al., 2019).

van Praag, Shubert, Zhao, & Gage (2005) compared the performance of older mice (19 months old) with unlimited access to a wheel for 45 days to a group of younger mice (3 months old) and to a group of sedentary elderly controls in a Morris Water Maze. The active elderly mice did not perform much differently than the younger controls, but they did significantly outperform their sedentary counterparts. The physically active elderly mice were found to have significantly higher levels of new-born neurons in the dentate gyrus compared to the age-matched control group. These results indicate that physical exercise might influence cognition through neural growth in old age, especially seeing as physically active

animals have been observed to have higher levels of BDNF mRNA in the dentate gyrus (Kramer et al., 2006).

Knowing that BDNF can induce neuroprotective and neurogenerative effects in the hippocampus, Kirk and colleagues (2011) created a study where the effects of physical exercise on the ageing human hippocampus were investigated. They recruited two groups of healthy older adults, between the ages of 55 and 80 years. One group was given an aerobic exercise program while the other was assigned a set of stretching exercises. Both groups were to complete their respective exercises three days a week for one whole year. Through neuroimaging, the individuals who had been assigned stretching exercises were observed to have suffered an average loss of 1.4% of their hippocampus. This is within the expected rate of decline for the age group in question (Kirk et al., 2011). The subjects who had been assigned aerobic exercise, however, showed an average rate of hippocampal growth of 2% throughout the year they had been exercising. This neurogenerative effect was limited to the anterior part of the hippocampus including the uncus, dentate gyrus, subiculum, pre/parasubiculum, CA3, CA2 and CA1. The study also reported BDNF-serum levels corresponding to the changes that were reported in the hippocampus. These results indicate that consistent aerobic exercise over time can increase the BDNF levels in the ageing hippocampus to the extent that some of the age-related atrophy is attenuated or even reversed (Kirk et al., 2011).

In a 2010 literature review by Knaepen and colleagues baseline samples of serum BDNF levels in healthy individuals as well as individuals with a disability or chronic condition were investigated. The authors discovered a tendency of great variation between findings, ranging from 1.5 +/- 0.5 nanograms per milliliter to concentrations of 30.5+/-6.9 nanograms per milliliter (Knaepen et al., 2010). Based on these values, it seems like there

exists great variation between BDNF levels. This could be attributed to either differences between protocols used in the different studies, or it could be attributed to BDNF levels being vulnerable to individual variations. Either way, the gap between the different results underline the possibility that several factors affect the production and expression of BDNF that we are yet to identify. It is also important to note that this review looked at BDNF levels across the life span, with a diverse array of age groups. One of the studies included (Laske et al., 2010), reported that the average baseline serum BDNF levels in their control group of 20 women (58.9 +/- 6.6 years) was 30.5 +/-6.9 ng/ml. Håkansson and colleagues (2017) conducted a study that that aimed to compare baseline serum BDNF levels of an older adult sample (N =19, age 65-85) to the BDNF levels in the same sample after an acute exercise-session. Researchers found a significant change in BDNF levels from 19.2+/-1.17 ng/ml at baseline to 22.5+/-0.99 ng/ml immediately after the exercise session. Based on results from similar studies, Håkansson and colleagues, accounted for gender differences in their data set but found none. Seeing that previous studies on healthy older adult samples display a vast variety in results, more research on baseline and post-exercise BDNF levels in healthy older adult populations is needed. Even though neither of the aforementioned studies in this section directly measure the effect BDNF may have on the ageing brain, investigating the circulating BDNF levels does give a valuable insight into how age may influence the brain's ability to produce this neurotrophic factor. Previous studies have reported a tendency of peripheral serum BDNF levels being negatively correlated with age (Bus et al., 2012; Lommatzsch et al., 2005; Minelli et al., 2011; Shimada et al., 2014; Ziegenhorn et al., 2007). Several factors may influence BDNF-production, and age does seem to be one of them. However, there is another widely researched factor that does seem to significantly impact an individual's capacity for BDNF-production, namely an individual's genetic make-up.

The Val66Met Polymorphism

The regulation of BDNF-emissions throughout the lifespan is regulated at a genetic level. The most common genetic modifier of BDNF in the human genome is the "Single Nucleotide Polymorphism" (SNP) commonly called Val66Met or rs6265 (Notaras, Hill & Buuse, 2015). Substituting either one or two valine (Val) to methionine (Met) at codon 66 in the pro domain of the cell is what causes this polymorphism. The area where the substitution of Val to Met occurs is responsible for modifying the sorting and secretion of the BDNF-protein into the synaptic cleft and regulated pathways. In both humans and animals, a substitution of one or two Val alleles to Met, has generally been believed to lead to a decrease in activity-dependent BDNF-secretion in the brain (Notaras et al., 2015).

The frequency of the Val66Met polymorphism has great geographical variation. In Europe about 30% of the population are carriers of this polymorphism. Around 27% of Caucasian-European populations carry one Met allele, while carriers of two Met alleles are much less common with only about 3% of Caucasian-European populations being carriers of a Met66Met polymorphism (Montag et al., 2014). This number rises when testing Asian populations for the same genetic variation, and an estimated 44% of Asian populations carry a variation of the Val66Met SNP. Sub-Saharan and some indigenous American populations exhibit strikingly low frequencies of this SNP, with a statistical likelihood of carrying any form of this SNP being close to zero percent (Grigorenko et al., 2012).

Individuals with one or two Met-alleles have in some studies been observed to have smaller hippocampal volumes and impaired memory performances compared to controls without this polymorphism (Caldwell Hooper, Bryan, & Hagger, 2014). Because of this, it is the hippocampal memory and learning functions that have been investigated most closely in relation to this SNP, including episodic and spatial memory (Dincheva, Glatt, & Lee, 2012).

Carriers of the Met-allele have been consistently shown to achieve lower scores on the part of the revised Wechsler Memory Scale that measures episodic memory compared to controls, though these findings have not always reached statistical significance (Dincheva et al., 2012). Some studies (Goldberg et al., 2008; Ho et al., 2006) have also observed lower rates of verbal recall in Met-carriers, indicating that homozygous Val carriers display a greater propensity for remembering verbally presented semantic information (Montag et al., 2014). These studies have primarily been conducted on younger demographics, and do not include adults in the second half of life. The impact of the Val66Met SNP on cognition is far from established, and the results have so far needed to be replicated to carry any weight in the current scientific debate.

As BDNF is an important modulator of both neuroprotection and neuroplasticity, any disruption to the emission of this protein carries important implications. Elzinga and colleagues (2011) reported that Met-carriers generally had higher BDNF-emissions measured in the serum of the blood compared to Val66Val-carriers, but that these values were highly vunlerable to adverse life-experiences. If a Met-carrying individual had experienced childhood abuse, recent stressful life events, or both, they exhibited lower serum BDNF levels than Val66Val-carrying individuals with similar life experiences. Based on this the Val66Met polymorphism has been theorized to carry a higher risk of cognitive deficits and neuropsychiatric disorders such as depression and certain anxiety disorders (Notaras et al., 2015). Seeing as many of the positive benefits reaped by physical activity may be caused by increased levels of BDNF, it seems plausible that this polymorphism may also attenuate these benefits which may in turn lead these individuals to be more vulnerable to neuropsychiatric disorders (Ieraci, Madaio, Mallei, Lee, & Popoli, 2016).

Val66Met and Physical Exercise

Even though physical exercise has been consistently shown to increase BDNF-emissions in the brain, the effect that the Val66Met polymorphism exerts on these emissions has still not been widely researched in humans. Ieraci and colleagues (2016) suggest looking at animal models instead. In their study they compared the effects of physical exercise in wild-type $BDNF^{val/val}$ mice to the effects of physical exercise in knock-in $BDNF^{met/met}$ mice. Both genotypes had one sedentary control group. The two active groups went through a 4-week period of free voluntary exercise by running in a wheel and both groups were observed to take part in similar amounts of exercise. Neurogenesis and BDNF-expression in the hippocampus were measured after the 4-week period using the western blot method. The Novelty Suppressed Feeding and Forced Swimming Test were used to assess the effects physical exercise had on anxious and depressive behavior. The Novelty Suppressed Feeding Test measures an animals' ability to eat in a novel environment. The idea is that more anxious animals will hold off on eating for longer than those who have a lower propensity for anxious traits (Ieraci et al., 2016). The Forced Swimming Test measures depressive traits, as the animals (rats or mice) are placed in an inescapable cylinder in the water. The animals' active attempts to escape the cylinder (or lack thereof) is the scale which is used to measure depressive traits. Passive animals who give up quickly are scored as higher in depressive behavior while those who attempt to escape for longer will be considered to exhibit less depressive behavior (Yankelevitch-Yahav, Franko, Huly, & Doron, 2015). Even though these tests are widely used to measure depressive and anxiety-like symptoms throughout animal studies (Blasco-Serra, González-Soler, Cervera-Ferri, Teruel-Martí, & Valverde-Navarro, 2017), it is important to note that they are indirect measures of psychological phenomena that might have their own unique human complexities that we cannot expect to replicate in animal studies.

The reasoning behind testing for anxious and depressive traits is the previously mentioned statement that physical exercise may attenuate certain depressive and anxiety-like symptoms. The results of this study indicate that the $BDNF^{val/val}$ mice reaped greater benefits from exercising than their $BDNF^{met/met}$ counterparts did. Even though physical exercise led to an increase in BDNF levels in both groups, the $BDNF^{met/met}$ mice showed lower levels of BDNF mRNA in both active and sedentary groups, specifically in the dentate gyrus of the hippocampus. The $BDNF^{met/met}$ mice also were less prone to exhibit the antidepressant and-anxiety-like effects that were demonstrated in the homozygous $BDNF^{val/val}$ mice. This could indicate that the Val66Met polymorphism can be a cause of a decreased capacity for BDNF-expression in the dentate gyrus which in turn leads to a reduction of neurogenesis and plasticity. This might further display itself behaviorally as a disability to suppress anxious and depressive behaviors (Ieraci et al., 2016).

In 2012, Hopkins, Davis, VanTieghem, Whalen and Bucci conducted a study investigating the effects of continuous exercise versus an acute exercise session in sedentary young adults, while also accounting for their BDNF-allelic status. The results of this study are in line with the study discussed above, though not identical; carriers of one or two met-alleles experience lower activity-dependent BDNF emissions, which seemed to cancel out the positive cognitive effects physical exercise has on the brain. Cognitive improvement was seen only in groups homozygous of the Val allele who continually exercised throughout four weeks. This study, though, did not find any correlation between the met-allele and impact on mood or anxiety-like behaviors. This could be due to the exercise protocol, the fact that the participants were originally a sedentary representation of the population, or that the neural networks in mice and humans differ to such an extent that what will be affected by BDNF-emissions in rodents could very well not be affected in human counterparts.

Val66Met in older adult Populations

Though results have been somewhat inconclusive, it does seem like those who have lower activity dependent BDNF-secretion due to being either heterozygous or homozygous to the Met-allele experience some form of alteration to their memory functions compared to Val homozygotes (Brooks et al., 2014; Kennedy et al., 2015). Despite this, there is still a great deal of controversy related to how this polymorphism manifests itself in old age (Canivet et al., 2015; Erickson et al., 2012). Researching the influence of carrying one or two Met-alleles in older adult populations has been a challenge, because the main brain areas affected by this SNP are the same brain areas that are most easily affected by ageing in the first place (Kennedy et al., 2015). The following section will attempt to elucidate the research that has been done on older adult populations, and what these may imply for future research.

In 2014, Brooks and colleagues, published a research article presenting findings from a healthy sample from Sweden consisting of 367 individuals between 70-75 years of age. They found that subjects carrying at least one Met-allele predicted better working memory performance as well as increased hippocampal volumes. Additionally, they found reduced brain volumes in the right occipito-temporal gyrus, the right orbitofrontal cortex, and the right thalamus. These areas are all associated with attentional and arousal-networks in the brain. Based on these findings, the authors propose that even though Val-homozygotes outperform Met-carriers during most of the life-span, this effect is flipped with old-age. Even though Met-carriers in the second half of life have a smaller brain volume overall, the areas most affected by atrophy are those connected to processing emotional stimuli, which has been shown to cause interference in memory-performance (Brooks et al., 2014). These results suggest the possibility that Met-carriers experience less “interference” than their Val-counterparts during memory processing, thereby boosting their performance. One of the earlier literary reviews on this topic (Erickson et al., 2012) that examined the relationship

between the Val66Met polymorphism, old age and cognitive performance concluded that research done on the Val66Met SNP in older adults is not done consistently enough. The definition of "older adult" ranges from around 60 years of age all the way up to 80 years. This 20-year span will inevitably include a great deal of individual and age-related variations (Erickson et al., 2012). It seems like a streamlined framework for research with a clearer separation between age groups is needed for more accurate results and satisfying conclusions.

