THE EFFECT OF EXERCISE INTENSITY AND EXERCISE ENVIRONMENT ON BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND PHYSIOLOGIC PARAMETERS IN YOUNG MALE SKIERS

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ABSTRACT

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Introduction. A strong connection exist between physical exercise and brain health. The crucial mediator of these benefits seems to be the brain derived neurotrophic factor (BDNF). Exercise has been shown to acutely increase the amount of circulating BDNF in blood. The amount of the rise of BDNF levels may be exercise- intensity dependent and the skill demands of the performed exercise might also play a role in the amount of BDNF produced. Also other substances, like cortisol and IGF-1, might play a role in BDNF regulation. An interesting sport in the viewpoint of BDNF is Cross country skiing. This sport is demanding both physically and technically. During winter, the practicing of cross country skiing is highly dependent of the weather conditions. During warm winters, one option is training on a treadmill with roller-skis. With the developed technique of today, it is possible to mimic a real race track surrounding in a treadmill environment by using a virtual environment. The aim of this study was to examine the dynamics of BDNF, IGF-1, cortisol (COR) and lactate (LA) in response to a high intensity exercise and the effect of environment on BDNF. The aim was also to compare the physiologic responses of skiing on snow and roller skiing on treadmill with and without a Virtua environment.

Methods. Nine healthy elite-level male skiers volunteered as subjects in the study (mean ± SD age: 18.8 ±1.5 years, weight 72.4 ±4.9 kg, height 178.6 ±5.4 cm). All subjects participated in three training sessions: One skiing session on snow (S) and two roller skiing treadmill sessions, one in normal treadmill environment (NTE) and one in virtual (V) environment. All sessions consisted of three intervals with 4 min rest after each interval. The intensities of the intervals were 70% of HR maximum (LOW), 85-90% of HR maximum (HIGH) and all –out (100%, MAX). The speed of the treadmill in the roller skiing sessions was adjusted individually on the base of the speeds skied on snow. Heart rate (HR) and technique changes were monitored throughout the sessions and venous blood samples were collected before the first (PRE), and directly after every interval. BDNF, IGF-1, COR and LA were analyzed from the blood.

Results. On S, the BDNF values were significantly higher in PRE compared to NTE. In NTE, there was a significant rise in the BDNF between the PRE (19. 8 ± 5.7 ng/ml) and HIGH (23.3 ± 5.8 ng/ml, $p < 0.05$). COR was higher during S compared with V and NTE in PRE and when compared to NTE also in LOW, HIGH and MAX respectively. For IGF- 1, there was a significant rise from PRE (50.6 ± 12.5 nmol/l) to HIGH (56.4 ± 13.4 nmol/l) on S and from PRE (45.8 ± 10.4 nmol/l) to MAX (49.0 ± 10.8 nmol/l) in NTE. There was a significant difference in the IGF-1 values between every interval (LOW, HIGH and MAX) when comparing the S with both V and NTE. For LA, there was seen a significant difference between the MAX intervals when comparing S (13.4 ± 1.7 mmol/l) with V (8.8 ± 3.7 mmol/l) and NTE (7.0 ± 2.7 mmol/l, $p < 0.05$). Also HR was higher ($p < 0.05$) on S in MAX (181 ± 8 bpm) than on V (172 ± 10 bpm) and NTE (175 ± 7 bpm). A positive correlation between BDNF and IGF-1 was found in PRE both on S ($r=0.81$, $p<0.05$) and in NTE ($r=0.69$, $p<0.05$). For BDNF and COR, a negative correlation was found on S in PRE ($r = -0.68$, $p< 0.05$). Furthermore, a positive correlation of absolute changes of BDNF and COR from PRE to HIGH measurements in the snow measurements was found. ($r = 0.74$, $p< 0.05$). Also technique changes were significantly more frequent during S in every interval (25 ± 4 in LOW, 26 ± 4 in HIGH and 25 ± 5 in MAX) when compared to V (11 ± 2 in LOW, 11 ± 2 in HIGH and 11 ± 3 in MAX) and NTE (9 ± 2 in LOW, 8 ± 1 in HIGH and 8 ± 1 in MAX).

Conclusion. The main findings of this study were that BDNF is upregulated by high intensity exercise, but maximal intensity exercise might lead to a drop in the concentration of circulating BDNF. On S and NTE, there was found a positive correlation between BDNF and IGF-1 and further on S, both a positive and negative correlation with BDNF and COR. IGF-1 might thus be an upregulator of BDNF and the effect of COR in turn might be condition dependent. Chronic stress causing high COR levels might be negative to brain health and BDNF, but acute stress caused by intense exercise might in turn cause upregulation of BDNF. But it is likely, that there exist also individual differences in the dynamics of these substances. The harmful effect of chronically elevated COR levels in response to intense training periods can possibly be suppressed by IGF-1, since IGF-1 might upregulate BDNF production during intense training periods. Finally, it seems that maximal intensity skiing on snow might be physiologically more demanding than roller skiing on treadmill.

Keywords: Brain-derived neurotrophic factor, Cortisol, IGF-1, cross country skiing, roller skiing, treadmill, virtual environment, technique changes

TIIVISTELMÄ

Ruostekoski, Anni. 2017. Intensiivisen intervalliharjoituksen ja harjoitusympäristön vaikutus aivoperäiseen hermokasvutekijään ja fysiologisiin vasteisiin nuorilla mieshiihtäjillä. Liikuntabiologia, Jyväskylän yliopisto, Liikuntafysiologian Pro Gradu- tutkielma, 64s.

Johdanto. Tutkimukset ovat osoittaneet, että liikunnalla ja aivojen hyvinvoinnilla on selkeä yhteys. Liikunnan aivoille suotuisten vaikutusten välittäjäaineena näyttää toimivan aivoperäinen hermokasvutekijä (BDNF). Liikunnan on osoitettu akuutisti nostavan veren BDNF- pitoisuutta ja BDNF- pitoisuuden nousu näyttää olevan riippuvainen liikunnan intensiteetistä, sekä taito- vaatimuksista. Myös kortisoli ja IGF-1 saattavat liittyä liikunnan aikaisen BDNF- tuotannon säätelyyn. BDNF- tuotannon näkökulmasta maastohiihto on mielenkiintoinen liikuntamuoto sen fysiologisten ja teknisten vaatimusten vuoksi. Talvisin lajin harrastaminen on pitkälti riippuvainen lumitilanteesta, ja vähälumisten talvien aikana vaihtoehto lumella hiihtämiselle on rullahiihto juoksumatolla. Tämän päivän kehittyneen tekniikan avulla matolla hiihtoon voidaan yhdistää virtuaalinen, oikeaa hiihtoympäristöä matkiva ympäristö. Tämän tutkimuksen tarkoituksena oli selvittää intensiivisen harjoituksen vaikutuksia BDNF:ään, IGF-1:seen, kortisoliin ja laktaattiin sekä harjoitusympäristön vaikutusta BDNF:ään. Tarkoituksena oli myös verrata lumella hiihdon ja juoksumatolla virtuaaliympäristössä sekä ilman virtuaaliympäristöä tapahtuvan rullahiihdon fysiologisia vasteita.

Menetelmät. Tutkimuksessa koehenkilöinä toimi yhdeksän kansallisen kärkitason nuorta mieshiihtäjää (KA ± KH: Ikä 18,8 ±1,5 vuotta, paino 72,4 ±4,9 kg, pituus 178,6 ±5,4 cm). Kaikki koehenkilöt suorittivat yhteensä kolme intensiivistä intervalliharjoitusta: Yhden harjoituksen lumella (L) ja kaksi rullahiihtoharjoitusta matolla, yhden virtuaaliympäristössä (V) ja yhden normaalissa ympäristössä (N). Kaikki harjoitukset koostuivat kolmesta intervallista, joiden välissä oli 3 minuutin palautus. Intervallien intensiteetit olivat 70% maksimisykkeestä (LOW), 85-90% maksimisykkeestä (HIGH) ja maksimi (100%, MAX). Nopeudet mattohiihtoihin määritettiin kaikille koehenkilöille yksilöllisesti lumella hiihdettyjen aikojen ja nopeuksien perusteella. Sykettä ja tekniikanvaihtoja mitattiin läpi koko harjoituksen ja laskimoverinäyte otettiin ennen harjoitusta (PRE) sekä jokaisen intervallin jälkeen. Verinäytteestä analysoitiin BDNF, IGF-1, kortisoli sekä laktaatti.

Tulokset. BDNF oli PRE- näytteessä lumiympäristössä merkittävästi korkeampi (23.2 ± 4,8 ng/ml) kuin normaalilla (N) matolla (18.7 ± 5,0 ng/ml). N- matolla BDNF nousi merkitsevästi välillä PRE– HIGH (19. 8 ± 5.7 ng/ml vs. 23.3 5.8 ng/ml, $p < 0.05$). Kortisoli oli korkeampi lumella PRE- näytteessä verrattuna molempiin mattomittauksiin (N ja V). Lumen ja normaalin maton välillä ero säilyi myös kaikissa intervalleissa (LOW, HIGH, MAX). IGF- nousi lumella merkitsevästi PRE (50.6 ± 12.5 nmol/l) - HIGH (56.4 ± 13.4 nmol/l) välillä ja normaalissa mattoympäristössä PRE (45.8 ± 10.4 nmol/l) – MAX (49.0 ± 10.8 nmol/l) välillä ($p < 0.05$). IGF-1 arvoissa oli merkitsevä ero lumiympäristön ja molempien mattomittausten välillä kaikissa intervalleissa (LOW, HIGH, MAX). Laktaatti oli MAX-intervallissa merkittävästi korkeampi lumella (13.4 ± 1.7 mmol/l) kuin Virtuaali (8.8 ± 3.7 mmol/l) ja normaalissa mattoympäristössä (7.0 ± 2.7 mmol/l, $p < 0.05$). Myös syke oli lumella korkeampi MAX- intervallissa (181 ± 8 bpm) kuin virtuaali (172 ± 10 bpm) ja normaaliympäristössä (175 ± 7 bpm, $p < 0.05$). BDNF:n and IGF-1:n välillä löytyi positiivinen korrelaatio PRE mittauksessa sekä lumella ($r=0.81$, $p<0.05$) että normaalimatolla ($r=0.69$, $p<0.05$). Kortisolin ja BDNF:n välillä puolestaan löytyi lumella PRE-mittauksessa negatiivinen korrelaatio ($r = -0.68$, $p< 0.05$), kun taas BDNF:n ja kortisolin absoluuttisissa muutoksissa löytyi lumella positiivinen korrelaatio PRE- HIGH välillä ($r = 0.74$, $p< 0.05$). Tekniikanvaihtoja tehtiin lumella merkittävästi enemmän (LOW: 25 ± 4, HIGH: 26 ± 4 ja MAX 25 ± 5) kuin virtuaaliympäristössä (LOW: 11 ± 2 ,HIGH: 11 ± 2 ja MAX:11 ± 3, $p< 0.05$) ja normaaliympäristössä (LOW: 9 ± 2, HIGH: 8 ± 1 ja MAX: 8 ± 1, , $p< 0.05$).

Johtopäätökset. Tutkimustulosten mukaan veren BDNF- pitoisuus nousee korkean intensiteetin harjoituksen seurauksena, mutta maksimaalisella intensiteetillä tehty suoritus voi laskea veren BDNF- pitoisuutta. Tutkimuksessa löytyi positiivinen yhteys BDNF:n ja IGF-1:sen välille, kun taas kortisolin ja BDNF:n välille löytyi sekä PRE- mittausten negatiivinen että harjoituksen aikainen positiivinen korrelaatio. Voidaankin todeta, että IGF-1 saattaa vaikuttaa positiivisesti BDNF:n sääntelyyn, kun taas kortisolin rooli saattaa vaihdella kortisolitasojen nousuun johtavasta tilanteesta riippuen. Todennäköistä on myös, että kortisoli-BDNF akselin toiminnassa esiintyy yksilöllisiä eroja. Voidaan myös todeta, että maksimaalinen hiihto lumella saattaa olla fysiologisesti kuormittavampaa kuin rullahiihto matolla.

Avainsanat: Aivoperäinen hermokasvutekijä, kortisoli, IGF-1, maastohiihto, rullahiihto, juoksumatto, virtuaaliympäristö, tekniikanvaihdot

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ABBREVATIONS

ACTH	Adrenocorticotropic hormone
AEA	Anandamide
AMPA	A-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
AMPK	AMP-activated protein kinase
BDNF	Brain derived neurotrophic factor
BBB	Blood-brain barrier
CaMK	Calcium/Calmodulin -activated kinase
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
COR	Cortisol
CREB	cAMP response element-binding protein
EPSP	Excitatory postsynaptic potential
FNDC5	Fibronectin type III domain-containing protein 5
HIIT	High- intensity interval training
HR	Heart Rate
IGF-1	Insuline-like growth factor 1
LA	Lactate
LTP	Long time potentiation
MAPK	Ras/mitogen-activated protein kinase
mRNA	messenger Ribo Nucleic Acid
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NT	Neurotrophin
PCG1-α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
p75	pan-neurotrophin receptor
RPE	Rate of perceived excertion
TrkB	Tyrosine receptor kinase B
VDCC	voltage-sensitive calcium channel
5-HT	Serotonin.

CONTENT

1 INTRODUCTION

Previous research has shown that a strong connection exist between physical exercise and brain health. The crucial mediator of these benefits seems to be a molecule included in the neurotrophin- family; the brain derived neurotrophic factor (BDNF). BDNF is mainly produced in the hippocampus in the brain, and it plays an important role in various aspects of developmental and adult brain plasticity, including proliferation, differentiation, and survival of neurons, neurogenesis, synaptic plasticity, and cognitive function. (Huang et al 2014.) In addition to the beneficial effect of BDNF on brain plasticity and cognitive function, it might also be involved in many central metabolic pathways including glucose and fatty acid metabolism (Pedersen et al 2009).