The findings regarding the rs6265 SNP and its effects on memory are not without controversy. A 2015 study by Canivet and colleagues, investigated the effects the Val66Met polymorphism may exert on memory in the ageing brain. This study assessed the link between physical activity and episodic memory in groups categorized by BDNF polymorphism and activity level; Val active, Val inactive, Met-carrier active, and Met-carrier inactive. Episodic memory was measured through the delayed recall of the Logical Memory II subtest of the Wechsler Memory Scale (Powel, 1988). They found that higher rates of physical exercise led to better episodic memory performance only for the subjects who were Val-homozygous. This was interpreted to indicate that BDNF-emission by physical exercise is more effective in Val-homozygotes.

Kennedy and colleagues, (2015) noted that most of the studies done on Val66Met and memory had been mainly concerned with verbal memory tests. In an attempt to rectify this, they composed a study where the participants (N =116, ages 20-92) were assessed on four specific types of memory. Investigating memory functions within such a large range of ages could give an indication toward how memory functions of Val-and Met carriers respectively vary throughout the lifespan. The difference in scores between groups on the California Verbal Learning Test, which measures item memory (Donders, 2008), yielded the most notable results in this study. It seems that Val homozygotes generally utilize a semantic

clustering strategy throughout the life span. This mnemonic strategy is generally considered to be the most effective on this kind of task, yielding higher results than other strategies such as serial clustering. In the Met-carriers on the other hand, the researchers saw a sharp decrease in using semantic clustering as a memory strategy with advancing age and serial clustering became the most common strategy (Kennedy et al., 2015). This finding is an interesting one, not only because it demonstrates how a genetic polymorphism can affect the ageing brain, but also because it implies that not only do Met-carriers exhibit poorer memory function; their self-initiated selection of memory strategies may also be affected. This gives us an indication that BDNF may also influence neural structures outside of the hippocampus (Kennedy et al., 2015). The researchers also found significant differences on the prospective memory test between Val-homozygotes and Met-carriers, where Met-carriers saw a much sharper age-related decline in their scores than Val-carriers did. They also identified a small effect of BDNF-genotype in subjective memory complaints, where Met-carriers reported higher rates of subjective memory complaints through the life-span than their Val-homozygote counterparts (Kennedy et al., 2015).

These contradicting results underline the fact that the effect the Val66Met-polymorphism exerts throughout the life span, and especially in old age is still poorly understood. Further research is needed to establish the direction of the interaction between physical exercise, BDNF-genotype and age. Only once these relationships have been established does it seem prudent to look at the possible implications this may have for memory functions.

proBDNF and mBDNF

Even though BDNF is largely referred to as one single “entity” throughout the literature, it actually exists in two distinct forms with different ways of interacting with the

central nervous system (Ding, Ying, & Gómez-Pinilla, 2011). As any other protein, the synthetization of BDNF happens through the transcriptional process called proteolytic cleavage (Maisonpierre et al., 1991). The BDNF-gene produces a precursor protein, called proBDNF that is stored in either the dendrites or axons of the neuron. proBDNF then undergoes cleavage by a tissue-type plasminogen activator in either the extra-or intracellular space which produces the mature form of the BDNF protein, often referred to as mBDNF, or simply BDNF (Ding et al., 2011). The activity-dependent secretion of BDNF is a mixture of both pro-and mBDNF, and proBDNF is found in both healthy and pathologic subjects (Miranda et al., 2019). Initially, proBDNF was thought to be merely a passive precursor protein to the mature form of BDNF, but several studies have proven this to be incorrect (Lee, Kermani, Teng, & Hempstead, 2001; Luo et al., 2019). In fact, proBDNF seems to exert effects that are biologically opposite to the ones mBDNF exert (Lee et al., 2001). Binding to p^{75NTR}-receptors proBDNF induces Long Term Depression, synaptic retraction and controlled cell death (Erickson et al., 2012). This can be interpreted as proBDNF acting as a regulatory agent to the proliferative effects of mBDNF. In 2011, Ding and colleagues, conducted an experiment where rats went through a seven-day regiment of exercising in their wheel each day. Voluntary wheel running was in this study found to increase the rate of maturation of proBDNF to mBDNF, indicating that physical exercise may be a homeostatic regulator of BDNF-processing where an increased conversion rate of pro to mBDNF could lead to increased neuroplasticity in certain areas of the brain (Ding et al., 2011). Many studies investigating the effects of BDNF throughout the years have either only measured for mBDNF levels or not distinguished between the precursory BDNF protein and the mature one (Erickson et al., 2012). Therefore, the interaction between the two is still poorly understood and under-researched.

Aims and Hypotheses

Aims

Based on the theoretic background provided above, a few things become clear. First, BDNF carries interesting implications for older adult populations. A deeper understanding of this biomolecule in the aging human brain could help us understand how best to use its neuroprotective and neurogenerative effects to attenuate the age-related atrophy of certain neural structures. Second, physical exercise seems to be a cost-effective way to increase BDNF serum levels in the blood, which in turn indicates rising BDNF levels in the brain. Looking at physical exercise and its interaction with BDNF can give us insight into which forms of exercise, if any, have the best neuroprotective effects, leading to a cost-effective way to get ahead of age-related neural and cognitive decline. As older adult populations tend to be overlooked both in research as well as in the healthcare system, researching cost-effective ways to help this group can lead to great socioeconomic benefits. At the genetic level, looking at the Val66Met polymorphism can give important insights into individual differences in BDNF levels in healthy older adult populations. The effects this polymorphism has on neural networks, cognitive functioning and susceptibility to psychiatric disorders throughout the lifespan is still unclear but gaining a basic understanding of how it affects healthy populations seems important in order to progress. Lastly, BDNF-research in humans, and especially in the age groups 60 and above, is incomplete. Attaining a basic understanding of BDNF at the molecular, genetic and demographic level in older adult populations will be crucial in order to move forward within this field of research. This can lay down a foundation for further research on how BDNF can benefit this population.

With this in mind, two sets of hypotheses have been formulated. The first set will investigate serum BDNF levels in an older adult sample at baseline. The second set will

examine the effect one aerobic exercise session will have on serum BDNF levels in the same sample.

Hypotheses

Baseline serum BDNF levels

Hypothesis 1: Participant age will influence individual baseline serum BDNF levels, where higher age will be associated with lower serum BDNF levels.

Hypothesis 2: The genetic polymorphism Val66Met will influence individual baseline serum BDNF levels.

Effect of Exercise on serum BDNF levels

Hypothesis 3: One single session of Physical Exercise will result in an increase of peripheral serum BDNF levels in our subjects compared to their baseline levels.

Hypothesis 4: One single physical exercise session will result in a peripheral increase of serum proBDNF levels.

Hypothesis 5: Differences in genetic status will influence participant response to the exercise intervention, where Met-carrying participants should have a lower response to the acute exercise session than Val-homozygous participants

Method

Background

This thesis is a part of the randomized controlled trial *Physical Exercise Augmented Cognitive Behavior Therapy for Older Adults with Generalised Anxiety Disorder* (PEXACOG). The aim of PEXACOG is to improve existing treatment for older adults who suffer from generalised anxiety disorder, as the established treatment protocols have proven

less effective in older age groups compared to working-age groups undergoing the same treatment.

The approach of PEXACOG is to augment cognitive behavior therapy with physical exercise to enhance the effect of cognitive behavior therapy. A variety of mechanisms involved in this expected improvement is investigated. 70 older adults aged 65-70 years are randomized into one of two treatment conditions. One group receives cognitive behavior therapy in combination with physical exercise, while the other group receives cognitive behavior therapy in combination with weekly telephone contact. Participants are measured on biological, physiological, psychological and neuropsychological measures, as well as an fMRI investigation. Measurement points are at baseline, after five weeks of intervention, post-treatment and after 6- and 12 months. Data from the patient group will be compared with data from a control group consisting of 70 healthy participants matched with the patient group on age, gender and level of education. For full study protocol, see Stavestrand and colleagues (2019).

The participants in this thesis are part of the control group of the PEXACOG project. See appendix A figure A1 for a visualization of the overlying PEXACOG research project.

Participants

Healthy adults between the ages 60-75 years old were included in this study. A total of 10 exclusion criteria were set:

(1) Any form of substance abuse three months prior to testing (including cannabis). (2) Using benzodiazepines or antipsychotic medication. (3) Having experienced any psychiatric or behavioral disorders previously or presently. (4) Physical ailments to the degree of not being able to participate in physical exercise. (5) Displaying any symptoms of severe depression as determined by the Mini International Neuropsychiatric Interview (M.I.N.I;

Sheenan et al., 1998). (6) A prior history of manic or psychotic episodes. (7) Currently engaging in any form of psychotherapy. (8) A history of transient ischemic attacks or stroke. (9) A score of 25 or lower on the Mini Mental State Evaluation (MMS-E; Folstein, Folstein &McHugh, 1975). (10) Engaging in moderate physical exercise any more than 60 minutes per week divided on two exercise sessions.

Measures

Screening Procedure

The process for participation in this study consisted of one phone interview and two separate days of attendance at the outpatient clinic at Solli DPS. The phone interview acted as a preliminary screening of a participants' ability to participate in the study and comprised of a check-list of nine items with yes/no answers (see appendix A table A1). If a participant answered "no" on all the questions they were invited for a more extensive interview at Solli DPS with one of the project coordinators. This appointment acted as a second, more in-depth screening designed to ascertain that the potential participant did not fulfill any of the exclusion criteria. The duration of this interview was estimated to last about 60 minutes per subject. If a subject passed this interview their primary physician would be contacted to give verbal confirmation of the medical soundness of including the individual in the study. If no exclusion criteria were met, and their doctor confirmed the safety of participation, the participant was invited back to Solli DPS for testing. See figure 1 for an overview of the screening and test procedure.

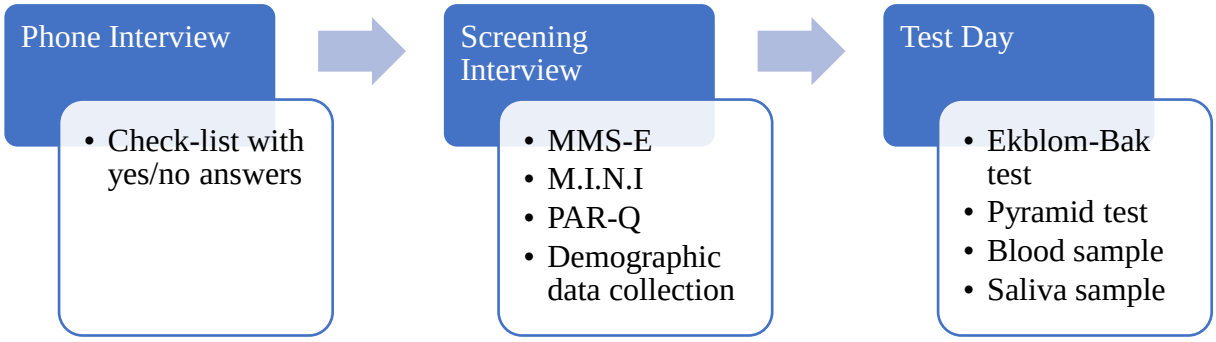

Figure 1. The inclusion process for participants in the control group for PEXACOG

Clinical Interviews

As the focus of this study was to investigate subjects with a healthy, ageing brain, it was important to ensure the absence of current mental illness and cognitive deficits. The Mini International Neuropsychiatric Interview (M.I.N.I; Sheehan, Janavs, et al., 1998) and the Mini Mental State Examination (MMS-E; Folstein et al., 1975) were both administered by a licensed psychologist. The M.I.N.I is a structured psychiatric interview for evaluation of Axis 1 psychiatric disorders in DSM-IV and ICD-10. Any potential participant with a history of mental illness as detected by the M.I.N.I. were excluded from participation. The MMS-E is a screening tool for cognitive function, which was used to check for symptoms of dementia and other signs of cognitive decline. There was also a short interview used to collect demographic data about each subject (see Appendix A, table A2).

Questionnaires

A Norwegian translation of the Physical Activity Readiness Questionnaire (Cardinal, Esters & Cardinal, 1996) was utilized during the second screening interview (see appendix A table A3) to assure that it was medically safe to include each participant and for them to complete the assigned exercise session. The subjects were then asked to describe their physical activity throughout a typical week, giving the project coordinator an opportunity to evaluate whether the subject met the inclusion criteria regarding levels of physical activity.