Exercise is shown to acutely increase the amount of circulating peripheral BDNF in blood (Huang et al 2014). There is some evidence that the amount of the rise of peripheral BDNF levels might be exercise- intensity dependent and recent research indicate that particularly high-intensity interval exercise protocols might be the most effective in increasing blood BDNF-levels (Marquez et al 2015). BDNF also seems to be in strong connection and interplay with other molecules and hormones connected to exercise. These substances include for example IGF-1 (Carro et al 2000), cortisol (Rojas-Vega et al 2006, Heyman et al 2011) and possibly lactate (Schiffer et al 2001). However, there are so far no studies examining the connections of all these exercise affected substances on BNDF during an intense training session in young elite athletes.

The type and skill demands of the performed exercise might also play a role in amounts of BDNF produced some research showing that cognitively more demanding exercise might be the most effective in increasing peripheral BDNF (Oztasyonar 2017). Also multiple other stimuli, including light, osmotic stimulus and electrical stimuli have been shown to alter BDNF-gene expression (Blinder et al 2001). But to date, there are no studies on the effects of an either special visual or virtual exercise environment on BDNF regulation during intense exercise.

Nordic cross country skiing is a popular sport, particularly, in the Nordic countries. Cross country skiing sets high demands both on the cardiovascular and metabolic systems (Rusko 2003). It is also a demanding sport from the technical perspective (Sandbakk et al 2011). During

winter, the practicing of the sport is highly dependent of the weather and snow conditions. During warm winters, practice has to be made either on artificially made snow tracks or indoor tracks. One option is also training on a treadmill with roller-skis. Performing exercise by roller -skiing on a treadmill has some benefits including the ability to control the intensity of the exercise accurately and the stable training conditions. It is also possible, with the developed technique of today, to mimic a real race track surrounding in a treadmill environment by using a virtual screen showing a race- or practice track. This could be a practical tool to use for example for skiers to get familiar with race tracks and practice on them without the need to travel long distances to the actual race tracks.

Usually cross-country skiers perform exercise evaluation tests in a laboratory by running or roller-skiing on a treadmill. But research has shown, that there are differences in cardio-respiratory responses when comparing the performance done by running or roller-skiing. (Vergès et al 2006.) This difference in physiologic responses in different exercise modalities is of great importance for athletes and coaches when planning, for example, the training intensities. However, to date, there is not that much information about the physiologic differences between skiing on snow and roller-skiing on a treadmill.

Therefore, there are two separate study areas and purposes in this study: The first aim is to examine the dynamics and possible relation between BDNF, IGF-1, cortisol and lactate during a high intensity session and compare the effects of different exercise environments on the dynamics of BDNF. The second aim is to compare the differences of skiing on snow and roller skiing on treadmill.

2 BRAIN, NEUROTROPHINS AND EXERCISE

During and after exercise, the secretion of many different substances and hormones in the body is altered. These substances have the power of inducing cellular signal pathways and processes which in turn have multiple effects all around the body. Many of these induced reactions have an effect on the metabolism, but some of them also affect the most complicated organ in the whole body, the brain. A member of the neurotrophin (NT) family, the brain derived neurotrophic factor (BDNF) is one molecule, which have gained recently a lot of attention in the area of exercise physiology. This molecule is known to have a great impact on neuroplasticity, memory and learning, and is of great importance during the prenatal maturation of the brain. (Chao 2003.) The lack of BDNF is also linked to several diseases like Alzheimer's disease, schizophrenia, and diabetes (Egan et al 2003, Tonoli et al 2014). But why exercise physiologists are interested in this molecule, are the findings of the effect of exercise on the levels of BDNF and the involvement of BDNF in several metabolic pathways linked to exercise (Fiuza-Luces et al 2013).

During the past few years, a growing number of research has shown that exercise seem to have an impact on the BDNF- levels (Huang et al 2014). The interesting question which have arised after these findings is that could exercise be a possible treatment of diseases affecting the brain and metabolism? Another interesting, but still an undiscovered area is the use of BDNF as a marker of athletic performance. Indeed, BDNF seem not only to have an effect in the brain, but also in several metabolic processes, which also might have the power to affect the athletic performance. The structure and function of BDNF is discussed more deeply in the following sections as well are two other molecules, insulin like growth factor 1 (IGF-1) and cortisol. Both IGF- and cortisol are speculated to have an impact on the activation and expression of BDNF.

2.1 Neurotrophins

The neurotrophin (NT) family includes five members of different neurotrophins, which are the nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), neurotrophin 6 and brain derived neurotrophic factor (BDNF) (Barbacid 1994). All the neurotrophins have important roles in the developing mammalian brain and nervous system and during

development, all the specific neuronal populations require the presence of some of these factors. The actions of the neurotrophins are mediated by two different receptortypes, a high affinity tyrosine receptor kinase (Trk) and a low-affinity pan-neurotrophin receptor (p75). (Vaynman et al 2003.)

The different neurotrophins are expressed in the different parts of the brain both during development and adulthood. The activity of the neurotrophins is regulated by many different kinds of stimuli including extreme sensitivity to electrical activity. (Poo 2001.) It has been shown that, for example, seizure activity induces a quick increase in NGF and BDNF messenger RNA levels in both hippocampus and the cerebral cortex. In other words, neurotrophins are synthesized and released in an activity-dependent manner. (Chao 2003.) In animal models, it has been shown that blockade of visual input quickly downregulates the synthesis of BDNF mRNA in visual cortex (Poo 2001). Finally, also physical exercise has been shown to cause increases in serum BDNF and the amount of increase might be exercise intensity dependent (Huang et al 2014).

2.2 Brain-derived-neurotrophic factor: Structure and function

BDNF was discovered in 1982 when it was for first time purified from pig brain (Barde et al 1982). The BDNF coding gene is in humans located in chromosome 11p14 (Liu et al 2005) and has four 5′ exons (exons I-IV) with different promoters, and one 3′ exon (exon V) that encodes the mature BDNF protein. The four different promoters allow a multiple regulation of the BDNF expression in different brain areas. (Metsis et al 1993.) BDNF mainly acts by binding on its major receptor tyrosin kinase B, (TrkB), but it can also bind to other receptors like p75, which acts like a co-receptor for the Trk- receptor (Vaynman et al 2003). BDNF is first synthesized as precursor protein called proBDNF that has low ability to activate TrkB. Biologically active BDNF is generated by enzymatic cleavage of proBDNF by plasmin and matrix metalloprotease 9. (Rothman et al 2012.) BDNF is most widely expressed in different brain areas including for example hippocampus and hypothalamus, but also in non-neural tissues like thymus, lungs, kidneys and muscle-tissue (Koppel et al 2009). It has been shown, that BDNF is able to cross the blood-brain-barrier (BBB) and may thus be transported both from the central nervous system (CNS) to the periphery and vice versa (Pan et al 1998).

The transcription of BDNF is driven by activity dependent induction. This means that BDNF coding genes are activated by neuronal activity and multiple different stimuli causing this activity can initiate the transcription of BDNF. (West et al 2001.) Examples of such kind of activities are, for example, sensory input, i.e. light, to the visual cortex (Castrén et al 1992) and visual cortex memory tasks (Tokyama et al 2000). A crucial element in activity dependent induction of BDNF gene transcription in neuronal tissue are calcium- ions, which in response to an action potential and neuronal membrane depolarization enter the cytoplasm. The influx of Ca^{2+} ions drives an activation of many different signaling molecules including the calcium-sensitive adenylate cyclase, calcium/calmodulin-activated kinases (CaMK), and Ras/mitogen-activated protein kinase (MAPK). Calcium can enter the cell via different routes, but the BDNF gene transcription is preferentially driven by calcium influx through voltage-sensitive calcium channels (VSCC). Other types of calcium channels are ligand-gated ion channels of the N-methyl-D-aspartate-type (NMDA) and a -amino-3-hydroxy-5-methyl-4-isoxazolepropionate-type (AMPA) glutamate receptors. (West et al 2001.)

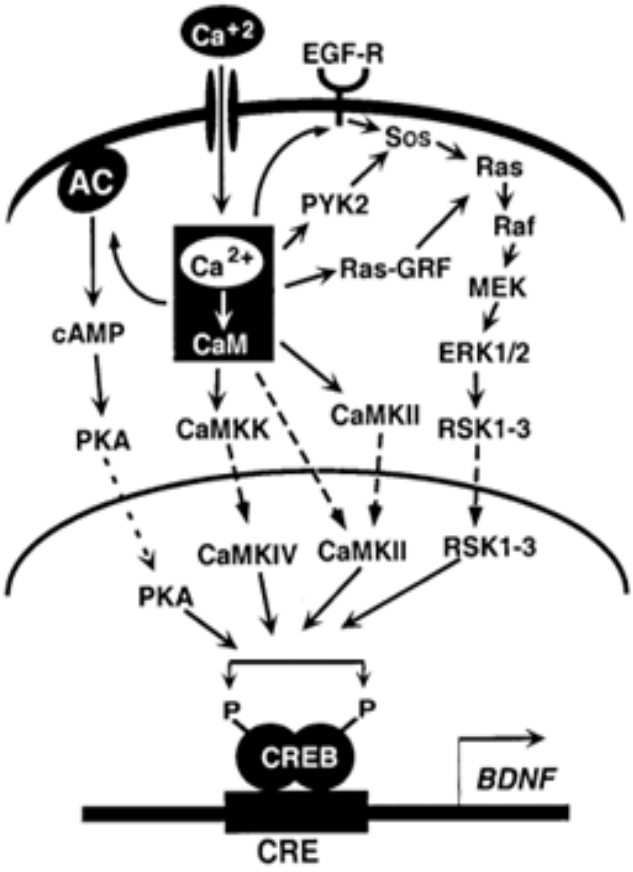

Figure 1. The calcium dependent activation of BDNF transcription. Ca^{2+} ions enters the cytoplasm and activates several kinase cascades. Finally, the phosphorylation of CREB initiates the transcription of BDNF. (West et al 2001)

In the nucleus, the calcium regulated kinase cascades then phosphorylates an important transcription factor for the BDNF synthesis, cAMP response element-binding protein (CREB). The phosphorylation of CREB then initiates the actual synthesis of new BDNF mRNA. (Figure 1, West et al 2001.) The transcribed BDNF protein is then transported in vesicles into the presynaptic terminal and dendrites from which it is released in response to glutamate receptor activation. (Marosi & Mattson 2014.)

The local BDNF production then activates the major receptors of BDNF, TrkB and p75. When TrkB is activated by BDNF, it has the capability of activating

cellular signaling pathways leading to activation of transcription factors. These factors regulate the expression of proteins involved in neuronal survival and plasticity as well several metabolic pathways like energy utilization and food intake which will be discussed later. (West et al 2001.)

2.3 BDNF, learning and memory

In the brain, BDNF has the power to regulate many different factors including neuronal cell survival, axonal and dendritic growth and guidance, synaptic structure and connections, neurotransmitter release, long term potentiation (LTP) and synaptic plasticity (Chao 2003). When thinking of memory and learning, particularly the connection of BDNF and long term potentiation (LTP) is interesting. LTP is strongly linked to long term learning and memory. It is one of the physiological mechanisms that has been explained to cause long term memories and also exercise related motor learning. Long term potentiation happens in certain neurons when they are exposed to continuous electric activity. This continuous activity can increase the excitatory postsynaptic potentials (EPSPs) in postsynaptic neurons manifold and these changes can persist for several hours. (Wackerhage 2014, 266.) The induced long term potentiation in neurons has in turn the power to increase the level of BDNF mRNA (Poo 2001). The increased amount of BDNF mRNA further enhances the formation of LTP and thus strengthens the synaptic currents in the neurons (Chao 2003). It is further shown that lack of the BDNF gene causes abnormal LTPs in mice (Hall et al 2000). Taken together it seems that BDNF has an important role in long term learning and memory functions in the brain.

LTPs has been shown to be induced both in hippocampus and motor-cortex (Wackerhage 2014, 268). Interestingly, since hippocampus is the part of the brain were higher level thinking occurs and motor cortex in turn is strongly linked to motor related skills and motor learning (Guyton & Hall 2000, 669). And in fact, there are speculations that BDNF could be able to facilitate LTP- dependent motor learning (Mang et al 2013). What comes to memory, decreases in BDNF- levels may lead to impairment of both episodic and spatial recognition memory (Egan et al 2003). In addition, lack of BDNF might also affect the behavior. In the study of Lyons et al (1999), BDNF- mutagenic mice with low levels of BDNF showed enhanced aggressiveness and the aggressive behavior is likely due to the dysfunction of serotonergic (5-HT) neurons in the central nervous system. Because BDNF has been shown to have trophic effects on

serotonergic (5-HT) neurons in the central nervous system, the lack of BDNF may thus effect the function of these neurons and the elaboration of behaviors that depend on these nerve cells. (Lyons et al 1999.)

2.4 BDNF and exercise

As discussed earlier, neuronal activity has the power of inducing BDNF mRNA synthesis in the neurons in response to neuronal activity. But it seems that neuronal activity is not the only trigger to elevate the activity of BDNF transcription. Indeed, according to several studies it looks like exercise, especially aerobic exercise, elevates the peripheral BDNF levels in humans. (Knaepen et al 2010, Huang et al 2014.) Elevated BDNF levels are found both after acute exercise sessions and during and after longer training periods. Interestingly, the rise in BDNF levels that is found after aerobic exercise is not seen as widely, if at all, after strength training. (Knaepen et al 2010, Huang 2014.) It seems that only if the strength training protocol is intense enough, there might be an upregulation of BDNF (Knaepen et al 2010).

It also seems to be that the intensity of the aerobic exercise plays a crucial role when thinking about the amount of rise in peripheral BDNF levels. Recent research indicate that high intensity interval (HIIT) training sessions might evoke larger BDNF output compared with continuous lower level intensity aerobic training. (Marquez et al 2015.) In their study, Marquez et al (2015) had a group of healthy men to perform two different intense interval sessions. One session consisted of a HIIT protocol in which the participants performed intervals of 1 min at 90% of maximal work load, alternating with 1 min rest at 60 W for a total duration of 20 min. The other session was a continuous high intensity protocol in which the resistance was set at 70% of maximal work rate and participants cycled continuously at the same intensity for 20 min. They found out that both types of intense training sessions caused an upregulation in BDNF compared with rest-values the HIIT protocol being even somewhat more effective in causing BDNF upregulation. (Marquez et al 2015.)

In response to exercise, the amount of BDNF is elevated both in the brain, periphery and within the muscles, but the origin of the measured elevation of BDNF in blood in response to exercise is not that clear. Rasmussen et al (2009) found evidence in their research with rowers that the brain would contribute to 75-80% of the rise in circulating BDNF and this would mean that

approximately 20-25% of circulating BDNF would stem from a peripheral source. Current research indicate that even if BDNF transcription is elevated in the muscles in response to exercise, it seems that BDNF is not transported to the circulation from the muscles (Matthews et al 2009). Yet, the fact that muscles do not release BDNF into the circulation is not a clear fact and particularly the muscle contractions during heavy exercise needs further research. The possible exercise intensity dependence of the BDNF expression and release in response to exercise is interesting and has evoked discussion among researchers of the mechanisms that would cause the larger BDNF expression during high-intensity exercise session compared with lower intensity sessions (Marquez et al 2015). The mechanisms of upregulation of BDNF in response to exercise will be discussed more deeply in next chapter.

In addition of the intensity of the exercise, also the modality and cognitive demand of the exercise might have an impact on the upregulation rate of BDNF. Oztasyonar (2017) compared the effectiveness of a running training session and combat sport training sessions in evoking BDNF-response. In this study, it showed out that high concentration and attention requiring combat sport sessions led to higher serum BDNF levels after the training sessions compared with an intensity matched running session (Oztasyonar 2017).

In a rewiev of 24 different BDNF and exercise related studies, Knaepen et al (2010) investigated the basal BDNF concentrations in healthy subjects and the levels of peripheral BDNF in athletes. The range in the 24 studies ranged from 1.5ng/mL to 30.9ng/mL showing a great variation on peripheral BDNF levels among healthy non-athlete subjects throughout the 24 studies. (Knaepen et al 2010). In case of athletes, it might happen that highly trained athletes actually have lower basal serum concentrations of BDNF. This has been speculated to indicate a higher BDNF clearance rate in athletes leading to lower levels of peripheral circulating BDNF. (Knaepen et al 2010.)

It seems obvious that exercise has an effect on the peripheral BDNF- levels. But there rises some important questions when talking about the connection of BDNF and exercise. These include for example: What are the mechanisms that cause the upregulation of BDNF by exercise, especially high intensity exercise? And what are the main sources of BDNF in response to exercise? These questions will be discussed in the next section.

2.5 Upregulation mechanisms of BDNF in response to exercise

The mechanisms that cause the upregulation of BDNF in response to exercise and particularly to high intensity exercise are not completely understood, but there exist both human and rodent studies that have given some suggestions of how the upregulation of BDNF gene transcription in response to exercise works. In the study of Vaynman et al (2003), it is shown that voluntary wheel running exercise in rats not only causes elevation of BDNF levels in the hippocampus, but also the means through which BDNF can exert its actions on brain health and neural plasticity. This means that there appears to happen an increase also in the main receptor of BDNF, TrkB, in response to exercise. In addition, exercise also increased the expression of other molecules associated with BDNF transcription: Calcium and cAMP response-element-binding (CREB) protein and synapsin I. CREB and synapsin I in turn are able to modify neuronal function by regulating gene-transcription and affecting synaptic transmission and therefore are important to brain function, learning and memory. (Vaynman et al 2003.)

A more recent finding of Wrann et al (2013) demonstrates a connection of exercise, BDNF, PCG1-alpha and FNDC5, a myokine secreted from the muscles. Interestingly, FNDC5 is also expressed in the brain and is known as irisin in its secreted form. Irisin in turn is found to be an upregulator of BDNF gene transcription in the brain (Wrann et al 2013). In their experiments with rats Wrann et al (2013) demonstrated that 30 days of voluntary wheel running caused an activation of a PCG1-alpha/FNDC5/BDNF pathway in rats, which lead to increased amount of BDNF in the hippocampus. (Wrann et al 2013.) There is also some evidence that exercise would elevate the activity of tissue type plasminogen activator (tPA), an enzyme that converts plasminogen to plasmin in the hippocampus. Plasmin in turn cleaves the precursor protein proBDNF to mature BDNF and thus the upregulation of plasmin would also enhance the upregulation of active BDNF. (Rothman et al 2012.) Also the upregulation of serotonin (5-HT) in response to exercise has been speculated to be a possible signal and upregulator of BDNF during exercise. It has been reported, that exercise training increases 5-HT turnover in the cerebral cortex and that 5-HT receptor antagonists can modify the ability of exercise to upregulate BDNF expression. (Marosi & Mattson 2014.) The complicated and multiple

pathways possibly causing upregulation or downregulation of BDNF are demonstrated in Figure 2.

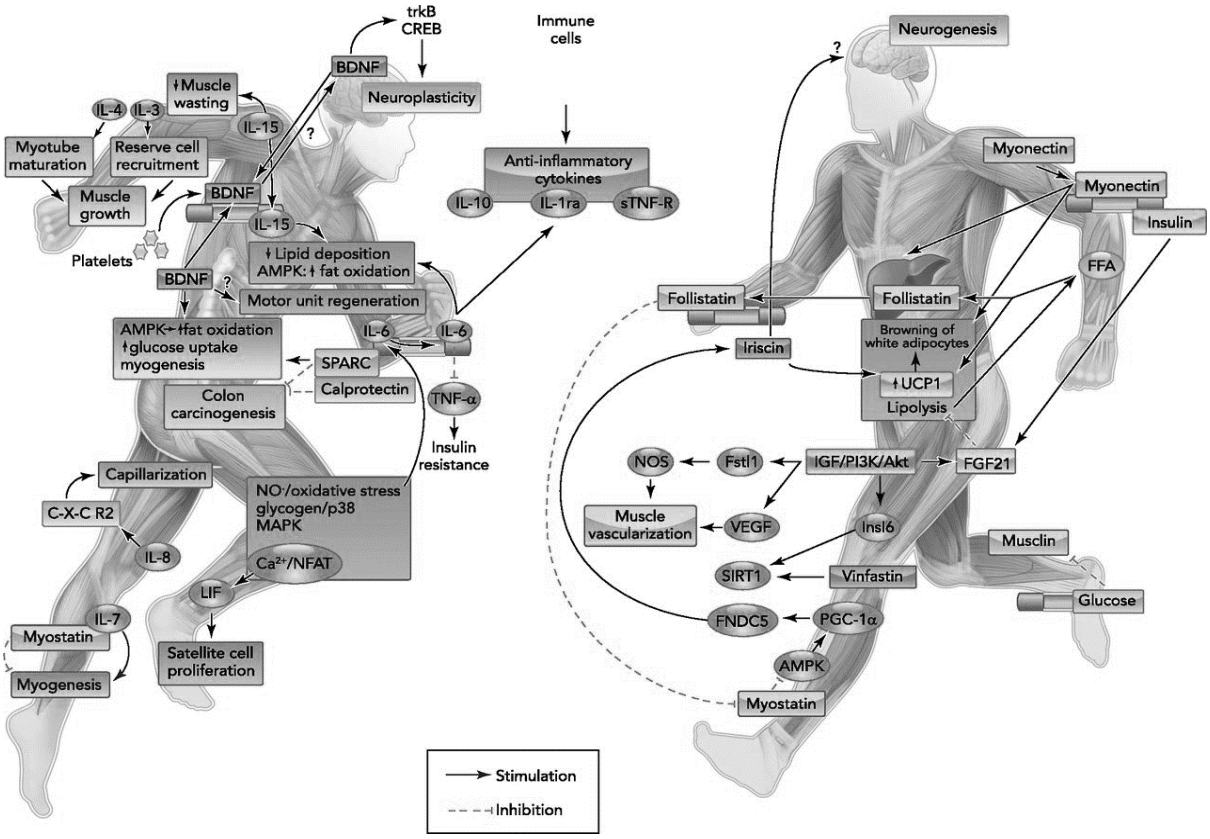

Figure 2. The complicated cell signaling routes in response to exercise and how BDNF affects the body and is affected by other substances. In the picture it is shown, AMPK-PCG-1 signaling cascades are activated in response to exercise, and these cascades are connected to BDNF-upregulation. (Figure source: Fiuza-Luces et al 2013)

Another system that might play role in the upregulation of BDNF in response to exercise is the endocannabinoid system. The human brain is capable of producing various endogenous substances with opiate like behavior and one of the substances in the endocannabinoid family, anandamide (AEA), might be able to upregulate the synthesis of BDNF in response to exercise. (McArdle 2015, 448-450.) In a study with cyclists, Heyman et al (2011) showed that intense cycling (60 min at 55% followed by 30 min at 75% W (max)) increased the plasma levels of AEA in the blood. They also found out that in the end of the exercise bout, AEA and BDNF concentrations were positively correlated ($r>0.66$, $P<0.05$). This finding suggest that AEA upregulation during exercise might be one of the factors involved in exercise-induced increase in peripheral BDNF levels. (Heyman et al 2011). The researchers also suggested that AEA production during exercise might be triggered by cortisol upregulation since they also find

correlations between AEA and cortisol and because corticosteroids are known to stimulate endocannabinoid biosynthesis. (Heyman et al 2011).

When talking about high intensity exercise, another molecule possibly playing a role in upregulating BDNF transcription is lactate. A couple of studies have investigated the connection between blood lactate and BDNF, and some of them have found evidence that blood lactate would be involved in the upregulation of BDNF (Schiffer et al 2011). In their study with male athletes, Schiffer et al (2011) saw an increase in both peripheral LA and BDNF levels after intravenous sodiumlactate infusions in rest. However, there also exist contradictory results what comes to the relationship of lactate and BDNF (Schiffer et al 2011). Rojas-Vega et al (2006) showed in their study with male athletes that in an incremental ramp test to exhaustion lactate and BDNF did not show any correlation (Rojas-Vega et al 2006). Thus, the role of lactate in BDNF regulation during intense exercise seems to need further research.

2.6 BDNF and metabolic pathways

It has been shown that BDNF gene transcription is activated both by neuronal activity in the brain and also by physical exercise. It is well known that brain, especially hippocampus, is a source of BDNF and BDNF is from the brain released to the bloodstream and periphery of the body (Russo-Neustad et al 2000). In the brain, BDNF plays important roles what comes to enhancement of neuronal survival, brain plasticity, learning and memory (Chao 2003). But recent research has also come up with evidence that another source of BDNF, highly linked to exercise, would be the muscles. (Matthews et al 2009.) It is further shown that besides brain health and neural function, BDNF would also be involved in several metabolic pathways and health benefits linked to exercise (Pedersen et al 2009).

Matthews et al (2009) were the first to demonstrate in their experiments with rats and cell cultures that BDNF indeed is produced also by skeletal muscle cells in response to exercise. They suggested that BDNF would be produced within the muscle by muscle cells, but it would not be secreted outside the muscle to the periphery. Rather, the muscular BDNF would act locally and contribute to the multiple health benefits associated with exercise, of which one would be the enhancement of fat oxidation in an AMP-activated protein kinase (AMPK) dependent fashion. (Matthews et al 2009.)

The AMPK regulates both long term and short term adaptations to exercise and metabolic activity when activated in the muscle by exercise. It increases, for example, the mitochondrial biogenesis by increasing the expression of PCG-1α and enhances expression of GLUT4. (Mooren & Völker 2005, 274.) The AMPK-PCG-1 activation in response to exercise is also one mechanism that might cause BDNF upregulation by promoting the PCG1-alpha FNDC5-axis as mentioned earlier (Wrann et al 2013). The AMPK activation also leads to increased carbohydrate catabolism by AMPK promoting GLUT4- mediated glucose uptake into skeletal muscle and at the same time inhibiting glycogen synthesis. Finally, AMPK also promotes fatty acid uptake into skeletal muscle and mitochondria leading to upregulation of fatty acid oxidation. The last long term effect of AMPK is the formation of new capillaries termed angiogenesis. (Wackerhage 2014, 99-100.)

In addition to the possible enhancement and activation of AMPK and PCG1-alpha pathways in response to exercise- induced transcription of BDNF, there exist evidence that BDNF also might be involved in the control of body weight, food intake and energy homeostasis (Xu et al 2003). In rats, deficit of BDNF and its main receptor TrkB in ventromedial hypothalamus (VMH) caused obesity and hyperphagia and these states were reversed by infusion of BDNF to the brain. In the same experiment, it was further shown that melanocortin receptor MC4R-mediated signaling stimulates expression of BDNF in the VMH and the results suggest that BDNF is one of the molecules that mediate the function of the hypothalamus in the control of energy balance. (Xu et al 2003.) In humans it is further shown that mutations in the BDNF and TrkB- genes can cause obesity and certain kinds of eating disorders (Lebrun et al 2006). Caloric restriction and alternating day fasting has also seen to impact the BDNF levels positively leading to better protection of neurons against dysfunction and neurological disorders (Rothman et al 2012). In animal models it is further shown that BDNF is able to enhance the effect of insulin in diabetic mice and this way lower the blood glucose concentration (Nakagawa et al 2002).

Taken together, there seem to exist several different signal cascades that may mediate the exercise driven upregulation of BDNF transcription. The control of energy homeostasis and energy intake also seem to be closely connected with BDNF levels. If there is one dominating signal pathway or do the pathways co-operate together what comes to BDNF, exercise and energy balance still remains unknown. There also seem to be different possible sources to the exercise dependent circulating BDNF brain and the muscle possibly acting as the main sources.