Seeing as previous literature has suggested that being very physically active or athletic may alter BDNF-emissions, our subjects were not included if their physical activity exceeded 60 minutes of moderate physical activity or exercise per week.

Physiological Measures

All physiological tests were done on one single test day at Solli DPS. The Ekblom-Bak Submaximal Cycle Stationary Bike Test (Ekblom-Bak, Björkman, Hellenius, & Ekblom, 2014) using a Monark stationary bike, and the Five-Minute Pyramid test (Andersson, Lundahl, Wecke, Lindblom, & Nilsson, 2011) were used to estimate maximum oxygen uptake (VO_2 max) in each subject. The Five-Minute Pyramid Test was included as a part of a larger test battery related to the main study that this thesis is a part of, and not directly relevant to this paper. The Ekblom-Bak was both utilized as a measure of VO_2 max, and also as a part of the physical exercise session. VO_2 max is considered to be a reliable way to estimate physical fitness. These physical tests were done under the guidance and supervision of either a licensed physiotherapist or an occupational therapist.

Intervention

The Ekblom-Bak test was followed by 20 minutes of cycling, which together entailed the exercise intervention that was expected to increase serum BDNF emissions in our participants. The Maximum Heart Rate (HRmax) was calculated with a formula (Tanaka, Monahan, & Seals, 2001) to ensure that our participants upheld the correct intensity levels throughout the exercise session. Heart rate (HR) was measured in all the participants by a pulse belt and an accompanying pulse watch (Polar FT4 pulse watch). The test leader registered the heart rate each minute throughout the 20-minute cycling session following the Ekblom-Bak test to ensure an intensity level above 65% of the participant's maximal heart rate. Blood was drawn from subjects at two points in time; the first before any form of exercise had commenced, and the second, ideally, right after the participant had finished the

Ekblom-Bak test and 20 minutes of consecutive cycling. See appendix B table B1 for an overview of the time-lapse between the end of the exercise session and the time of blood-sample collection. The blood samples were used to assess serum BDNF levels by utilizing the human proBDNF and mBDNF DuoSets. Genetic material was collected by one single saliva sample which was analyzed using the TaqMan method. To ensure that our results were not influenced by diurnal variations, precautions were taken so that all blood samples were drawn at similar times in the morning hours.

Figure 2. The progression of the test day for participants of the PEXACOG control group at Solli DPS.

Statistical Analyses

Statistical analyses were run using the IBM SPSS program. First, we ran a Pearson product-moment correlation coefficient to see whether the VO2max scores in the two physical tests (the Five-Minute Pyramid Test and the Ekblom-Bak test) were correlated with each other, ensuring the correlation between the two tests. We then controlled for physical fitness and baseline BDNF levels in our participants by a Pearson product-moment correlation coefficient. This was done by investigating the relationship between the VO2max scores on both the Five-Minute Pyramid Test and the Ekblom-Bak test respectively in relation to baseline serum BDNF levels.

A Pearson product-moment correlation coefficient was then used to investigate the relationship between age and baseline BDNF levels. An independent *t*-test was utilized to look for differences in baseline BDNF levels in Val66Val and Val66Met-carriers, as well as a two-way analysis of variance (two-way ANOVA) to examine the impact of age and genetic

status on baseline BDNF-values across our participants when divided into two age groups (60-67 years and 68-75 years).

To investigate the impact the exercise session may have had on BDNF levels, a repeated measures ANOVA was used. This was to look at the effect of the exercise intervention on BDNF values overall, as well as the impact genetic status may have on these values. Additionally, a paired samples *t*-test was used to examine proBDNF levels pre and post exercise.

After the initial analyses were completed, three participants (participants 4, 23 and 26) were found to have waited more than the recommended (Kirk and colleagues, 2011) ten minutes after the exercise session had ended before their blood was drawn (see appendix B table B1). It was decided to run a post hoc analysis to investigate how much these delayed tests impacted the results from the initial analyses. The three subjects were removed from the dataset, and a repeated measures analysis of variance was run. The same variables were investigated as in the previous repeated measures ANOVA; the effect of one exercise session on serum BDNF levels, as well as the impact genetic status may have had on the effect of exercise on serum BDNF levels.

Data Management and Security

Each participant, at inclusion, was given an anonymous, unique ID-number. This number was then consistently used as to identify the participant throughout their participation. Excluded subjects were also given an ID-number. The name of the participant and the ID-number were never stored in the same place, except for in one single document. This document was stored on a separate domain on a secure research server and was only available to project coordinators. All other data regarding each participant was stored on the regional secure research servers of Solli DPS outpatient clinic.

Biological material was stored in a research-specific biobank that followed the guidelines of the Regional Committee for Medical and Health Research Ethics procedures. All data will be anonymized after a time limit of five years has expired.

Ethical Considerations

This study is approved and registered by the Norwegian Regional Committee for medical and health research ethics (REK) as number 2015/2189 as well as being registered at clinicaltrials.gov. All participants signed a form of consent which contained information regarding participant privacy and guidelines for data storage. In order to ensure a safe work-out session for each participant, their doctors were contacted to give a medically informed consent to their participation. No patient information was exchanged between any doctor and the research group, simply an affirmative or a negative answer was required to determine a participant's ability to participate.

Throughout the screening process for this study, several potential disorders could be uncovered, such as symptoms of severe depression or cognitive impairment. At such an occurrence, one of the project coordinators would exclude the subject from participation on the study, and then refer them to further assessment or suitable treatment.

Results

Descriptive Analyses

Preliminary analyses were run to investigate the demographic composition of our data set consisting of 26 individuals. 19 women (73.1%) and 7 (26.9%) men were included. The youngest participant was 62 years old while the oldest was 75 years. Overall, the majority of our sample (61.5%) were in the older age group (68-75 years), with 16 individuals, while the younger age group (60-67 years) comprised of 10 individuals (38.5%). 18 individuals (69.2%) were Val66Val carriers and 8 individuals (30.8%) were Val66Met carriers. We also found that

all baseline blood samples in all subjects were drawn between 09:13 and 10:02 while all post-exercise samples were drawn between 10:07 and 10:57. We had no Met-homozygous individuals in our sample. Table 1 gives an overview of these variables and the distribution across our sample.

Table 1

Demographic composition of our data set across gender, age group and genetic status

		N	%
Gender			
	Female	19	73.1%
	Male	7	26.9%
Age Groups			
	60-67	10	38.5%
	68-75	16	61.5%
Genetic Status			
	Val66Val	18	69.2%
	Val66Met	8	30.8%

As this sample was a control group for a larger study, measures were taken to ensure the absence of psychiatric illness and physical fitness in our participants. Table 2 provides an overview of several variables across three levels; inclusion, testing and intervention.

Our participants were screened with the MMS-E (Folstein et al., 1975) to strive to ensure absence of age-related cognitive decline (see table 2 for mean scores on the MMS-E). The included participants had no previous history of mental illness as determined by the M.I.N.I.

During the test day, several factors were measured. Systolic and diastolic blood pressure was measured to ensure that it was safe for each participant to go through with the physical exercise. Body Mass Index (BMI) was also noted based on each participants height and weight, as well as average performance on the two submaximal tests; the Five-Minute Pyramid Test and the Ekblom-Bak test.

During the exercise intervention, ensuring that our subjects were exercising with an intensity of a minimum of 65% of maximum heart rate was important. Average heart rate

values are included in table 2. 23 of our participants held an intensity of 65% of their maximum heart rate or higher. Three individuals did not reach this intensity, and their individual heart rate values are illustrated in appendix B table B2. These participants were still included in our data analyses, as aerobic exercise still is likely to elicit a serum BDNF-response, despite not reaching the intended intensity (Kramer et al., 2006).

Table 2

Descriptive Statistics for continuous variables

	N	Min.	Max.	*M*	*SD*
Age	26	62.00	75.00	69.00	4.01
Systolic Blood Pressure	24	111.00	177.00	137.75	16.89
Diastolic Blood Pressure	23	64.00	87.00	81.70	8.53
Body Mass Index	26	19.50	32.70	24.38	2.73
MMS-E	26	28	30	29.54	0.58
Relative VO2max - Pyramid test	24	17.80	34.90	28.10	4.42
Relative VO2max - Ekblom-Bak	26	19.80	43.00	30.50	5.60
Average max HR	26	155	164	160	3.04
Average 65% of max HR	26	101	107	104	1.97
Average HR through 20 minutes of Cycling	26	84	143	115.28	11.99

Table 3 provides an overview of the overall serum BDNF levels at baseline and post-exercise in addition to serum proBDNF levels at the same points in time. As becomes evident from table 3, our proBDNF values were difficult to detect in the laboratory and only eight participants had detectable proBDNF levels both pre and post exercise.

Table 3

BDNF- and proBDNF levels across our sample at baseline and post exercise

	N	Min.	Max.	*M*	*SD*
BDNF ng/ml Baseline	26	8.90	23.50	15.75	4.01
BDNF ng/ml Post Exercise	26	9.70	27.70	16.33	4.71
proBDNF ng/ml Baseline	9	.02	3.10	1.21	1.08
proBDNF ng/ml Post Exercise	12	.01	10.90	2.13	2.97

Baseline Serum BDNF levels

Figure 3 illustrates the individual differences between baseline BDNF levels in our participants. These individual differences at baseline will be further investigated below in relation to factors such as physical fitness, age and genetic status.

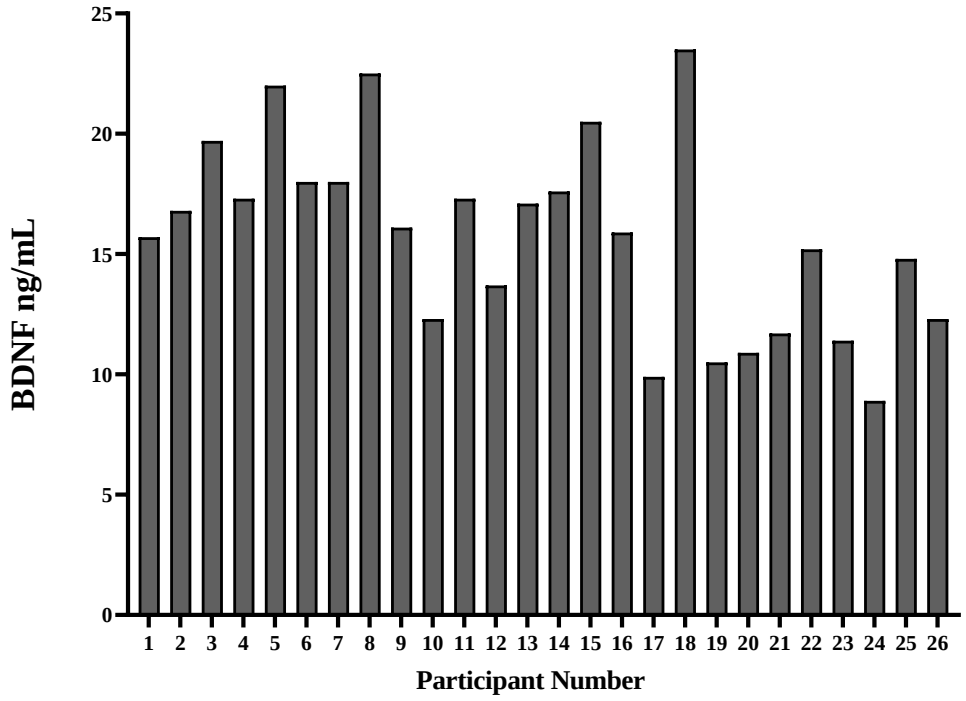

Figure 3. Serum levels of Brain Derived Neurotrophic Factor (BDNF) measured in nanograms per milliliter (ng/ml), in each individual at baseline.

Physical Fitness and Baseline Serum BDNF levels

An independent analysis of the correlation between the relative VO2max scores on the Ekblom-Bak test and the Five-Minute Pyramid Test was run as a Pearson product-moment correlation coefficient. This was done to ensure that both tests can be said to reliably correlate with one another. We found a strong, positive correlation between the two tests, $r = 0.55$, $n = 24$, $p < 0.005$, with high scores on the Five-Minute Pyramid Test being associated with high scores on the Ekblom-Bak test, indicating that the two tests are highly correlated.