3 THE INTERACTION BETWEEN BDNF, IGF-1 AND CORTISOL

The amount of many different molecules changes markedly during and in response to exercise. The changes are strongly linked to the type and intensity of exercise performed, and these changes further emphasizes both short- and long term adaptations to exercise. In the previous section, the signaling cascades triggered by exercise and capable to also activate BDNF transcription were discussed. In the next section, some common molecules linked to exercise and BDNF transcription activation will be taken into discussion. Some of the numerous molecules linked to BDNF and exercise are IGF-1 and cortisol, which seem to have the power to influence the expression and activity of BDNF. The interaction of BDNF, IGF-1 and cortisol will be discussed in the next section.

3.1 Cortisol

Cortisol, also known as hydrocortisone, is an important glucocorticoid secreted from the adrenal cortex (Guyton & Hall 2000, 921). It has several important tasks in the metabolic activities of the body: The release of cortisol from the adrenal cortex stimulates gluconeogenesis (formation of carbohydrate from proteins and other substances), reduces the protein stores in essentially all body cells except those in liver and promotes fatty acid mobilization from the adipose tissue. (Guyton & Hall 2000, 928-929).

Besides the effects of cortisol to many metabolic-linked activities in the body, it also plays an important role in resisting stress and inflammation. Almost any type of stress causes a marked increase in cortisol secretion of the adrenal cortex. A stress- situation first stimulates the secretion of ACTH from the anterior pituitary gland, and the increased amount of ACTH then stimulates the secretion of cortisol. (McArdle 2015, 424.)

What comes to exercise and training, prolonged and excessive endurance training continued for a longer time may lead to higher cortisol release in response to corticotropin-releasing hormone (CRH) activity. This may be due to a decrease in corticotropic sensitivity to the negative feedback signal leading to a higher release of cortisol. (Heuser et al 1991.) It is further shown that also acute exercise, particularly intensive training sessions leading to accumulation of

lactate cause an increase in plasma cortisol. Similar effects on cortisol are not found after acute lower intensity short duration exercises. (Rojas-Vega et al 2006.)

3.2 Insulin like growth factor 1 (IGF-1)

Insulin like growth factors (also known as somatomedins) are small proteins, which have the power to promote growth in many different tissues. These proteins are called insulin like growth factors, because their effect on growth are similar as is the effect of insulin. (Guyton & Hall 2010, 901.) At least four insulin like growth factors have been isolated, but by far the most important of these is somatomedin C (also known as insulin-like growth factor-1, or IGF-I). The concentration of IGF-1 in the plasma closely follows the rate of growth hormone (GH) secretion and thus these two growth promoting factors have a close connection with each other. (Guyton & Hall 2000, 901.) In fact, it has been postulated that most, if not all, of the growth effects of growth hormone result from IGF-1 and other insulin like growth factors, rather than from direct effects of growth hormone on the bones and other peripheral tissues. (Guyton & Hall 2000, 901.) IGF-1 is secreted from the liver in the response to growth hormone, but it can also be stored and released independently of GH from other tissues including muscle and bone (Copeland & Heggie 2008).

When released to the bloodstream, IGF-1 attaches strongly to a carrier protein in the blood. As a result of this strong attachment, IGF-1 is released slowly from the blood to the tissues unlike the actual growth hormone, which in turn is released from the blood to the tissues rapidly. The slow release of IGF-1 to the tissues greatly prolongs the growth-promoting effects of the bursts of growth hormone secretion. (Guyton & Hall 2010, 902.) The majority of IGF-I in circulation is bound in a binding protein (IGFBP-3) and an acid-labile subunit. IGF-I bound to IGFBP-3 is considered a functional pool and become activated of physiological stress like exercise (Copeland & Heggie 2008).

Both acute exercise (Schwarz et al 1996, Copeland & Heggie 2008) and longer training periods (Jeon & Ha 2015) have shown to increase the amount of IGF-1 in blood. It seems to be that both moderate and intense interval exercise causes upregulation of circulating IGF-1 levels, but particularly intense exercise seem to cause upregulation in the IGFBP-3. (Schwarz et al 1996, Copeland & Heggie 2008). In their study, Copeland and Heggie (2008) found an increase both

in IGF-1 and IFGBP-3 after both continuous 20min aerobic exercise on 60-65% of VO2max and a 20min interval training session consisting of intervals of 60 seconds on 80-85% of VO2max alternating with 40 second active rest. For longer training periods, Jeon & Ha (2015) investigated the effect of an 8 week training period on IGF-1 levels for a group of young male students. The training group trained for 3 times/week and after the training period there was found a significant rise in their IGF-1- levels when compared to the control group who did not do exercise. Circulating IGF-1 levels have also been said to positively correlate with fitness level and muscle strength, and they might thus play a role in stimulating training adaptations. (Copeland & Heggie 2008.)

3.3 The interaction between BDNF, IGF-1 and cortisol in response to exercise

In rodent models, it has been shown that exercise induced rise in peripheral IGF-1 levels might be able to stimulate BDNF expression in the brain (Carro et al 2000). Carro et al (2000) showed in their experiment with mice, that circulating IGF-1 can travel from the periphery to the brain, and there stimulate the neuronal expression of BDNF and thus facilitate long-lasting changes in neuronal activity. This means that among other regulators also IGF-1 might possibly be a mediator of exercise induced brain plasticity and an upstream mediator of BDNF gene regulation. In exercise related studies, Jeon & Ha (2015) showed that a longer exercise period leads to upregulation of basal levels of both BDNF and IGF-1 in young men.

When it comes to the interaction of BDNF and cortisol in response to exercise, the research findings are slightly conflicting. Early animal studies evaluating the connection of glucocorticoids and neural function have demonstrated that stress causing elevation in cortisol levels might reduce the BDNF mRNA levels in dentate gyrus and hippocampus (Smith et al 1995). In exercise related studies with human subjects, this link of cortisol suppressing the BDNF upregulation during exercise has in contrast not been demonstrated. Rojas-Vega (2006) found in their study with eight young recreational male athletes both an acute elevation in BDNF levels and a more prolonged elevation in cortisol levels after an incremental ramp test to exhaustion and in their study they did not find any correlation between BDNF and cortisol. The upregulation of cortisol seems thus not acutely affect negatively the BDNF response in response to intense exercise. (Rojas-Vega et al 2006.)

For acute exercise-induced cortisol responses, there are also some evidence that cortisol might actually be an upregulator of BDNF during intense exercise and the linkage between cortisol and upregulation of BDNF is speculated to endocannabinoid mediated. (Heyman et al 2011) In their study with eleven well trained young male cyclists, Heyman et al 2011 found a significant upregulation of BDNF, the endocannabinoid anandamide (AEA) and cortisol in response to a 90min high intense cycling session. The session consisted of 60min cycling at 55% Wmax and and 30min cycling at 75% Wmax. The researchers found a correlation between BDNF and the endocannabinoid molecule anandamide (AEA) suggesting that AEA would be an upregulator of exercise induced upregulation of BDNF. Since also a positive correlation with plasma AEA and cortisol was found in the present study, the researchers speculated that cortisol would be a trigger for the upregulation of AEA, which in turn would upregulate the synthesis of BDNF in the brain. (Heyman et al 2011.)

When talking only about IGF1 and cortisol, one interesting research finding can be discussed what comes to the training status of an athlete. There exist evidence that the IGF-1/cortisol ratio could be a marker of training status or recovery state (Nassib et al 2016). In young boxers, five weeks of intensive training decreased markedly the IGF-1/cortisol ratio and this change was due to the increase in cortisol. After one week of tapering, the ratio increased due to the increase in IGF-1. (Nassib et al 2016.) Thus, measurement of the IGF1/cortisol ratio might be used as a tool when monitoring the training status and need for recovery for an athlete.

In summary, there seem to be numerous factors that induce the transcription of the BDNF gene. These factors include among others neuronal activity and exercise, particularly high-intensity exercise. BDNF can in turn contribute to many different aspects and mechanisms in the body including brain plasticity, learning and memory, behavior as well many metabolic pathways, control of body weight and energy intake. Taken all these actions into account, it has to be admitted, that BDNF is a really powerful molecule and insufficient amounts of it might cause serious healthproblems and diseases. Because of the wide actions of BDNF around the whole body there has even been some speculations among the scientist of the link of athletic performance and BDNF as a future doping molecule. (Ulucan 2016). In Figure 3, a summary of different habits and environmental issues and their effects on BDNF are listed.

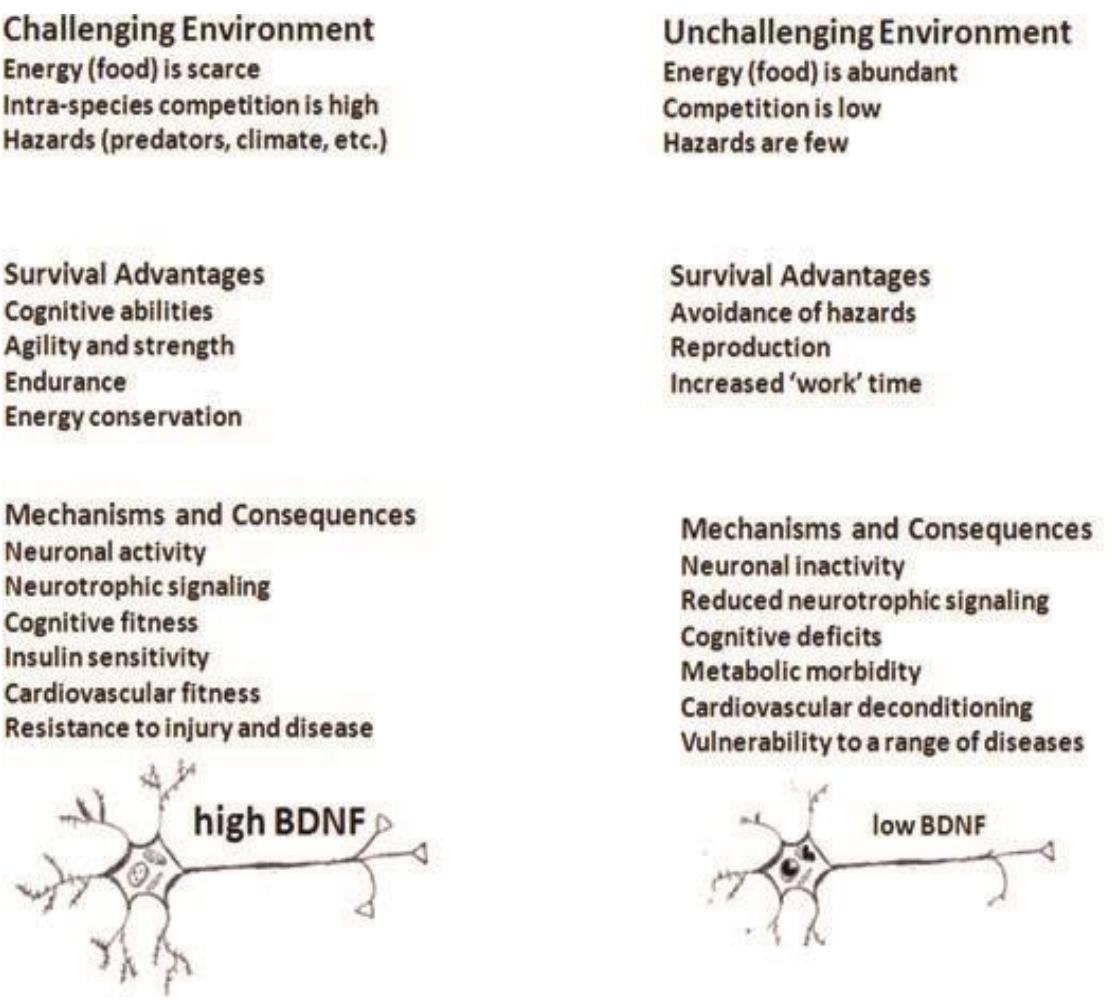

Figure 3: How the environment and living habits may impact the levels of BDNF. In the column to the left, it is listed beneficial surroundings and habits for supporting brain health and BDNF upregulation and the positive effects of these on health. To the right, it is shown in turn the unbeneficial environments and habits for brain health and amounts of BDNF. (Figure: Rothman et al 2012).

4 CROSS COUNTRY SKIING

Cross-country skiing is a demanding sport, which on a competitive level requires huge amounts of endurance training on different intensities. Among elite cross country skiers, the largest amount of training is usually performed in the low intensity training zone 1 (approximately 75% of total training), whereas 15-20% of the total training amount consist of very high intensity training (zone 3). The boundaries of the intensity zones are set to the ventilatory thresholds 1 (VT1) and 2 (VT2) meaning, that 75% of the training occurs under VT1 and 15-20% over the VT2. The zone between the VT1 and VT2 is termed the lactate accommodation zone. This type of training distribution is known as polarized training model. (Seiler & Kjerland 2006.) In addition to the large training amounts, cross-country skiing is also technically a very demanding sport requiring adaptation of skills to both different techniques, terrains and speeds (Sandbakk et al 2011). Thus, the both metabolically and technically demanding nature of the sport makes it very interesting when thinking about BDNF.

4.1 Physiology of cross-country skiing

Cross-country skiing is an endurance sport with high demands on the aerobic energy capacity. It also requires a lot from the whole muscle-skeletal and cardiovascular systems. One of the most important single physiological determinant of cross-country skiing performance is considered to be maximal oxygen uptake (VO_2max). The maximal oxygen uptake depends on the ability of the blood to bind and transport oxygen, the ability of the heart to pump blood (maximal cardiac output) and finally the muscles to utilize oxygen to energy production. (Rusko 2003, 1-3) In addition to the high physiologic demands, the varying terrains and speeds also makes cross-country skiing a technically demanding sport (Sandbakk et al 2011).

Due to the demanding nature of the sport, cross-country skiing also requires a lot of energy. This energy is obtained from the food (carbohydrates, fats and proteins) and stored in the body as ATP, glycogen and fat. The body uses energy in the form of ATP and because the ATP storages of the cells are really small the body has to synthesize more ATP from its energy storages. The body is able to synthesize more ATP from muscle phosphocreatine (PCr), by glycolytic breakdown of glycogen and oxidative breakdown of carbohydrates, proteins and fats.

The main energy source and way to synthesize ATP chosen by the body depends highly on the intensity and duration of the exercise and the body can synthesize energy both aerobically and anaerobically. (McArdle 2015, 162 – 168).

4.2 Sprint skiing

During a cross-country ski race or intense training, the source and type of energy production can vary a lot depending, for example, on the distance and duration of the race or exercise and also by the terrain. In short (1km/2min) and intense sprint races or trainings, the contribution of aerobic vs. anaerobic energy production is about 50/50 and the longer the duration of the race or exercise, the higher is also the fraction of aerobic energy production. (Rusko 2003, 5) A normal sprint race event involves four high-intensity heats typically lasting around 3min/heat. The heats are separated by relatively short resting period. (Hébert - Losier 2016) It has been considered that during sprint races the maximal aerobic capacity is often exceeded and the total work rate in sprint skiing on uphill terrain may be as high as 160% of peak aerobic power (Sandbakk et al 2011) Skiing on such high intensities leads to accumulation of lactate, which is a byproduct of the anaerobic energy production pathway. The lactate is transported by blood to the less active muscles and heart to be used in the oxidative energy production or to liver to be further synthesized again to glucose. (McArdle 2015, 162- 168).

4.3 Cross-country ski skating techniques

Cross country ski skating is traditionally divided into four different techniques. These techniques have somewhat different names depending on country and part of the world, but in most widely coaches and researchers talk either of gears, of “V’s” or simply of the common English explaining names of the techniques. The term “V” comes from the position of the skis, which is a “V” during the skiing movement. Some researcher call the different styles as “Gears” or call the techniques simply how they look like. (Millet et al 2003).

In “V”- terms, the four most commonly known skating techniques include V1, V2, V2 Alternate, and free skating without poles. The V1 technique, which also is known as gear 2 or “Offset”, is generally used in upphills and consists of an asymmetrical and asynchronous pole plant combined with a skating stroke on one side but not on the other side. The V2 technique,

or gear 3/One skate, in turn is used in faster conditions, flat terrain and gentle sloping or short upphills. The V2 technique is symmetrical with a pole plant on each leg stroke. The V2-Alternating, or gear 4/two skate, again is a very fast technique in gentle sloping downhills or really fast conditions on flat terrain. It consists of a symmetrical pole stroke on every other leg skating stroke. The last technique most commonly used is known as "No pole, free skate or gear 5". This technique, like its name indicates, consists only of leg strokes and the arms and poles are pendling in the air to gain more speed to the movement. The division and illustrations of the four most common ski skating techniques are listed in Figure 4. It has been shown that the different techniques have different cost of energy and the appropriate selection of the technique impacts the energy cost of the skiing depending on the conditions and slopes. (Millet et al 2003, Kvamme et al 2005, Sandbakk et al 2015).

Figure 4. The four most common ski skating techniques, how they are called and how they look like when illustrated. To the column in left, the numbers indicate the number of the gear.Source: (http://crosscountryskitechnique.com/name-skate-skiing-technique/, McKenney, 2017)

4.4 Comparisons of skiing on snow and roller skiing with different training modalities

Quite a lot research has been made comparing the physiologic responses of skiing or roller skiing to responses of other training modalities. These comparisons include for example running (Vergés et al 2006, Larson 2006) and alpine skiing (Stöggl et al 2016). According to previous research, there seem to exist a difference in the physiologic responses between the different training modalities when compared to skiing or roller skiing. (Stöggl et al 2016, Larson 2006, Verges 2006). Also differences in physiologic responses are found between different skiing techniques. (Sandbakk et al 2015).

Roller skiing and running are some of the most popular off season training modalities among cross-country skiers and they are also widely used in exercise testing. (Larson 2006). But according to previous research findings, it seems that testing done by running does not give accurate training threshold values which could be used to monitor ski- or roller-ski training sessions and the training intensities (Vérges et al 2006). Roller skiing has been considered a more sport specific training and testing modality for cross country skiers compared to for example running, but still there is a lack of specific comparisons of physiological responses between skiing and roller skiing. (Larson 2006). Sandbakk et al (2011) did make a comparison of one uphill section of a sprint race both on snow and treadmill skiing and found a strong positive correlation both in performance and kinetics (Sandbakk et al 2011).

There also exist some data and comparison on the kinesiology between skiing on snow and roller skiing on asphalt. In a case study with one elite skier, Suchý & Kračmar (2008) showed a kinesiological correspondence and well-matching activation of major muscle groups when comparing V2 technique skiing on snow and roller skiing on asphalt. A minor difference in the activation pattern and timing of activation of m. gluteus medius was found when comparing the whole cycle of skiing locomotion on asphalt and snow (Suchý & Kračmar 2008).

Similar results are found also in very recent research made with sit-skiers. In their study, Rosso et al (2016) compared natural sit - skiing on snow and sit skiing on ergometer. The researchers compared speeds, pole forces and EMG for triceps, pectoralis, erector spinae and rectus abdominis between natural sit- skiing and simulated sit- skiing on ergometer. The results of this study revealed that natural sit skiing and simulated sit skiing are very similar what comes to

force production and muscle activation. (Rosso et al 2016.) But to date, there does not exist any actual comparison of the possible physiological differences between skiing on snow and roller skiing on treadmill in an alternating terrain.

5 RESEARCH QUESTIONS AND HYPOTHESES

In earlier studies it is clearly proved that BNDF is a very powerful molecule affecting many things and pathways in the human body. Therefore, it is of great importance to gain more understanding of the pathways and metabolites affecting this little powerful molecule, as well as the effects of certain intensities of exercise to the dynamics of the substance. It is also important to examine the possibility of enhancing the expression of BDNF with the exercise environment. What comes to cross country skiing, it is important to both coaches and athletes to gain knowledge of how well the commonly used training form roller skiing physiologically matches skiing on snow.

The aims of the current study can be divided into two parts: The part related to the physiologic blood markers examined (BDNF, IGF-1, cortisol and LA) and the skiing related part comparing skiing on snow and roller skiing on treadmill. The research question are following:

Question 1: How do the serum concentration of BDNF, IGF-1 and cortisol alter and respond during a high intensity training session?

Hypothesis 1: *BDNF upregulation has been seen in response to high intensity exercise (Marquez et al 2015) as well as IGF-1 ((Schwarz et al 1996) and cortisol upregulation (Rojas-Vega et al 2006). Thus, it can be expected that all the substances are upregulated in response to high intensity exercise.*

Question 2: Are there any linkages or correlations between BDNF and IGF-1, cortisol or lactate during a high intensity training session?

Hypothesis 2: There are some previous research showing, that IGF-1 can possibly be an upregulator of BDNF (Carro et al 2000). When it comes to cortisol, there exist research showing cortisol to have a negative impact on BDNF (Smith et al 1995) and no effect of cortisol on BDNF levels (Rojas-Vega et al 2006). The same is true for lactate; lactate has been shown to be both an upregulator of BDNF (Schiffer et al 2011) and to have no correlation with exercise induced BDNF upregulation (Rojas-Vega et al 2006). Thus, it can be expected IGF-1 and lactate to be upregulators of BDNF and cortisol to be a downregulator of BDNF.

Question 3: Are there any differences in BDNF regulation in different exercise environments?

Hypothesis 3: In previous research it is shown that more cognitively demanding tasks (Oztasyonar 2017), visual imput (Castrén et al 1996) and memory tasks in a virtual environment (Tokyama et al 2000) would enhance the expression of BDNF in the brain. Thus, it can be expected that the virtual environment could cause a higher upregulation of BDNF in the brain compared with a normal treadmill environment.

Question 4: Are there any physiological differences between skiing snow and roller skiing on treadmill in the same terrain and with the same intensity?

Hypothesis 4: *There is no previous research comparing the physiologic differences between skiing on snow and roller skiing on treadmill. However, roller skiing is shown to be quite similar to skiing on snow in the perspective of kinesiology (Suchý & Kračmar 2008) and thus also the physiological responses can be expected to be similar.*

6 METHODS

6.1 Subjects

Nine high level young male skiers with a several year background of competitive cross-country skiing acted as subjects in the research. Prior to testing each subject provided his written consent to participate in the study. The age of the subjects was 18.8 ±1.5 years, the weight 72.4 ±4.9 kg and height 178.6 ±5.4 cm.

6.2 Research set-up

The measurements took place in April 2016 and May 2016. Prior to the measurements, the test protocol was approved by the ethical committee of the University of Jyväskylä. All the subjects performed altogether three high intensity interval training sessions, one skiing session on snow in April and two roller skiing sessions on treadmill in May. The skiing style was skate in all the sessions. The subjects were asked to make similar preparations (training, food and fluid intake) before all three sessions and the subjects were also asked to avoid vigorous training the day before the measurements. Each subject was scheduled to make all the three different training session at the same point of day to avoid diurnal variations of the blood markers.

The snow session. The snow session consisted of three intervals on the 1.2 km long sprint track in Vuokatti, Finland. Before the session as a warm up and familiarization, all the subjects skied through the whole track together with the leader of the research project with a slow pace. The first actual interval was performed on the intensity of approximately 70% of maximum HR (LOW), the second interval approximately 85-90% of maximum HR (HIGH) and the last interval was an all-out effort interval (MAX). The subjects controlled the intensity by themselves. Rest between the intervals was 3 minutes and the intervals lasted between 3.5 - 4.5 minutes each. Venous blood samples were collected to measure levels of BDNF, IGF-1 and Cortisol before the session after the warm up lap (PRE) and immediately after the first (LOW), second (HIGH) and third (MAX) interval. Lactate blood sample was collected from the fingertip at the same time points. The research set up is illustrated in figure 5. In the snow session, the subjects used their own skiing equipment and had also waxed their skis by themselves. The weather was humid and quite warm with an outside temperature of 0 to 3

degrees Celcius during the sessions. The heart rate, speed and GPS data were measured throughout the whole session using Polar heart rate sensor (Polar V800, Polar Electro, Kempele, Finland).

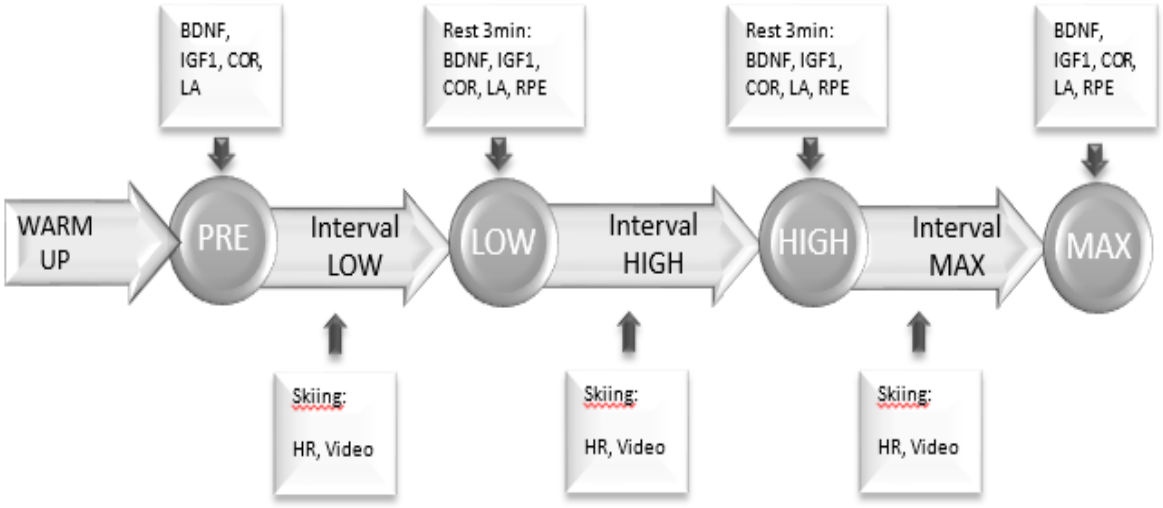

Figure 5: The research set-up. The arrows indicates the warm up and intervals, and the circles the resting periods. In the boxes are shown the measurements and recordings done either during rest or during the intervals.

Sector Analysis. After the interval session performed on the snow track, a sector analysis from each interval was made for each subject individually and this analysis gave the individual speeds for every subject prior to the treadmill interval session. In the sector analysis, the sprint track was divided into 11 sectors and for each sector an individual mean speed was calculated based on the speeds the subjects had been skiing on snow. Each of these 11 sectors had a constant incline in the treadmill sessions (Figure 6). In addition to the 11 actual sectors, 2 transition "safety sectors" with same speed for all subjects were added to the analysis. These sectors were added in the transition phases (uphill-downhill) to avoid dangerous situations during large changes in the speed between sectors. The sector analysis was made on the basis of the time, speed, GPS and altitude data collected by the heart rate monitor (Polar V800, Polar Electro Oy, Kempele, Finland) and the analyze was made from second by second-collected data exported from polar training software (Polar Flow, Polar Electro Oy, Kempele, Finland) to excel. The track profile and sector division of the snow track, as well as the inclines of the sectors on treadmill are illustrated in Figure 6.