Due to the association between physical fitness level and baseline BDNF levels in existing literature (Chan et al., 2008; Nofuji et al., 2008), we investigated this association in the current sample. This was done by using a Pearson product-moment correlation coefficient for physical fitness by analyzing individual relative VO2max score on the Five-Minute Pyramid Test and the Ekblom-Bak test in relation to baseline BDNF levels. The Five-Minute Pyramid Test and the Ekblom-Bak test were analyzed separately in regard to their correlation with baseline levels of BDNF.

Preliminary analyses showed that there were no violation of the assumptions of normality, linearity and homoscedasticity. We found no correlation between either the Five-Minute Pyramid Test and baseline BDNF levels ($r = .177$, $N = 24$, $p = 0.407$) nor the Ekblom-Bak test and baseline levels of BDNF ($r = -.176$, $N = 26$, $p = 0.389$). As such, we did not find that level of physical fitness was associated with baseline levels of serum BDNF in our sample.

Age and Baseline Serum BDNF Levels

We investigated the relationship between age as a continuous variable and baseline levels of BDNF measured in the serum of the peripheral blood stream by using a Pearson product-moment correlation coefficient. Preliminary analyses showed that there were no

violation of the assumptions of normality, linearity and homoscedasticity. There was a medium, positive correlation between the two variables, $r = .414$, $N = 26$, $p < 0.05$, where higher age is associated with higher baseline BDNF levels in the serum of the peripheral blood flow. See figure 4 for relationship between BDNF levels and age.

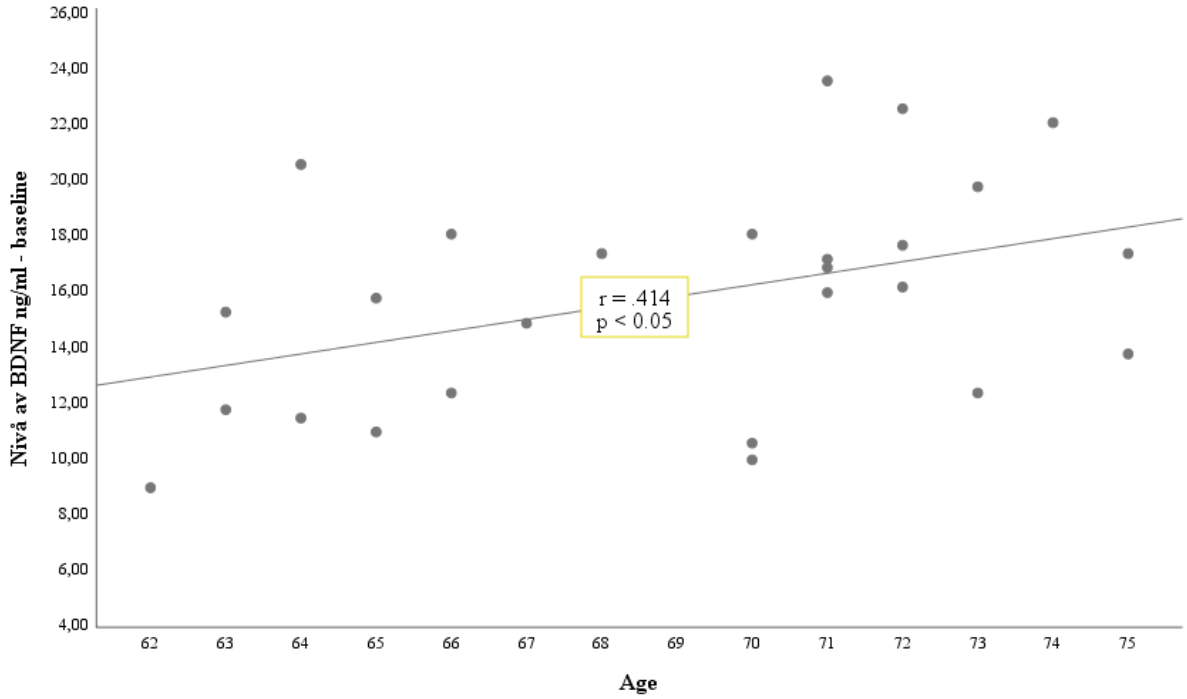

Figure 4. The individual variance in serum Brain Derived Neurotrophic Factor measured in nanograms per milliliter at baseline between the ages 62 to 75 years.

Age Group and Genetic Status

Figure 5 shows the baseline levels of serum BDNF across participants divided by their genetic status. We ran an independent samples *t*-test to check for differences in baseline BDNF levels between subjects with different genetic status (Val66Val and Val66Met respectively). No significant difference between Val66Val-carriers ($M = 15.77$, $SD = 3.54$) and Val66Met-carriers ($M = 15.73$, $SD = 5.18$; $t\ (26) = 0.021$, $p = 0.98$, two-tailed) was

detected. Cohen`s *d* was 0.01 which indicates a minimal effect size. This indicates that baseline serum BDNF levels are not mediated by genetic status.

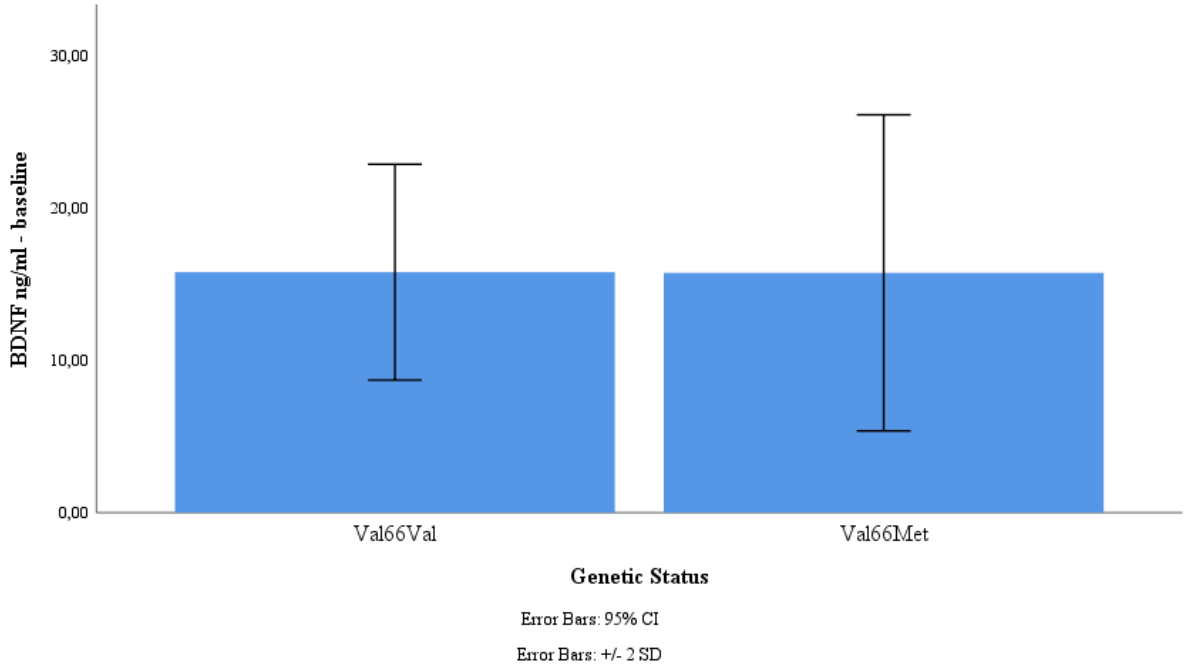

Figure 5. Mean variations in baseline levels of Brain Derived Neurotrophic Factor (BDNF) measured in nanograms per milliliter (ng/ml) between carriers of Val66Val and Val66Met respectively.

We then split the data set into two age groups and ran a two-way ANOVA to investigate the impact age and genetic status may have on baseline serum BDNF levels. No significant results were found for the main effect of age ($F = 3.76$, $p = 0.07$) nor for genetic status ($F = 0.83$, $p = 0.83$) on baseline BDNF levels. There was also no interaction effect between age and genetic status ($F = 0.27$, $p = 0.61$).

Despite the two-way ANOVA not yielding any significant results of the impact of age and genetic status on baseline serum BDNF levels, effect size analyses showed that there may be a relationship between rising age and higher serum BDNF levels. See table 4 for a comprehensive overview of the genetic distribution of participants across the two age groups with the associated effect sizes.

Table 4

An overview of genetic distribution across the two age groups, and their associated Cohen`s d

Age Group	Genetic Status	*M*	*SD*	Cohen`s *d*	*N*
60-67					
	Val66Val	14.15	3.15		6
	Val66Met	13.63	4.59		4
	Total	13.94	3.56	0.13[a]	10
68-75					
	Val66Val	16.58	3.56		12
	Val66Met	17.83	5.46		4
	Total	16.89	3.95	0.27[b]	16
Total				0.78[c]	
	Val66Val	15.77	3.54	0.72[d]	18
	Val66Met	15.73	5.18	0.83[e]	6
	Total	15.75	4.01	0.01[f]	26

[a] Cohen`s *d* between the two polymorphisms in the age group 60-67 years, [b] Cohen`s *d* between the two polymorphisms in the age group 68-75, [c] Cohen`s *d* between the two age groups, [d] Cohen`s *d* in Val66Val-carriers between the two age groups, [e] Cohen`s *d* in Val66Met-carriers between the two age groups, [f] Cohen`s *d* between Val66Val-and Val66Met-carriers.

The effect size between the two age groups showed a Cohen`s *d* of 0.78, which indicates a medium to strong effect size. Both genetic polymorphisms also exhibit medium or large effect sizes between the two age groups. Comparing our “younger” and “older” Val-homozygous subjects yielded a Cohen`s *d* of 0.72 (medium effect size), while Met-carrying participants across the same groups showed a Cohen`s *d* of 0.83 (high effect size). This indicates that age may still modulate baseline serum BDNF levels. See figure 6 for an

illustrated overview of average serum BDNF for the two polymorphisms across the two age groups.

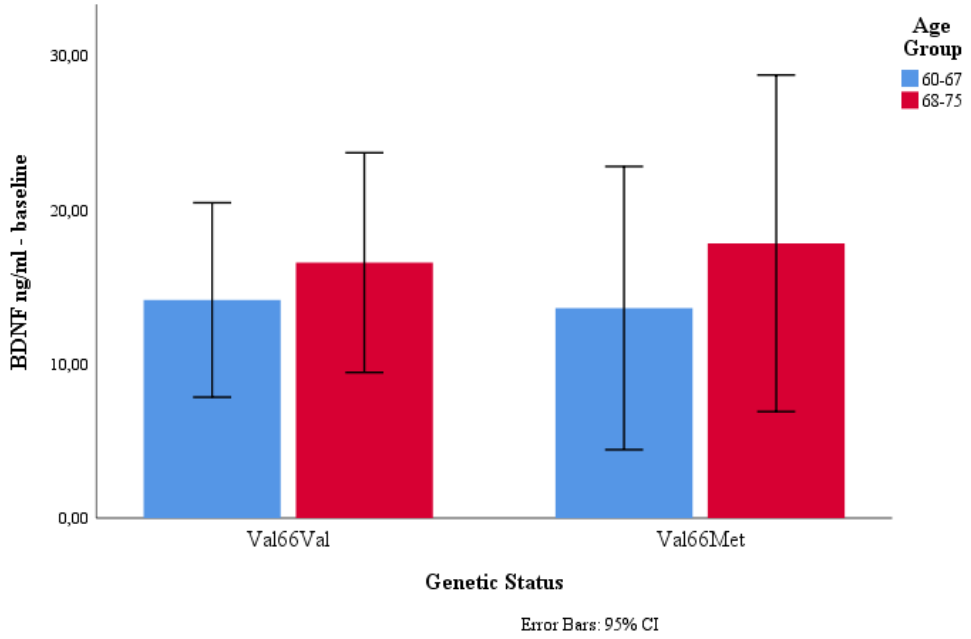

Figure 6. Average serum Brain Derived Neurotrophic Factor (BDNF) levels at baseline, measured in nanograms per milliliter (ng/ml), in Val66Val carriers and Val66Met carriers accounting for age group and genetic status (val66Val and Val66Met).

Based on our analyses there is no significant relationship between baseline serum BDNF levels and genetic status, nor between baseline serum BDNF levels and age group.

Effect of Exercise on BDNF

Individual Variations in serum BDNF levels Post Exercise While Accounting for Genetic Differences

A repeated measures analysis of variance was run, investigating the effect one single aerobic exercise session had on serum BDNF levels overall, as well as investigating the relationship between the genetic polymorphism and BDNF levels following the exercise intervention.

We did find a significant main effect of aerobic exercise on serum BDNF levels in our subjects ($F = 4.89$, $p = 0.04$), which showed a general rise in serum BDNF levels following one acute aerobic exercise session (see Figure 7). Comparison between baseline serum BDNF levels ($M = 15.75$, $SD = 4.01$) and post exercise serum BDNF levels ($M = 16.33$, $SD = 4.71$) yielded a Cohen`s d of 0.13, which indicates a minimal effect size of exercise on general serum BDNF levels. All in all, 14 participants (53.85%) experienced an increase of BDNF, while 10 participants (38.46%) experienced decreased BDNF levels in response to the exercise intervention. Two participants (7.69%) experienced no change. The average rise in serum BDNF levels across our participants is a 3.7% increase.

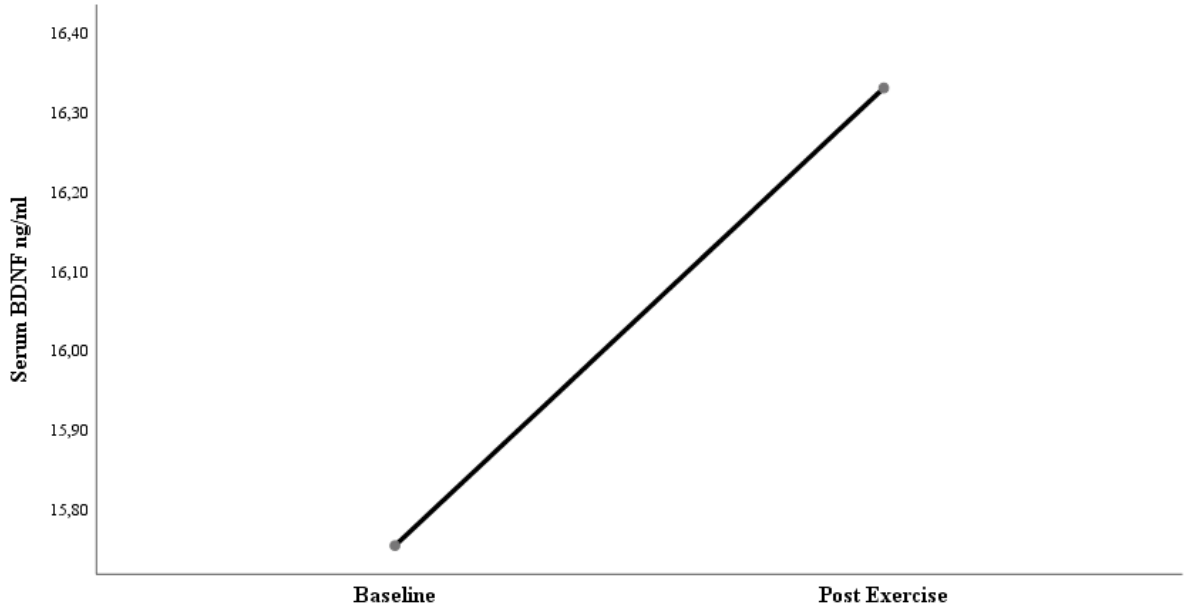

Figure 7. Average serum Brain Derived Neurotrophic Factor (BDNF) at baseline and after an exercise intervention measured in nanograms per milliliter (ng/ml) in all participants.

We found a significant interaction effect between exercise and genetic status ($F = 4.947$, $p = 0.036$), which shows us that a participant's response to the aerobic exercise

intervention is mediated by their genetic status. See figure 8 for the mean response in both groups, and figure 9 for individual responses across the two groups.

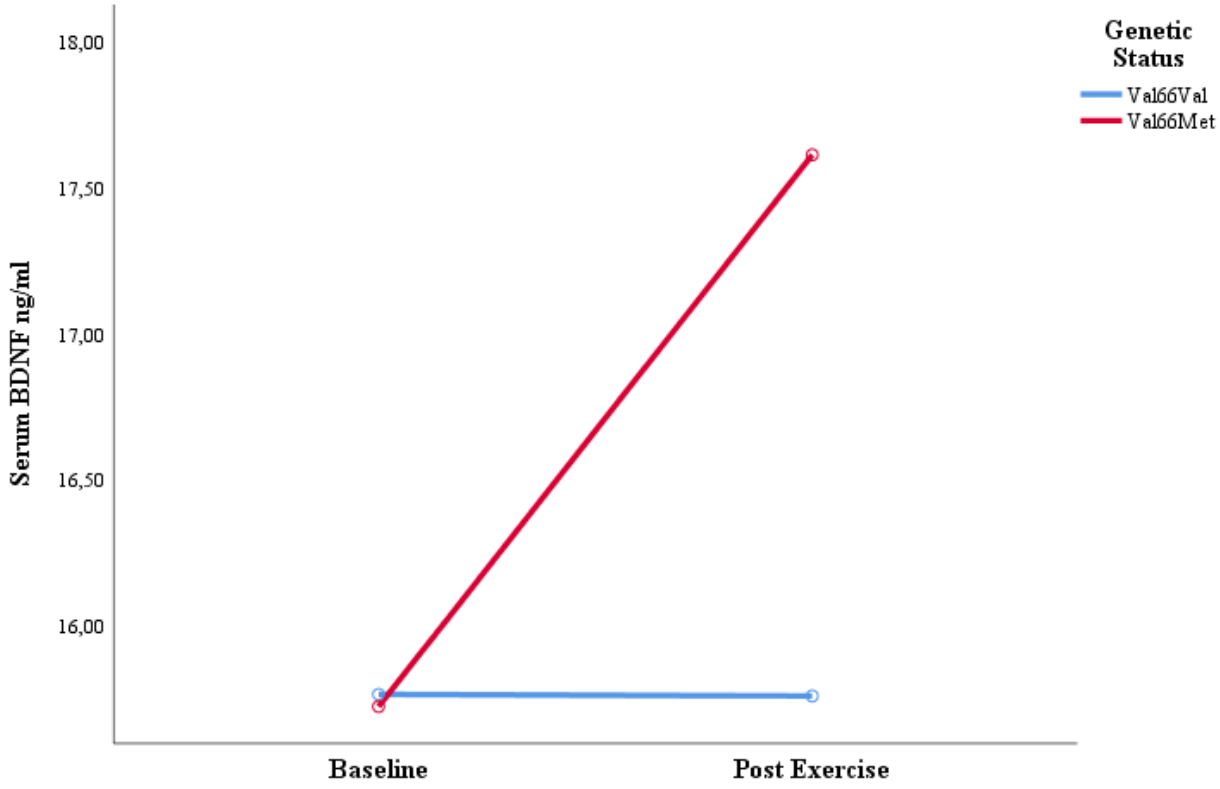

Figure 8. Average serum Brain Derived Neurotrophic Factor (BDNF) at baseline and after an exercise intervention in Val66Val and Val66Met carriers respectively, measured in nanograms per milliliter (ng/ml).

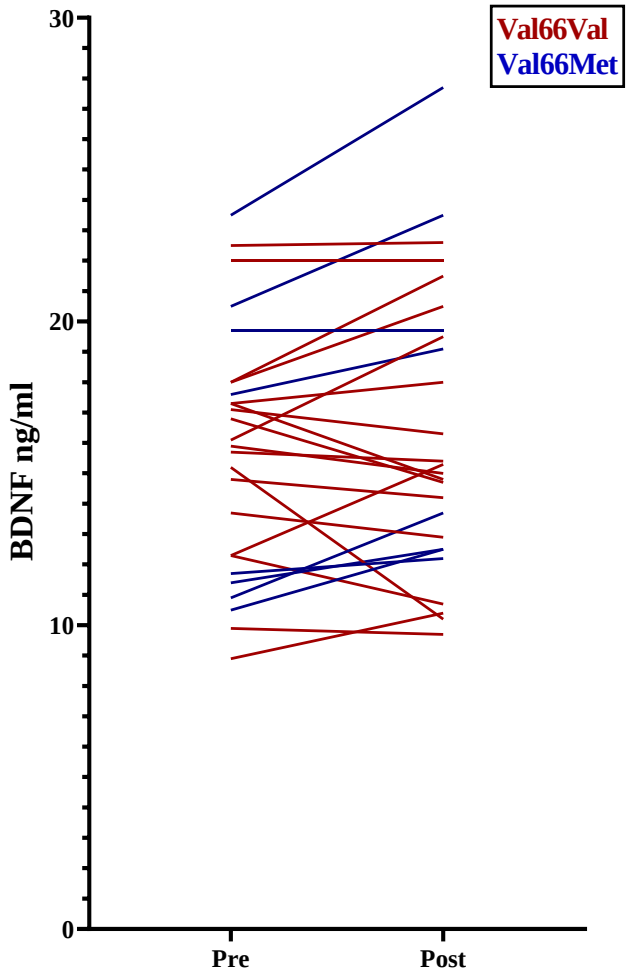

Figure 9. Individual serum Brain Derived Neurotrophic Factor (BDNF) serum levels pre and post exercise in Val66Val (red) and Val66Met (blue) carriers measured in nanograms per milliliter.

At baseline Val66Val-carriers (*M* = 15.77, *SD* = 3.54) and Val66Met-carriers (*M* =15.73, *SD* = 5.18) exhibited very similar mean levels of serum BDNF, with a Cohen`s *d* of 0.01 which indicates a minimal effect size, and thereby a very small difference between these two groups at this point. Post exercise there was an observable difference between the two genetic polymorphisms. Val66Val-carriers (*M* = 15.76, *SD* = 4.17) on average seemed to emit less serum BDNF in response to physical exercise compared to the Val66Met-carrying participants (*M* = 17.61, *SD* = 5.85). Cohen`s *d* between these two groups post exercise was 0.38 which indicates a small effect size. Effect size analysis within the two groups at the two

different points in time, yielded a result of 0.00 for Val66Val-carriers which indicates that there was no difference within this group before and after the exercise intervention. The analysis for Val66Met-carriers on the other hand yielded a Cohen`s *d* of 0.34 which indicates a small effect size for increase in BDNF serum levels post exercise intervention.

Our Val-homozygous participants overall exhibited a 0.6% decrease in serum BDNF levels post exercise. One individual (5.56%) showed no detectable response to the exercise intervention, seven participants (38.89%) in this group showed an increase in serum BDNF levels while ten participants (55.56%) showed decreased BDNF levels post exercise. Seven of eight (87.5%) Met-carriers exhibited increased serum BDNF levels post exercise.

Post Hoc Analysis: Individual Variations in serum BDNF levels Post Exercise While Accounting for Genetic Differences with Three Excluded cases

Due to discovering long time lags between physical exercise and the second blood sample, three participants (4, 23, and 26, see appendix B table B1) were excluded from further data-analysis. Two of the excluded participants were Val66Val-carriers, and they exhibited among the sharpest declines of serum BDNF-levels across all participants with a respective decrease of 1.6 ng/ml (sample taken 41 minutes post-exercise) and 2.5 ng/ml (sample taken 27 minutes post-exercise) of serum BDNF-levels. The last excluded participant was a Val66Met-carrier, and waited 21 minutes after the exercise session, but still saw a positive response in serum BDNF-levels post-exercise with a 1.1 ng/ml increase.

A new repeated measures analysis of variance was run post hoc without the excluded participants. There were also four participants with missing values in the reporting, and three with errors in reporting (see appendix B table B1). These were still included in the following analysis. Another repeated measures analysis of variance was run, investigating the impact these participant scores had on the dataset.

In this post-hoc analysis, we found a significant main effect of aerobic exercise on serum BDNF levels ($F = 6.04$, $p = 0.02$), which indicates a general rise in serum BDNF levels across all ($N = 23$) participants following one aerobic exercise session (see figure 10). Comparing baseline serum BDNF levels ($M = 16.03$, $SD = 4.08$) and post exercise serum BDNF levels ($M = 16.81$, $SD = 4.77$) gave a Cohen`s d of 0.20 indicating a small effect size. 13 participants (56.52%) experienced an increase, while 8 (34.78%) experienced decreased serum BDNF levels post exercise. Two participants (8.70%) experienced no change. Overall our participants experienced a 4.87% increase in serum BDNF levels post exercise.

Figure 10. Average serum Brain Derived Neurotrophic Factor (BDNF) at baseline and after an exercise intervention measured in nanograms per milliliter (ng/ml) in all participants from the post-hoc analysis, excluding three participants from the dataset.

We did not find a significant interaction effect between the exercise intervention and genetic status ($F = 3.65$, $p = 0.07$). Based on this post-hoc analysis there is no significant

relationship between serum BDNF-response to an exercise intervention and genetic status. See figure 11 for the mean response to exercise in both genetic groups.