Sector	Angle (°)	Distance (m)
1	2.8	0
2	0.0	153
3	4.4	334
4	9.5	413
5	- 3.9 (0)	435
6	0.0	558
7	4.4	833
8	0.0	958
9	4.4	1008
10	-5.9 (0)	1069
11	0.5	1223

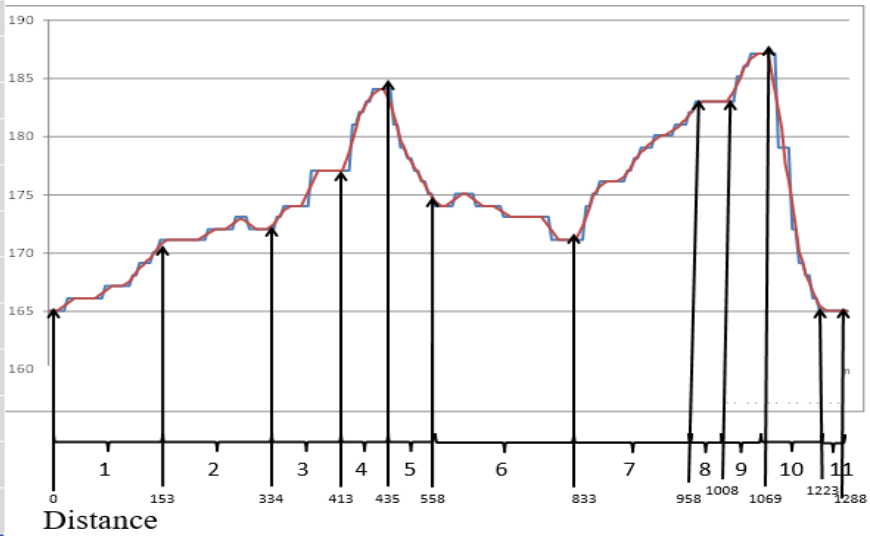

Figure 6. The track altitude profile, sector division and inclines of the treadmill on the treadmill session. The altitude curve and sector division of the on snow track is illustrated on right as well as the total distance of the on snow track. The inclines of each sector on treadmill can be seen on the left side of the altitude curve.

The treadmill sessions. The treadmill sessions were performed approximately four weeks after the snow sessions. Each subjects skied two sessions on treadmill, one in a virtual environment and one without the virtual environment in a normal treadmill surrounding. In the both treadmill sessions, the aim was to mimic the performances of the subjects on the snow sessions as closely as possible. In both of the treadmill sessions, the snow-track profile was mimicked by adjusting the treadmill to automatically follow the altitude changes of the real track and the speed of the treadmill was adjusted individually for each subject based on the sector analysis. What comes to blood sample collection and rest, the set up was identical to snow (Figure 5). Minor adjustments to the interval speeds though had to be made because of safety reasons on the top of the hills and in the beginning of the downhills. The downhills on the treadmill were passive the subjects holding on a rack in front of the treadmill and the treadmill having a 0- incline (Figure 7). All the subjects used the same pair of roller skis with standard wheels (Marwe 800

XC, wheel nr. 6, Polymer Components Finland Oy, Hyvinkää, Finland).

Figure 7. Downhill skiing position in the virtual treadmill session. The downhills were passive the subjects holding on a rack in front of the treadmill.

The Virtual Environment. All the subjects skied one roller skiing session on treadmill in a virtual environment (V). (Figure 8) This meant that the subjects had the track environment on a screen in front of them during the whole session. The environment was designed by Athene exergaming (Athene exergaming, CSE Entertainment, Kajaani, Finland).

Figure 8. The virtual treadmill environment.

The Normal Treadmill environment. The normal virtual environment (NTE) measurements where otherwise identical with the virtual environment measurements, but instead of the virtual track scenery the subjects saw the altitude profile, the speed and incline changes as a graph in front of them on a screen (Figure 9).

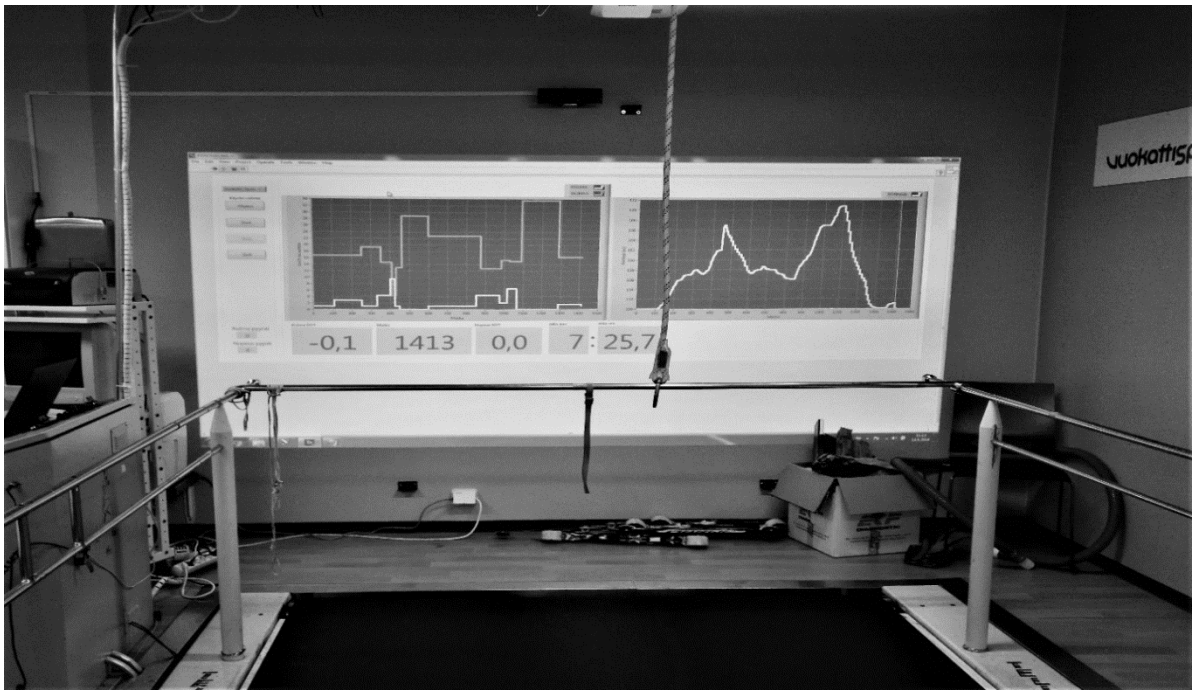

Figure 9. The normal treadmill environment. The speed and incline of the treadmill are shown on the screen to the left and the altitude curve of the snow track on the right in the picture.

Skiing technique changes. In the snow session, technique changes were analyzed from a portable video camera (GoPro, Calfornia, USA) which was placed in a belt on the lower back of the subjects. The camera was set to record the rear end of the skis and the techniques were determined by the changes of the locomotion of the skis. If one technique lasted more than 2 cycles, it was interpreted as a technique change. In the treadmill sessions, the camera was placed behind the treadmill, and the technique changes were analyzed in a similar manner. A picture from the view from the camera recording the technique changes is shown in Figure 10.

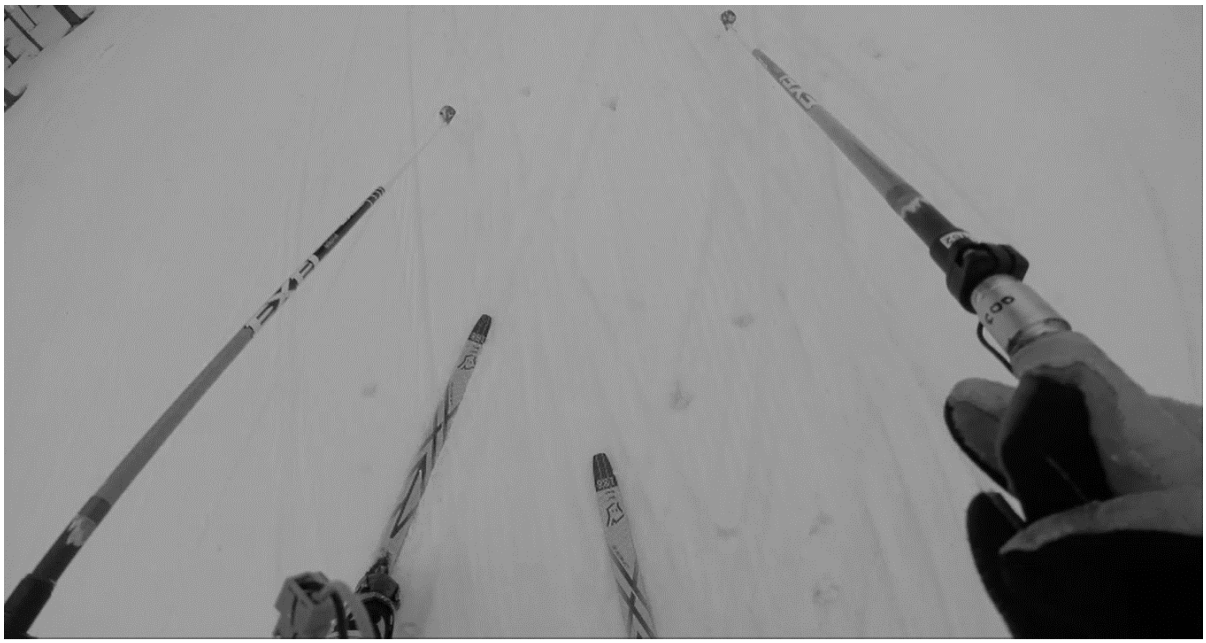

Figure 10. The picture from the Go Pro video recording during the interval. From the video it was possible to analyze the used technique and the amount of technique changes.

All the subjects performed their sessions on the same pointt of the day to minimize the diurnal variations of some of the blood markers. The two treadmill sessions were performed during one week so, that there was at least one day rest between the sessions for each subject. The subjects were asked to make similar preparations what comes to training, food and fluid intake prior to every measurement session.

6.3 Blood collection & analysis, heart rate and RPE

Blood collection. Brain derived neurotrophic factor (BDNF), IGF-1 and Cortisol were analyzed from the venous blood samples. The blood was collected in 7ml Venoject vacuum blood collection tubes and the samples were allowed to coagulate for 30min in room temperature after collection. After this, the samples were centrifuged for 10 minutes (3500 rpm) and after the centrifugation the serum (1ml) was collected in two minimeka tubes. The serum was then frozen down to -80 degrees Celsius for later analysis. The lactate samples were in turn collected from a fingertip – blood sample. The sample was collected in capillaries (20 µl) and were then put in Lactate hemolyzing solution cups (Biosen). Like the venous bloodsamples, also the lactate blood samples were frozen down to -80 degrees C for later analysis.

Blood analysis. The BDNF levels in the serum were measured using a sandwich ELISA Assay (Human BDNF ELISA kit, ScienCell Research laboratories, San Diego, California) and analyzed using Dynex DS 2 ELISA processing system (Dynex Technologies, Chantilly, Virginia). The sensitivity of the BDNF assay was < 2 pg/ml and coefficient of variation (CV%) < 16%. The IGF-1 and Cortisol were measured using chemiluminescense and analyzed with Immulite 2000 Xpi (Siemens Helathcare/DCPA, LA, USA). For IGF-1 and cortisol the sensitivities were 25.5 nmol/l and 5.5 nmol/l and coefficients of variation (CV%) 6.9% and 7.4 % respectively. Finally, lactate was measured amperometrically with the Biosen C-line Sport-analyzer (EKF, Diagnostics for life, United Kingdom) with a CV% of < 2%.

Heart rate and RPE. A mean heart rate was calculated for every subject using the polar training software and every interval from the data collected by the heart rate monitor. The RPE values were as well asked immediately after every single interval in every session.

6.4 Statistical Analysis

The data is presented as mean and standard deviation (mean ± SD). The SPSS software program (SPSS, Inc., Chicago, IL) was used for all statistical analyses. Due to small sample size, the non-parametric Wilcoxon matched pairs signed-rank test was used to compare lactate, heart rate and technique changes between the different sessions and different intervals. The same Wilcoxon signed rank test was also used when comparing the changes in BDNF, IGF-1 and cortisol values within and between the sessions. Correlation tests were performed for the different blood markers using the bivariate correlation test (Pearson's correlation). Statistical significance was set to be $p < 0.05$.

7 RESULTS

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All the results are presented as mean ± SD. Mean times for LOW, HIGH and MAX intervals on snow were 4.02 min ±14s, 3.49 min ± 9s and 3.41min ± 7s, respectively.

BDNF. In the snow measurements, the amounts of BDNF were significantly higher before the interval session in the PRE-blood sample reaching values of 23.2 ± 4.8 ng/ml on snow (S) compared to 18.7 ± 5.0 ng/ml in the normal treadmill environment (NTE) measurements ($p < 0.05$) . (Figure 11). During the interval session in NTE, a significant rise was seen in the BDNF between the PRE (19.8 ± 5.7 ng/ml) and the second (HIGH) interval (23.3 ± 5.8 ng/ml, $p < 0.05$). Between the second (HIGH) and third (MAX) interval, there was seen a significant drop in BDNF values in the normal treadmill environment (23.3 ± 5.8 ng/ml vs. 20.4 ± 7.6 ng/ml, $p < 0.05$). (Figure 12). No other significant changes were seen in the BDNF values in any other interval sessions or between the skiing conditions.