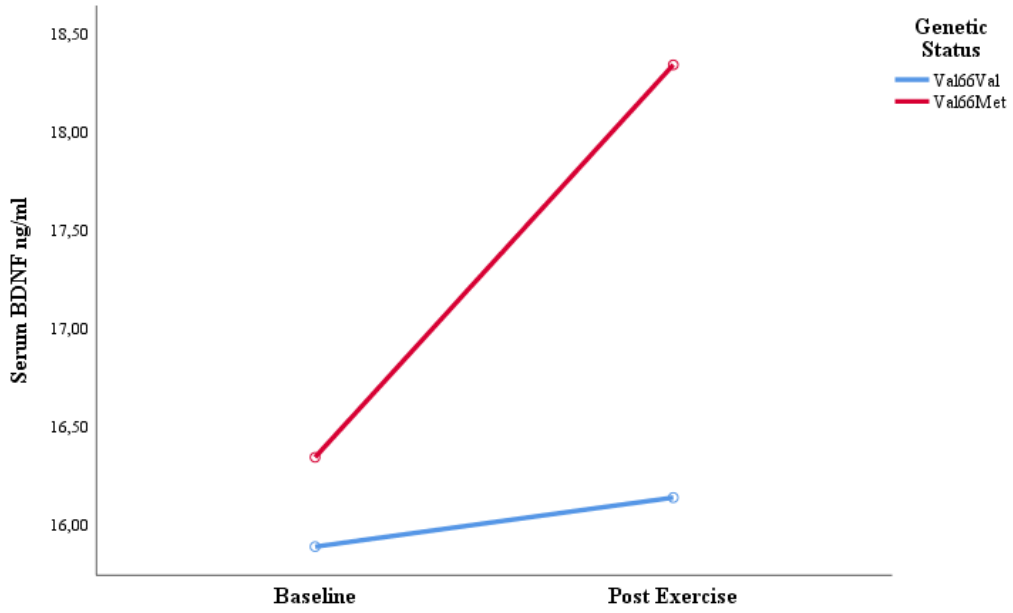

Figure 11. Average serum Brain Derived Neurotrophic Factor (BDNF) at baseline and after an exercise intervention in Val66Val and Val66Met carriers respectively, measured in nanograms per milliliter (ng/ml) in the post-hoc analysis where three participants were excluded.

At baseline, there was a small mean difference between Val66Val-carriers (M = 15.89, SD = 3.64) and Val66Met-carriers (M = 16.34, SD = 5.27) yielding a Cohen`s $d$ of 0.10, which indicates a minimal effect size between the two genetic groups at baseline. Post exercise the mean difference between our Val66Val-carriers ($M$ = 16.14, $SD$ = 4.22) and our Val66Met-carriers ($M$ = 18.34, $SD$ = 5.92) giving a Cohen`s d of 0.43 indicating a small effect size.

In this post-hoc analysis, Val66Val-carriers still seem to have a smaller BDNF serum response to the exercise than…. Effect size analysis within the Val-homozygous group at the two different points in time, yielded a Cohen`s d of 0.06, which indicates a very minimal effect size when looking at serum BDNF levels pre and post exercise. Analyzing Met-

carrying participants within the same parameters yielded a Cohen`s *d* of 0.36, indicating a small effect size.

Excluding the three participants, the Val66Val-carriers had, on average, a 1.57% increase in serum BDNF levels after the exercise intervention, while the Val66Met-carriers collectively exhibited an increase of 11.63% post exercise.

The Impact of Exercise on proBDNF Levels

As the levels of proBDNF were nearly mainly in the laboratory analyses, we experienced a great deal of missing data when analyzing proBDNF-levels across our sample. proBDNF in 8 subjects pre and post exercise was detectable, which means that 18 (69.23%) participants had proBDNF values that were undetectable in the laboratory. Having such a small sample size is likely to impact and invalidate any results we attain from analyzing this group, which is why running a data analysis of this hypothesis was decided against.

Discussion

The results from our data-analyses show that one aerobic exercise resulted in a general increase in serum BDNF levels our subjects. This increase was genetically mediated and Met-carrying individuals saw a much more consistent increase in serum BDNF levels after the exercise session compared to their Val-heterozygous counterparts. A post hoc analysis was run where three participants were excluded, which led to a change in significance levels in our overall results.

We also found that baseline serum BDNF levels increased with age in our sample. Genetic polymorphism did not seem to have any significant influence on baseline serum BDNF levels, and when further dividing our participants into two age groups (60-67 years and 68-75 years) there seemed to be no difference in baseline serum BDNF levels between the two groups. The correlation between age and baseline serum BDNF levels was only

significant when using age as a continuous variable, and not as two distinct age groups. proBDNF levels were hard to detect in the laboratory, which in itself is considered an interesting finding.

Baseline BDNF Levels

Serum Baseline BDNF Levels

We found great individual variations between our participants' baseline BDNF levels. The values ranged from 8.9 ng/ml to 23.5 ng/ml, with an average of 15.75 ng/ml across our 26 participants. These results are in accordance with previous studies on baseline BDNF levels in older adult groups. In the meta-review conducted by Knaepen and colleagues (2010) it was reported that extreme individual variations in baseline serum BDNF levels should be considered normal. Across the 16 studies they included in their review they reported individual variations ranging from 1.5 ng/ml to concentrations of 30.9 ng/ml. It is important to note that this literary review looked at studies investigating age groups from 20 to 70 years, and their results are therefore not exclusive to older age groups as the current study is. That may explain why the variations in our results span across a smaller range (8.9 ng/ml to 23.5 ng/ml) compared to those found in the review by Knaepen and colleagues (1.5 ng/ml to 30.9 ng/ml).

One of the studies included in the literary review discussed above (Laske et al., 2010) looked at BDNF levels in older adult women who had experienced an episode of depression during the last year. Their control group consisted of 20 women at an average age of 58.9 (+/-6.6) years and an average of serum baseline BDNF levels of 30.5 +/-6.9 ng/ml. This average is almost twice as high as the average we found in our group. This could be explained by several important differences between the sample groups used in each respective experiment. We used a mixed sample with both males and females, while Laske and colleagues (2010) only recruited women. The relationship between gender and BDNF levels has not yet been

established and gender should therefore be counted as a possible factor influencing serum BDNF levels in our sample. There were also certain diurnal variations between the two studies. While Laske and colleagues (2010) collected their baseline BDNF samples in the early afternoon (between 14:00 and 15:00) our baseline tests were drawn earlier in the day, with every baseline blood sample occurring between 09:13 and 10:02 in the morning. Many biological mechanisms in our bodies are diurnally regulated (Martin-Fairey & Nunez, 2014), and it seems reasonable to assume that BDNF production may also be prone to diurnal fluctuations, especially seeing as BDNF levels may be connected to the HPA axis (Nibuya et al., 1999). Our subjects also notably differ in age with an average age of 68.9, which is exactly 10 years older than those who participated in the study of Laske and colleagues (2010). Several studies (Bus et al., 2012; Lommatzsch et al., 2005) have noted that serum BDNF production seems to decrease with ageing. Based on this it seems like comparing individuals from the same age groups could yield a more accurate foundation for future discussion.

A more recent study, conducted by Håkansson and colleagues (2017), also looked at serum BDNF levels in an older adult sample (65-85 years, $N = 19$). Håkansson and colleagues is arguably the one existing study that is most similar to ours, as they compared serum BDNF levels in older adults pre and post an acute aerobic exercise session. This study looked at a slightly older cohort than we did and had a more balanced sample than we did in terms of gender. Similar to our study, blood-samples were drawn in the morning hours. They found a baseline average of serum BDNF to be 19.2+/-1.17 ng/ml. This average is still higher than the one we found in our sample, but it is much closer to our values than the studies previously mentioned. Small differences could be the source of our differing baseline BDNF levels. It is still not determined whether gender impacts BDNF levels in healthy groups. Additionally, the sample of Håkansson and colleagues was older than the one we investigated in the present

study. Seeing as both studies had small sample sizes it seems reasonable to assume that slight differences in the sample composition will have a rather significant impact on the final numbers.

Generally, our baseline serum BDNF levels seem to be in accordance with previous studies (Håkansson et al., 2017; Knaepen et al., 2010; Laske et al., 2010), exhibiting large individual variations that are probably affected by several factors that are yet to be identified. Factors such as (but not limited to) diurnal variation, physical fitness, gender and age and their role in relation to BDNF levels and production is yet to be determined. The fact that our sample size was quite small makes it seem plausible that some unknown factors will have an impact on our sample, which in turn will affect our results.

Controlling for Physical Fitness in Relation to Baseline BDNF Levels

Before we started analyzing our hypotheses, we controlled for the impact of physical fitness on baseline serum BDNF levels. No correlation was detected in our sample, indicating that physical fitness did not have any impact on the production of serum BDNF in this sample. There seems to be three plausible explanations to these results. The first is that physical fitness simply does not impact serum BDNF levels in healthy older samples. The second explanation is that our sample is simply too small to properly investigate the nuances of individual variations of BDNF levels associated with physical fitness. The third is that one exclusion criterium for participation in this study was to not exceed 60 minutes of moderate intensity physical activity per week, thereby barring the most fit subjects from participating in this study. This may very well have impacted the correlation analysis between physical fitness and baseline BDNF levels, and further looking into this possible link in a more varied group in terms of physical fitness could yield interesting results.

The Impact of Age on Baseline BDNF levels

Contrary to several previous studies (Minelli et al., 2011; Shimada et al., 2014; Ziegenhorn et al., 2007) and our own hypothesis, we found a positive, medium correlation between age and baseline serum BDNF levels, when we used age as a continuous variable. Which is to say that in our sample, baseline levels of serum BDNF increased with age, and our older participants generally exhibited higher baseline serum BDNF levels compared to younger participants.

Seeing as our average baseline levels of serum BDNF are lower than similar studies on older populations, it could be that our participants' serum BDNF levels would have fallen within the lower echelon of their respective age group had this been a larger study. Therefore, it is possible that this finding can simply be explained by the size of our sample, and that the fluctuations we detected may not have been significant had we tested a larger group of individuals. Despite this, our finding contributes to the underlining of how little is known about the fluctuations of BDNF levels throughout the lifespan and across individuals. So far, there does not seem to exist a standard "range of normalcy" for measuring BDNF levels, which makes the interpretation of such findings difficult.

Age, Polymorphism and Baseline BDNF levels

In our sample we had a total of 18 Val66Val-carriers (69.2%), 8 Val66Met-carriers (30.8%) and no Met66Met-carrying participants. These numbers are close to the average genetic distribution in European populations where 27% are Val66Met-carriers, 3% are Met66Met-carriers and 70% are estimated to be Val66Val-carriers (Montag et al., 2014). In this regard our sample seems to be a reasonable representation of the general public.

When further controlling for the impact the Val66Met polymorphism had on baseline BDNF levels in our participants, we split our sample into two age groups (60-67 years, n = 10

and 68-75 years, n = 16). There were four Met-carriers in each age group. Statistical analyses showed that neither age group nor genetic status had a significant impact on baseline BDNF levels.

Despite not finding any significant main or interaction effects of age group nor genetic status, looking at effect sizes between groups (see table 4 for an overview of mean values, standard deviations and effect sizes) showed that both genetic polymorphisms exhibited lower serum BDNF levels in the younger age group compared to the older age group at medium or high effect sizes respectively (0.72 for Val66Val carriers and 0.83 for Val66Met carriers). The overall difference between baseline BDNF levels of the two polymorphisms was close to zero (0.01), while the difference between the two age groups was much larger (0.78) indicating a medium to large effect size. This shows us that in our sample, it is the impact of age itself, and not the difference in polymorphism that mediates the differences of baseline levels between the two age groups.

Cohen`s *d* for the Val66Val-carrying participants between age groups indicated a medium effect size, while the Met-carriers yielded a slightly larger Cohen`s *d* between age groups, indicating a high effect size. This indicates that there may exist a larger difference between the baseline BDNF levels of our “younger” and “older” Met-carriers than between the Val-homozygous participants in the same age groups. The Val-homozygous subjects seemed to be placed more consistently in the middle of our dataset, while the Met-carriers showed a slight tendency of inhabiting the higher and lower fringes of the dataset (see figure 6). Seeing as the results were non-significant and that our sample was very small, with only four Met-carrying participants in each age group, this discussion is merely speculative. Despite this, these observed inclinations in our dataset seem reasonable to accentuate, as future research in larger samples may be able to elucidate these tendencies.