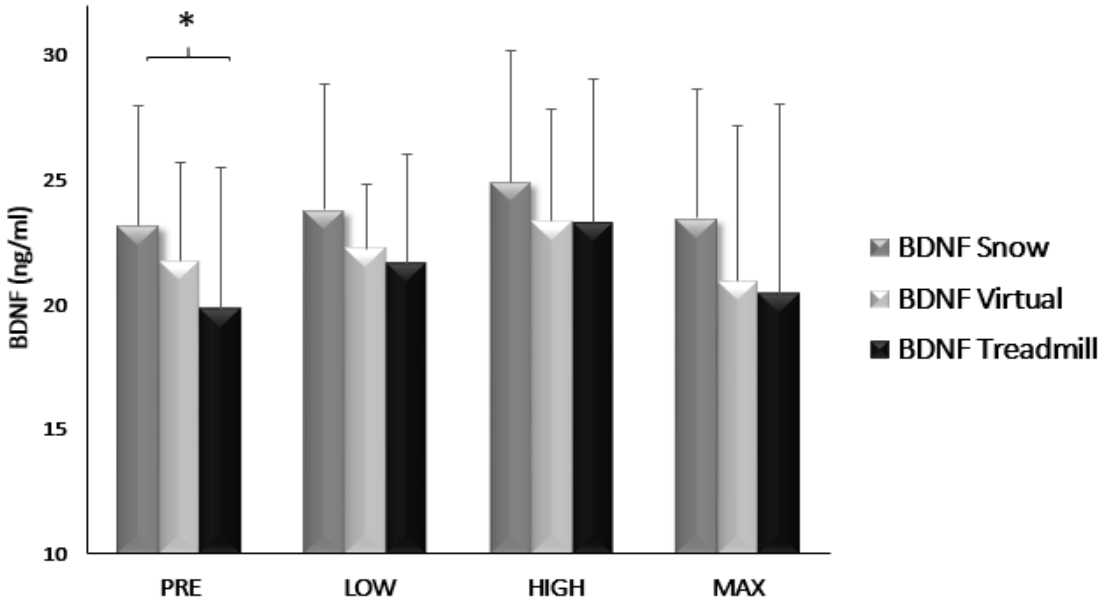

Figure 11. The mean (± SD) BDNF values in the different environments and intervals. The BDNF values were significantly higher in the snow measurements (dark grey staples) in PRE when compared to the normal treadmill environment (the black staples).

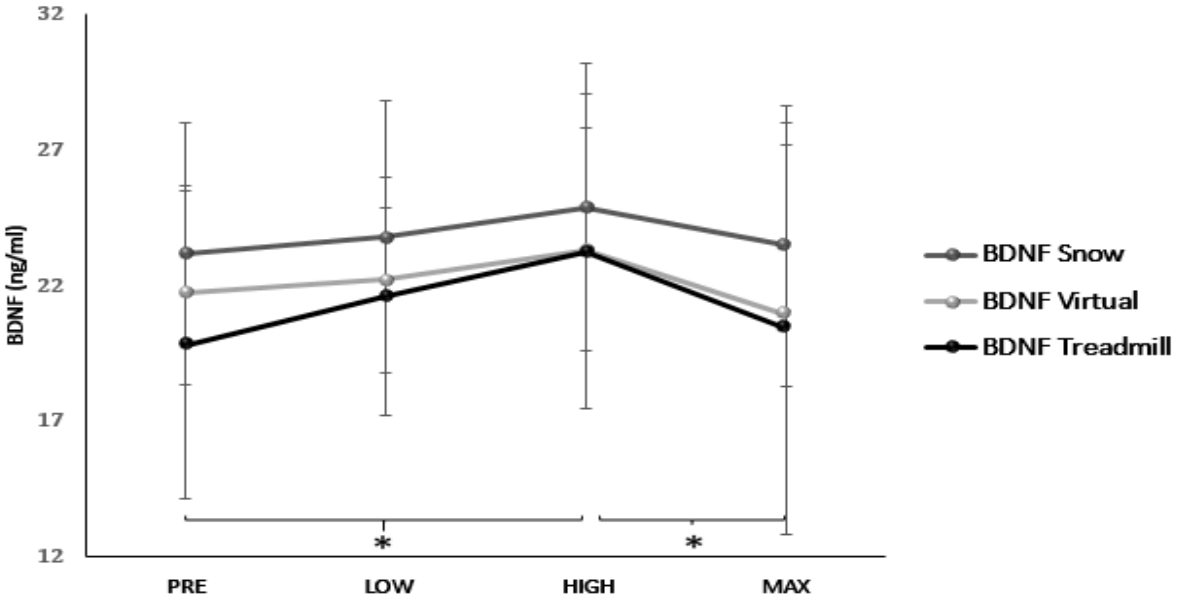

Figure 12. The changes in peripheral BDNF (mean ± SD) *during the sessions. In all the sessions, a clear trend can be seen showing peripheral BDNF upregulation up to the second interval, and a drop to the last interval. The changes were significant only in the normal treadmill environment t (Black line) from PRE to HIGH (* = p < 0.05) and from HIGH to MAX (* = p < 0.05).*

CORTISOL. As in case of BDNF, the cortisol values were significantly higher in the PRE-blood samples in the snow measurements reaching levels of 465 ± 102 mmol/l compared with 358 ± 101 mmol/l in the normal treadmill environment ($p < 0.05$).The cortisol levels were also significantly higher in the snow measurements after the first (LOW) interval (535 ± 149 mmol/) when compared with both virtual (397 ± 130 mmol/l) and normal treadmill (358 ± 101 mmol/l) environment measurements ($p < 0.05$). The significant difference between the cortisol values remained also between the snow environment measurements and normal treadmill environment after second "HIGH" (517 ± 150 mmol/l vs. 376 ± 97 mmol/l) and third "MAX" (541 ± 167 mmol/l vs. 404 ± 129 mmol/l) interval respectively ($p < 0.05$). The differences in cortisol values in the different sessions and intervals are illustrated in Figure 13. In the snow (S) measurement, there was a significant rise in cortisol levels from the pre- measurements (465 ± 102 mmol/l) to the first LOW interval (535 ± 149 mmol/l, $p < 0.05$, Figure 14)

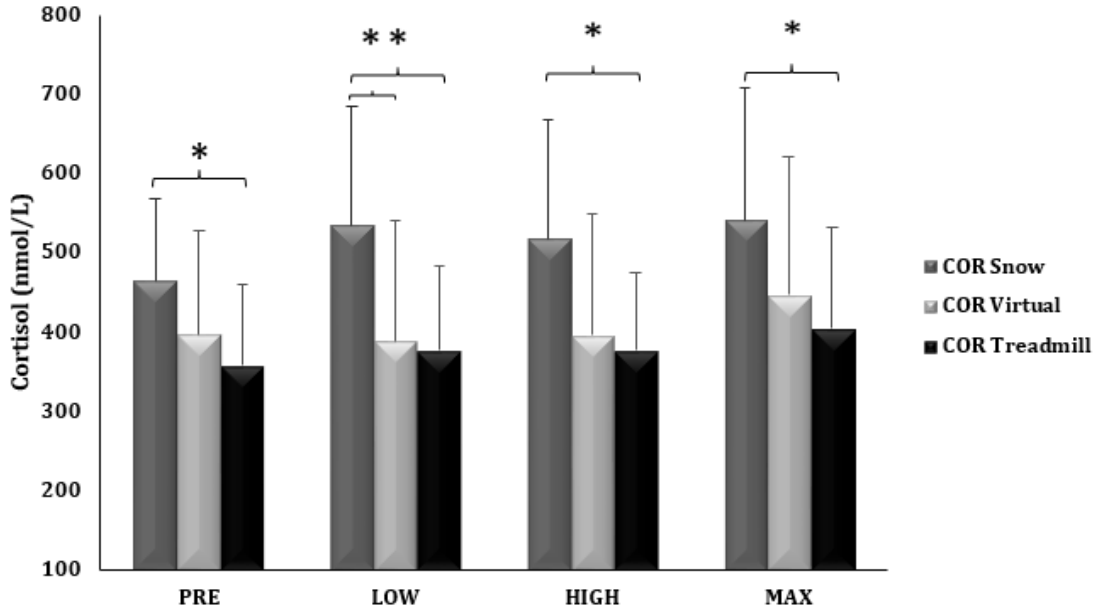

Figure 13. The mean (± SD) cortisol values in the different environments and intervals. From the figure it can be seen, that cortisol was significantly higher during the snow measurements compared with the normal treadmill session in all the measurement points (PRE, LOW, HIGH, MAX) and between snow and virtual environment in LOW (= p < 0.05)*

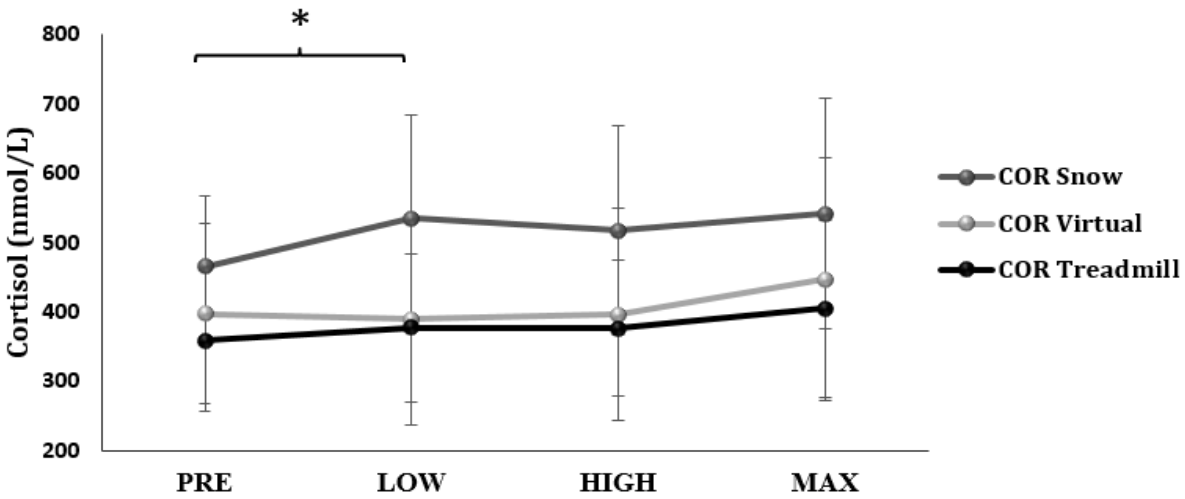

Figure 14. The change in cortisol mean (± SD) values within the sessions. In the snow measurements, there was seen a significant rise in cortisol levels from PRE to LOW (= p < 0.05). No other significant changes were seen for cortisol in any other sessions.*

IGF-1. The IGF-1 values did not significantly differ from each other in the PRE- measurements between the environments, but there was seen a significant difference in the IGF-1 values

between every interval (LOW, HIGH and MAX) when comparing the snow measurements with both the virtual and normal treadmill measurements. (Figure 15). The values were for "LOW" 52.9 ± 11.6 nmol/l (S) vs. 48.2 ± 13,4 nmol/l (V) and 48.1 ± 10.2 nmol/l (NTE), for "HIGH" 56.4 ± 14.4 nmol/l (S) vs. 49.7 ± 13.8 nmol/l (V) and 48.3 ± 10.9 nmol/l (NTE) and for "MAX" 55.6 ± 13.4 nmol/l (S) vs. 49.9 ± 13.0 nmol/l (V) and 49.0 ± 10.8 nmol/l (NTE) respectively ($p < 0.05$, Figure 15). In the snow (S) measurements there was seen a significant rise in the IGF-1 values from PRE (50.6 ± 12.5 nmol/l) to HIGH (56.4 ± 13.4 nmol/l) ($p < 0.05$). A significant rise in IGF-1 levels was also seen in the normal treadmill environment from PRE (45.8 ± 10.4 nmol/l) to MAX (49.0 ± 10.8 nmol/l). ($p < 0.05$). The within session changes of IGF-1 are illustrated in Figure 16.

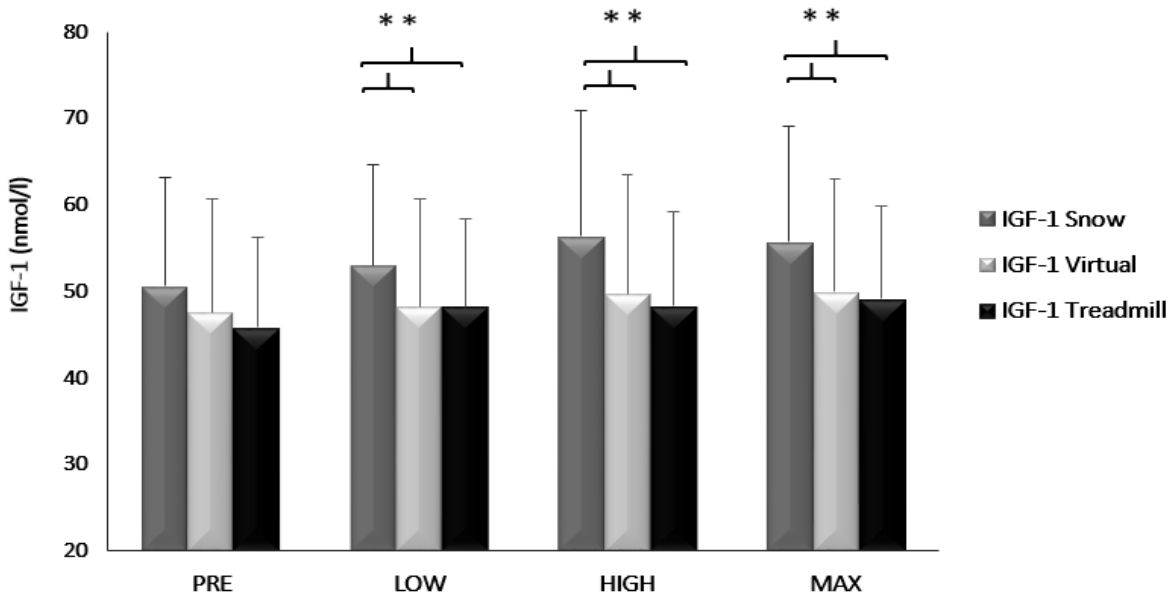

*Figure 15. The differences in IGF-1 (mean ± SD) values between the different environments. In the PRE-measurements, there were no differences, but a significant difference in IGF-1 values between Snow and both the treadmill environments were seen after all the intervals (LOW,HIGH, MAX, *= $p < 0.05$).*