Previous findings (Elzinga et al., 2011; Minelli et al., 2011) show that Met-carriers consistently had higher concentrations of serum BDNF compared to the Val-homozygous carriers. Elzinga and colleagues (2011) found that while Met-carriers generally had higher serum BDNF levels than Val66Val-carriers, this changed when accounting for early negative, life-events such as childhood abuse. Met-carriers seemed to be more vulnerable to such adverse conditions, and this was reflected in their serum BDNF levels. Therefore it could seem like Met-carriers may have a propensity for high serum BDNF levels, but that these serum BDNF levels are more vulnerable to stressors and adverse life-events than in Val66Val-carriers. This could help explain why our Met-carrying participants seemed to exhibit slightly greater variation between subjects; their serum BDNF levels may be more prone to stressful environmental stimuli. Val-homozygous individuals may be less disposed to environmental changes or stimuli impacting their serum BDNF levels. As these results were not significant, and only detectable through analyzing effect sizes, this should be considered a tentative discussion and further research is needed.

The Effect of Exercise on Serum BDNF levels

One Single Session of Physical Exercise Will Result in an Increase of Peripheral Serum BDNF Levels in our Subjects Compared to Their Baseline Levels.

We did indeed find a significant effect ($p = 0.04$) of exercise on BDNF levels across our sample, from an average of 15.75 ng/ml pre exercise to 16.33 ng/ml post exercise. This rise in serum BDNF levels is consistent with previous studies (Håkansson et al., 2017; Saucedo Marquez et al., 2015), and was therefore not a surprising finding. The rise in serum BDNF post exercise is equivalent to a 3.7% rise from baseline, overall in our 26 subjects. In the post-hoc analysis this effect became more significant ($p = 0.02$) from 16.03 ng/ml at baseline to 16.81 ng/ml post exercise, equivalent to a 4.9% increase across the 23 included participants. Comparing these results to the 2010 literary review by Knaepen and colleagues

(2010) our overall rise in BDNF levels both in the original analysis and in the post-hoc analysis is lower than the range they reported, which was ranging from 11.7% to 410%. It is important to note that this was the range reported in studies that *had* detected a significant change in serum BDNF levels from pre to post exercise. At least one of the studies included in the literary review was unable to report a significant response to exercise (White & Castellano, 2008). Looking at our own sample (see figure 9), there does seem to be great individual variation between the individual BDNF responses of our subjects. Taking a closer look on our data, 14 participants experienced elevated BDNF serum levels after the exercise intervention, while 10 participants showed lower BDNF serum levels post exercise compared to their baseline values. Two participants exhibited no detectable changes in serum BDNF levels pre and post exercise session.

Other than genotype, there are several plausible explanations for these individual variations between BDNF serum response to physical exercise. One explanation could be that the BDNF response to physical exercise has been reported to be transient (Knaepen et al., 2010), and if a blood sample is not collected within ten minutes at the most, BDNF levels may have regressed to baseline, or even lower (Saucedo Marquez et al., 2015). Per appendix B table B1, four participants' time of testing was not reported and three had an error in reporting these times. In the 19 remaining participants, three exceeded the recommended 10-minute time limit between the end of the exercise session and collection of blood sample, and the two participants with the longest time lapse (41 minutes and 27 minutes respectively) between the end of the exercise session and blood-sample collection saw a negative serum BDNF-response to the exercise session. These two participants had amongst the most marked negative response to the exercise session (-1.6 ng/ml and -2.5 ng/ml). Despite this, out of the 16 participants who were reported to have been measured within the recommended time-limit, six participants still exhibited decreased serum BDNF levels. It seems like delayed collection

of blood-samples could explain some, but far from all of the negative serum BDNF levels in our participants post exercise.

The intensity of the exercise session has also been reported to affect the release of peripheral BDNF (Knaepen et al., 2010). Even though the exercise session for this study was designed with previous BDNF-research in mind, small differences between exercise protocols have been shown to yield different results across studies (Knaepen et al., 2010). Because our participants were monitored throughout their 20-minutes of cycling and were generally exercising above 65% of their maximum heart rate, this does seem unlikely, but should still be considered as a possible factor. It should also be noted that the 65% of maximum heart rate was calculated through a mathematical formula, and not directly measured in each participant. This could therefore be considered a possible source of uncertainty in our results.

One last explanation for these results, is that several factors may influence an individual's capacity for activity-dependent BDNF-emissions. Previous research (Håkansson et al., 2017; Notaras et al., 2015; Shimada et al., 2014) has speculated that factors such as gender, age, physical fitness and genetic polymorphisms may influence BDNF-production in response to physical exercise, both centrally and peripherally. This is why we further analyzed our data to investigate whether there were any notable differences between Val66Val-carriers and Val66Met-carriers in their serum BDNF-response to the exercise intervention.

Individual Variations of BDNF Levels After One Exercise Session Could Be Explained by the Val66Met Genetic Polymorphism.

In the first analysis including all 26 participants, the effect of exercise was significantly stronger in our Met-carrying participants, where seven of the eight participants with this polymorphism showed increased BDNF serum levels post exercise. The last participant from this group showed no detectable change between the pre and post exercise

sample. Figures 8 and 9 illustrates that our Met-carrying participants were experiencing a much more consistent response to the exercise intervention, showing an average 11.95% increase in serum BDNF levels post exercise. The Val-homozygous participants' response was much more varied, and as a group they exhibited a 0.6% *decrease* in serum BDNF levels post exercise. This makes sense if one looks at the individual values across the Val66Val-sample. One individual showed no detectable response to the exercise session, seven subjects exhibited rising serum BDNF levels, and ten participants saw a marked decrease in serum BDNF levels post exercise. That is to say that a majority of the Val66Val participants actually had a negative BDNF response to the aerobic exercise session.

However, in the post-hoc ANOVA with three participants excluded due to excessive time-lag from exercise to blood sample, the significance of these findings was reduced to a non-significant level. Although the findings failed to be significant in the post hoc analysis, the tendency remains highly interesting. This post-hoc analysis underlines how small changes to an already small sample can significantly impact the results. Though the same tendencies became clear (see figure 11) the levels of significance changed, and the interaction effect of genetic status seized to be significant ($p = 0.07$). As we already had very few Met-carrying individuals in our original sample, removing one would inevitably have a noticeable effect on the results of our data analyses.

Looking at figure 11, though, the overall trend that Met-carriers have a more marked response to the exercise intervention still remains, with the accompanying effect size between Met-carriers pre and post exercise (Cohen`s $d = 0.36$) being larger than that between Val-homozygous carriers at the same points in time (0.06) which was very minimal, not even reaching a small effect size. Removing the three participants did give our Val66Val-carriers as a group a slightly more positive response to the exercise intervention, from a 0.6% decrease in the original analysis to an average 1.57% increase in this post-hoc analysis. Met-carriers

saw a very small decrease on average, from 11.95% in the original analysis, to 11.63% in the post-hoc analysis. Met carriers still saw a more marked response to the exercise intervention, compared to their Val-homozygous counterparts. Six out of seven participants in the Val66Met group saw an increase in serum BDNF levels, on the other hand, eight of our sixteen Val66Val-carriers still experienced decreased serum BDNF levels in response to the exercise session. Though Val66Val-carriers in this analysis saw an increase in their response to the exercise session, 50% of this group still experienced decreased serum BDNF levels. The Met-carriers saw a more consistently positive response to the intervention, with only one participant seeing no change and no participants exhibiting decreased levels post exercise. Though this post-hoc analysis changed the significance-levels of the analysis, it does not seem to have had a major impact on the tendencies observed in the original analysis of all 26 participants. The most important point to underline in this context seems to be that a small sample will be much more vulnerable to minor changes than a larger one.

These are very interesting findings, as previous research has implicated Met-carriers of being less susceptible to increased BDNF-production in response to physical activity (Hopkins et al., 2012). It is important to note that the majority of studies on this polymorphism has been conducted on younger age groups, be it in human or animal studies (Hopkins et al., 2012; Ieraci et al., 2016). In younger age groups it does seem like Met-carriers do experience an attenuated cognitive improvement to physical exercise when comparing them to Val-homozygous subjects (Caldwell et al., 2014). Throughout the literature this is attributed to the Met-carriers exhibiting lower activity dependent BDNF-emissions that in turn manifests itself as lower hippocampal volumes in these subjects.

The research done on the Met-polymorphism and its effect on the ageing brain has been much more inconclusive than that done on younger age groups. Some controversy remains as to how this polymorphism manifests in old age. Brooks and colleagues (2014)

presented the possibility that older adult Met-carriers actually had an advantage over Val-homozygous carriers in old age. They found that Met-carrying individuals had higher hippocampal volumes and scored higher on a working memory task compared to the Val-homozygous participants. The authors posed that Met-carriers were more protected from age-related cognitive decline. Canivet and colleagues (2015) on the other hand, found that active Val-homozygous subjects outperformed their Met-carrying peers in regard to episodic memory performance. Subjects were asked to report their general physical activity and were genotyped through buccal samples.

It is important to note that the participants of neither of the aforementioned studies were measured for peripheral BDNF levels. Neither study included an exercise protocol to measure physical fitness and no corresponding measurement of individual BDNF-response to physical exercise was taken. Therefore, the conclusion that the differences between memory scores could be attributed to Met-carriers exhibiting lower activity dependent BDNF-emissions was implied and not directly measured. Our results contribute to further the confusion within this field.

Our results also drive home a very important point; more basic research is needed to establish any connection the Met-allele may have with memory performance in old age. Since there seems to exist a greater variability in findings in studies on older adult groups compared to findings in younger groups, generalizing knowledge accumulated through research on young age groups to the older age groups does not seem ideal. Further research on older adults should therefore be carried out to explicate the relationship between genetic status, physical exercise, serum BDNF levels and memory in older populations.

Looking at our results it does seem like Met-carriers may experience a “flip” in old age, where their activity-dependent BDNF-emissions rise. This carries important implications

because BDNF is associated with improved neuroplasticity in areas of the brain that are most affected by age-related atrophy. Seeing as there is so little research on older age groups that takes genetic polymorphism, BDNF serum response, and methods to increase serum BDNF levels such as physical exercise into account, further research is necessary to establish the link between carrying a Met-allele and having a higher BDNF serum response to aerobic exercise.

One single exercise session will result in a peripheral increase of serum proBDNF levels.

In regard to this hypothesis we do not have any previous studies that are similar to our own to rely on in terms of comparing our results. It was striking how few of our subjects we were able to detect proBDNF in at all. Only 8 subjects exhibited pre and post exercise proBDNF levels that were measurable at all, which is to say that we were unable to detect levels or proBDNF in 69.23% of our sample. See appendix C table C1 for an overview of each individual with detectable proBDNF levels with additional information like gender and genetic status. Seeing as proBDNF research has mainly been done in animal studies (Ding et al., 2011) future research on this precursor protein in humans carries important implications as it seems to have a more active role in neuroplasticity than previously assumed.

Limitations

The most obvious limitation of this study is the size of our sample. Ideally a much larger sample should have been used to ensure the validity of our data and results. Even though we did see some very clear results as well as some interesting tendencies in our dataset, these results and tendencies should be considered with a certain amount of precaution until they can be replicated in a larger sample. The diminished significance in the post-hoc two-way ANOVA clearly demonstrates the vulnerability of a small sample size.

The second most important limitation of this study was that the protocols surrounding the 10-minute time limit between the end of the exercise session and taking blood samples

should have been stricter. Missing data and errors in reporting (see appendix B table B1) made it difficult to ascertain that the data we collected was valid and correctly reported. This led to a post-hoc analysis being necessary to ensure that the results were not skewed by delayed blood sampling. We decided to only exclude the three participants who we could say with the most certainty had waited too long between exercise session and collection of blood sample. This could itself impact our dataset as several values were missing or inaccurately reported (See appendix B, table B1). There is also a possibility that the three excluded participants were also a consequence of inaccurate reporting of exact points in time. Seeing as the sample used in this study was a part of a larger research project, these protocols should be updated to ensure the validity of future samples.

In addition to this the demographic composition of our participants could have been more balanced, both in terms of gender (we had a 73.1% share of females) and age (61.5% of our participants were in the older age group). It could also be argued that our participants were too young to be considered a fair representation of the "older adult" and that having a cut off at 75-years is limiting our data collection and may leave out part of the bigger picture when looking at BDNF emissions in old age. However, the genetic distribution in our sample did correspond closely to general European populations, which does point to a level of generalizability of our sample.

Another fact that should be noted is that our participants had been resting for approximately 15 minutes before venipuncture. Seeing as the knowledge about which factors influence serum BDNF emissions is not fully understood, this should be included as a factor that may have had an influence on baseline serum BDNF levels. We did not have a control group for our participants. This is, of course, because our participants were themselves the control group for a larger experiment. Having a control group for our participants (a group that were measured within similar times but without going through the exercise intervention)

could have ensured that we accounted for any fluctuations in serum BDNF levels that were due to other factors than the exercise session. One last limitation of this study is that the more physically fit applicants were excluded from participation, and certain nuances that may be attributed to physical fitness were thereby not possible to examine in this study.

One last note is that measuring BDNF levels in living humans is by nature an indirect way of measuring the BDNF-expression we assume is happening in the brain. Though Rasmussen and colleagues (2009) and Klein and colleagues (2011) have established that there seems to be a high correlation between BDNF-expression in the brain and peripheral BDNF levels in the blood or in cerebrospinal fluid, there is still an aspect of uncertainty that should be taken into consideration when interpreting the magnitude of peripheral BDNF levels.

Strengths

The sample used in this thesis is a part of the control group of a much larger clinical research project. This offers the advantages of the large infrastructure surrounding the encompassing project. Initial screening of each participant was extensive, as to ensure a range of both physically and psychologically healthy participants. Additional measures were taken to ensure that our subjects' physical fitness did not supersede a previously established form of "normal", as previous research has suggested that very high levels of physical fitness may influence BDNF levels and emission (Zoladz et al., 2008).

The physical exercise intervention was always conducted by either a licensed physiotherapist or occupational therapist. This ensured that each participant had close monitoring and exerted the effort necessary to follow the desired intensity for the intensity session. The physio/occupational therapists were also careful to conduct each exercise session in as similar manner as possible, to ensure the absence of confounding factors. The blood samples for each participant were all drawn within the same hour of the day. The baseline

blood sample in each participant was drawn between 09:13 am and 10:02 am, while the post exercise blood samples were all drawn between 10:07 and 10:57. Ensuring the absence of large diurnal variations between the samples is important, since we do not yet know how circadian rhythm may influence BDNF levels.

Taking all of these points together makes for a very uniform and stable handling of our sample group, eliminating many external factors that may influence the data collection and interpretation.

Future Directions

This study has replicated findings across several previous studies, that confirm that serum BDNF levels increase as an effect of aerobic exercise. The interaction effect between genotype and response to the exercise intervention, as well as the positive correlation between age and baseline serum BDNF levels need to be replicated in a larger, more balanced sample in terms of both age and gender. The lack of detectability of proBDNF levels in our subjects is unexpected and should also be further investigated in a larger sample. In addition to replicating the current findings, future research on BDNF in older populations have several possible venues of exploration.

Future research would benefit from expanding the age-range, allowing for observing BDNF emissions across a larger age-span. As people in modern society live long lives, valuable information could be overlooked by not including participants above the age of 75 years. This would ensure a more nuanced view of how BDNF may fluctuate across old age. Replicating this study in a larger group would also open for a broader understanding of what is considered “normal” BDNF serum values in older adults. For the time being, little is known about how this neurotrophic factor acts in humans, and even though it has very interesting implications regarding neuroplasticity, psychiatric disorders and cognition. Establishing a

solid framework seems necessary in order to move forward within this field. Including a larger range of physically fit older adults could also yield interesting results. Looking at older groups across differing fitness-levels could give important insight into how staying physically active in old age may influence peripheral BDNF levels.

As it does seem like BDNF-emissions and the HPA axis may be connected in some way, investigating cortisol levels in relation to baseline serum BDNF levels as well as genetic polymorphism could be very interesting. Further examining the interplay BDNF may have with the HPA axis through cortisol could give useful insight into how BDNF may influence mental health and development of psychiatric disorders, and whether this could be genetically modified through the Val66Met genetic polymorphism.

Future research could also benefit from investigating the possible connection between serum BDNF levels and genetic status in relation to neuropsychological tests and MR-testing. Seeing as BDNF is heavily associated with neuroplasticity in the hippocampus and thereby to certain aspects of cognition, examining this as directly as possible in older human subjects might further our insight into how the neuroplastic effects of BDNF may be modulated.

Conclusion

Results from this paper indicates that acute aerobic exercise in older adults leads to increased serum BDNF levels. Genetic status does seem to influence individual response to physical exercise. In our sample Val66Met-carriers exhibited a tendency toward a much more consistent increase in serum BDNF levels as a response to the exercise than our Val-homozygous participants. These results were influenced by the time that had lapsed between the end of the exercise session and the time of blood sample collection. Despite this being controlled for in a post-hoc analysis, the same tendencies were present throughout the results.

At baseline, we found a medium positive correlation between age and serum BDNF levels. No differences between serum BDNF levels in Val66Val-carriers and Val66Met-carriers were detected, nor any correlation between physical fitness and baseline levels of serum BDNF. proBDNF-levels proved to be surprisingly difficult to detect across our sample, and the sample with detectable values both pre and post exercise was too small to justify running any analyses.

These results and ensuing discussion underline that these connections need further research to be established, and even though they are interesting they should be replicated across larger samples before their implications can be thoroughly investigated.

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Appendix

Appendix A – PEXACOG Design and Questionnaires

Figure A1

Flow Chart excibiting Study Design of the PEXACOG project

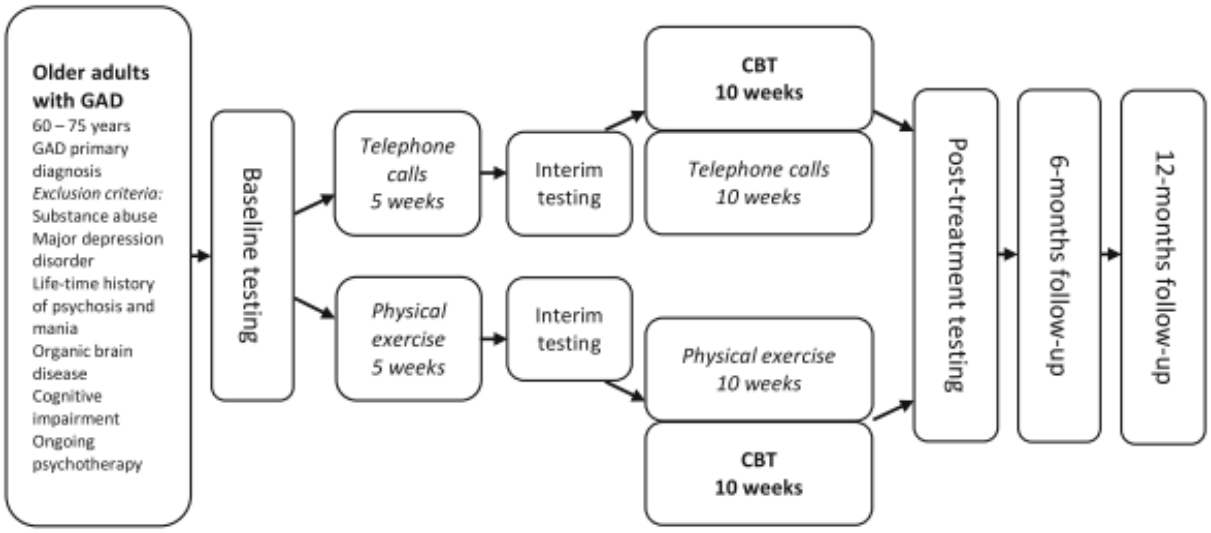

Table A1

Phone Interview; First Screening Interview

Alder	
Har du eller har du noen gang hatt en psykisk lidelse eller atferdsforstyrrelse?	
Har du noen gang opplevd å være manisk eller psykotisk?	
Har du fysiske plager som gjør det vanskelig for deg å delta i fysisk aktivitet?	
Har du vært operert i øyne eller hodet? Har du pacemaker? Har du cochleaimplantat?	
Bruker du briller? - Har du mulighet til å bruke kontaktlinser i forbindelse med MR-undersøkelse?	
Har du brukt narkotika, inkludert hasj de siste tre månedene?	
Bruker du vanedannende legemidler, slik som sobril, valium, vival, eller innsovningsmidler, slik som imovane eller stillnoct, jevnlig? Alkoholkonsum?	
Har du tidligere hatt slag eller drypp?	
Går du for tiden i psykoterapi?	

Table A2

Interview Questions for collecting of Demographic Variables

Kjønn	
Alder	
Sivil status	
Utdanningsnivå	
Utdanningslengde	
Lengde tid ute av arbeid	
Antall år i arbeid	
Antall barn	
Bor sammen med	
Bruker du tobakk?	

Table A3

Norwegian Translation of the PAR-Q Interview

Har legen din noensinne sagt at du har hjerteproblemer eller en hjertesykdom?	
Har du regelmessige smerter i hjertet eller brystet?	
Føler du deg ofte svak eller opplever episoder med alvorlig svimmelhet	
Hender det du mister balansen på grunn av svimmelhet?	
Har legen din noensinne sagt at blodtrykket ditt er for høyt?	
Bruker du for tiden medisiner for høyt blodtrykk eller hjertesykdom (f.eks vanndrivende tabeletter?)	
Kjenner du til om du har høyt kolesterolnivå i blodet?	
Har du besvimt i løpet av de siste 6 måneder?	
Har du sukkersyke (diabetes)?	
Har noen av dine foreldre, søsken eller barn fått hjerteinfarkt plutselig (før fylte 55 år for menn og 65 år for kvinner)?	
Har legen din noensinne sagt at du har problemer med ben eller ledd, for eksempel gikt, som har blitt forverret ved fysisk trening?	
Lider du av problemer med nedre rygg, som for eksempel kroniske smerter eller nummenhet?	
Har du for øyeblikket en uførhet (funksjonshemming) eller en smittsom sykdom?	
Finnes det en god fysisk grunn, som ikke nevnes i denne testen, til at du ikke burde delta i et aktivitetsprogram, selv om du selv ønsker dette?	

Appendix B – Descriptive tables related to the Exercise Intervention

Table B1

Overview of Time Elapsed Between End of Exercise Session and Collection of Blood Sample, with corresponding BDNF values and genetic status

ID	End of Exercise Session	Time of Second Blood Sample	Time Between end of Exercise Session and Blood Sample	Change in serum BDNF levels (ng/ml)	Genetic Status
1	Missing	10:18	Missing	-0.30	Val66Val
2	10:18	Missing	Missing	-2.1	Val66Val
3	10:28	10:30	2 minutes	0	Val66Met
4	09:58	10:25	27 minutes	-2.5	Val66Val
5	10:18	10:17	Error	0	Val66Val
6	10:23	10:22	Error	2.5	Val66Val
7	10:28	10:35	7 minutes	3.5	Val66Val
8	10:28	10:20	Error	0.1	Val66Val
9	10:28	10:35	7 minutes	3.4	Val66Val
10	10:13	10:20	7 minutes	3.0	Val66Val
11	10:28	10:28	0 minutes	0.7	Val66Val
12	09:58	10:07	9 minutes	-0.8	Val66Val
13	10:28	10:34	6 minutes	-0.8	Val66Val
14	Missing	Missing	Missing	1.5	Val66Met
15	10:18	10:19	1 minute	3.0	Val66Met
16	10:28	10:38	10 minutes	-0.9	Val66Val
17	10:28	10:33	5 minutes	-0.2	Val66Val
18	10:43	10:49	6 minutes	4.2	Val66Met
19	Missing	10:57	Missing	2.0	Val66Met
20	10:28	10:33	5 minutes	2.8	Val66Met
21	10:36	10:42	6 minutes	0.5	Val66Met
22	10:38	10:38	0 minutes	-5.0	Val66Val
23	10:28	10:49	21 minutes	1.1	Val66Met
24	10:18	10:27	9 minutes	1.5	Val66Val
25	10:28	10:33	5 minutes	- 0.6	Val66Val
26	09:28	10:09	41 minutes	-1.6	Val66Val

Table B2

Overview of Participants who did not reach the Recommended HR

Participant Number	65% of max HR	Average HR during 20 minutes of cycling
7	106	95
11	101	84
18	103	98

Appendix C – An overview of proBDNF data

Table C1

Demographic overview, proBDNF levels and changes in participants with detectable proBDNF levels pre and post exercise

Participant Number	Gender	Age	Polymorphism	Change in proBDNF levels post exercise (ng/ml)	Change in BDNF levels post exercise ng/ml
5	Female	74	Val66Val	0.50	0.00
7	Male	66	Val66Val	0.00	3.50
10	Female	73	Val66Val	- 0.10	3.00
14	Female	72	Val66Met	1.30	1.50
17	Female	70	Val66Val	0.00	-0.20
20	Female	65	Val66Met	0.80	2.80
21	Female	63	Val66Met	0.18	0.50
23	Female	64	Val66Met	0.30	1.10