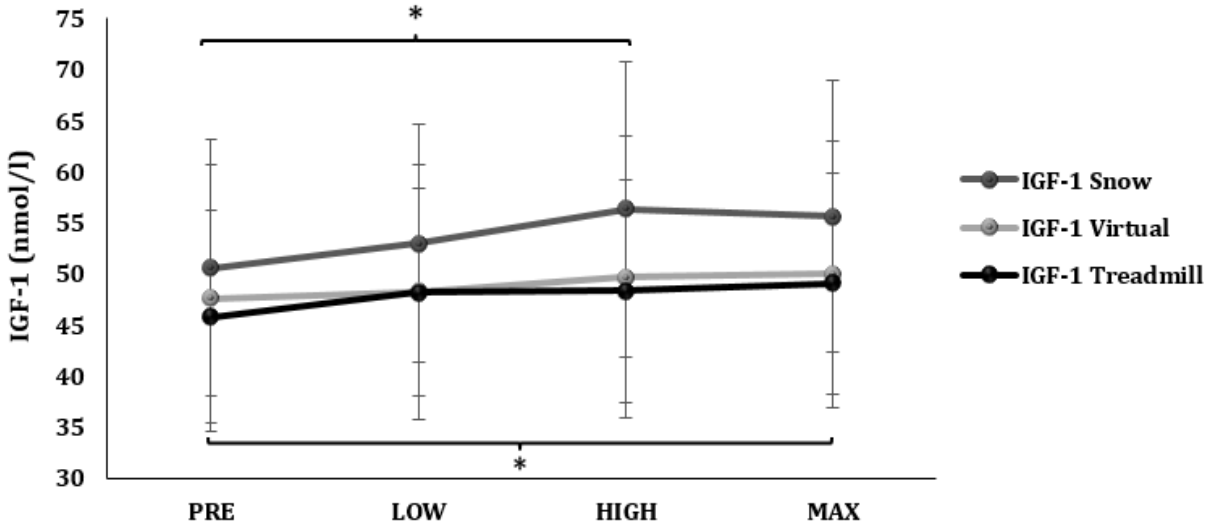

*Figure 16. The changes of IGF-1 (mean ± SD) values within the sessions. On snow, a significant rise was seen in IGF-1 values from PRE to HIGH (dark grey line, * = p< 0.05). In the normal treadmill environment, there was seen a similar trend IGF-1 showing a significant rise from PRE to MAX (black line, * = p< 0.05).*

Heart rate and lactate. A significant difference in blood lactate concentration was found after the MAX interval between snow and both the treadmill environments ($p < 0.05$) (Figure 17). Also the mean heart rate was higher on snow in MAX compared with both treadmill environments (181 ± 8 bpm on snow vs. 172 ± 10 bpm in virtual environment and 175 ± 7 bpm in normal treadmill environment respectively, $p < 0.05$) (Figure 18). No other significant differences were either found in heart rate or lactate between the different sessions. For RPE-values, no differences were seen between the different environments during the sessions and intervals (Figure 19).

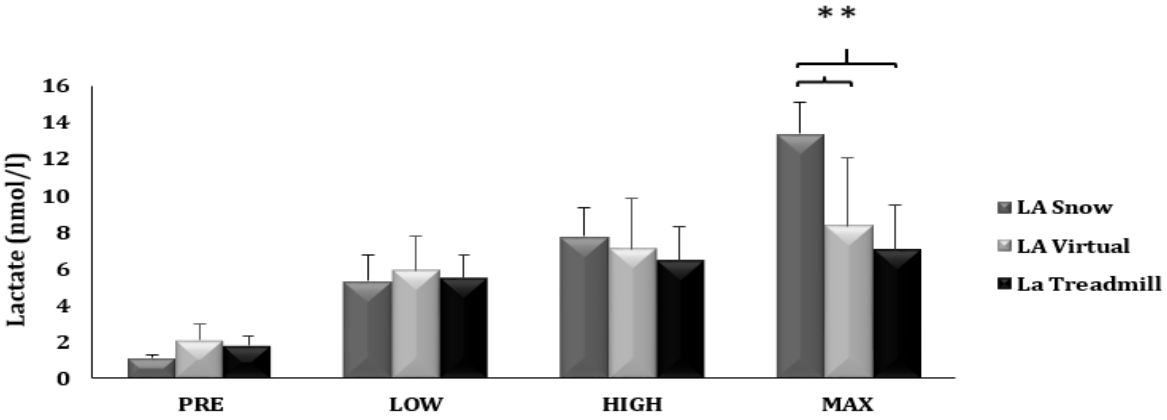

Figure 17. The mean (± SD) lactate values in the different sessions. A significant difference between snow and both the treadmill sessions was found in the MAX interval mean lactate being 13.4 ± 1.7 mmol/l on snow vs. 8.8 ± 3.7 mmol/l in virtual environment and 7.0 ± 2.7 mmol/l in normal treadmill environment (= p< 0.05).*

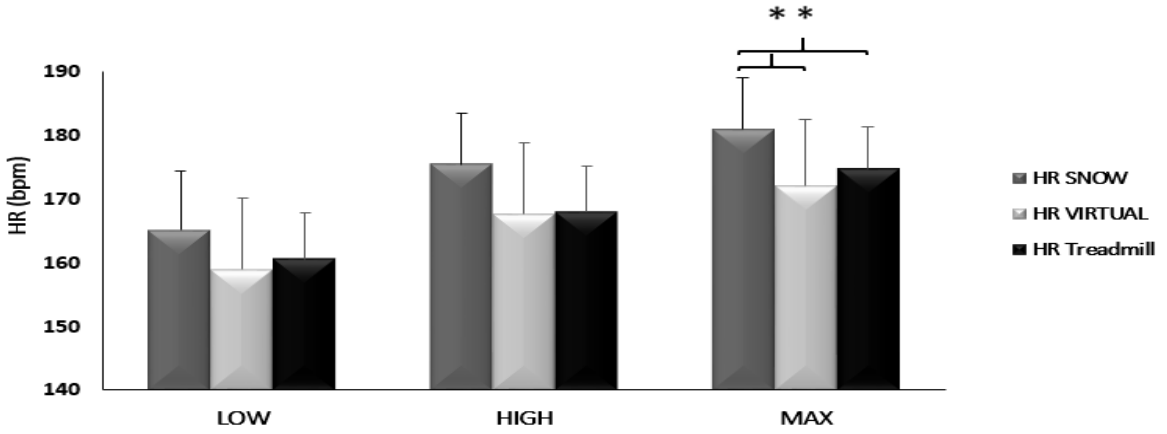

Figure 18. The mean (± SD) heart rate values in the different sessions. A significant difference between snow and both the treadmill sessions was found in the MAX interval (= p< 0.05).*

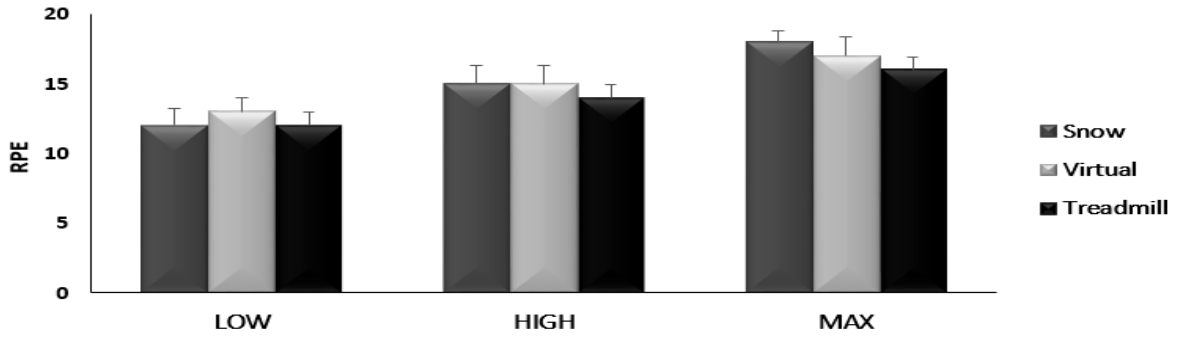

Figure 19. The mean (± SD) RPE values in the different sessions. No differences in RPE were seen between the different sessions.

Correlations. For BDNF and cortisol, a negative correlation was found in the snow measurements in the PRE- tests ($r = -0.68$, $p < 0.05$).The negative correlation of BDNF and cortisol in the PRE tests on snow is illustrated in Figure 20. No further correlations were found for BDNF and cortisol in any environments in the separate measurement points (LOW, HIGH, MAX). For the absolute changes of BDNF and Cortisol from PRE to HIGH measurements in the snow measurements a correlation was found. ($r = 0.74$, $p < 0.05$) and illustrated in Figure 21. However, when the correlation of relative changes from PRE-HIGH on snow was tested, no significant correlation was found. The graph showing the relative changes of BDNF and COR from PRE to HIGH is illustrated in Figure 22. Furthermore, to highlight the individual variations in the relative changes of COR and BDNF from PRE to HIGH, the values are illustrated in Figure 23.

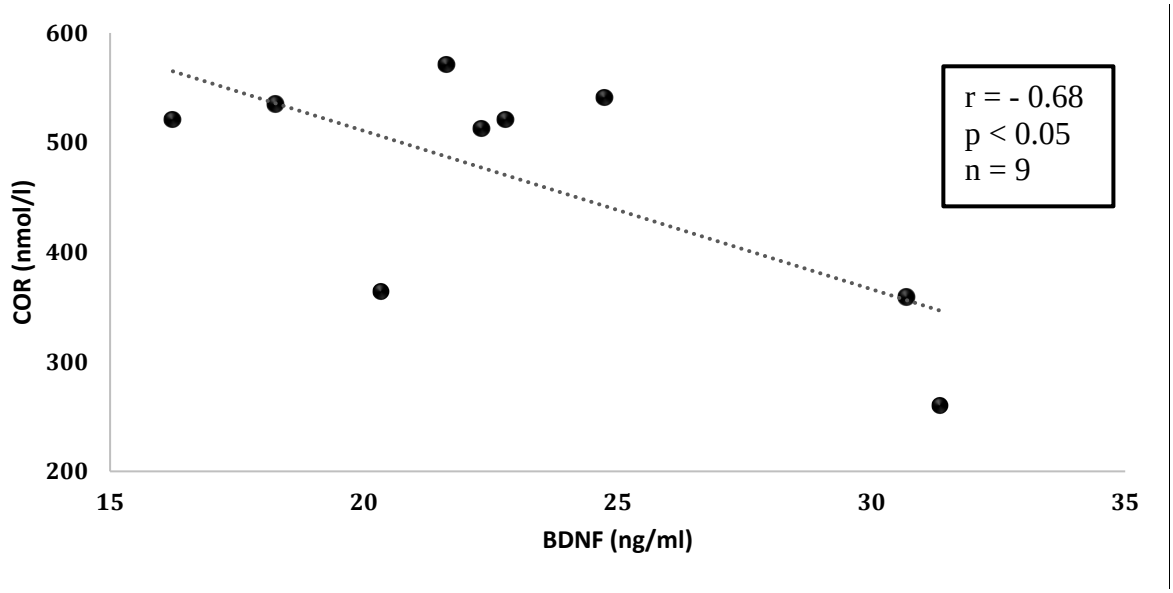

Figure 20. The correlation between absolute cortisol and BDNF values on snow in the PRE measurements. In the PRE measurements on snow, there was found a negative correlation between Cortisol and BDNF ($r = -0.68$, $p < 0.05$). This means that the higher the resting Cortisol values, the lower the resting BDNF values

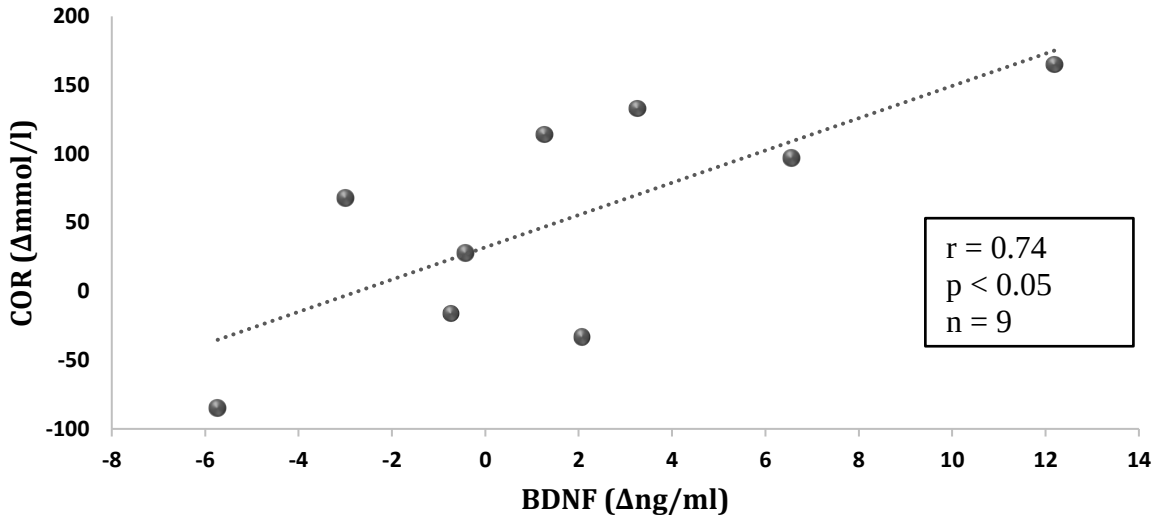

Figure 21. The absolute changes in BDNF and COR from PRE to HIGH. A positive change indicates, that the value rises from PRE to HIGH and a negative change indicates a drop in the measured value. The correlation coefficient for the Δ COR and Δ BDNF was $r = 0.74$, $p < 0.05$.

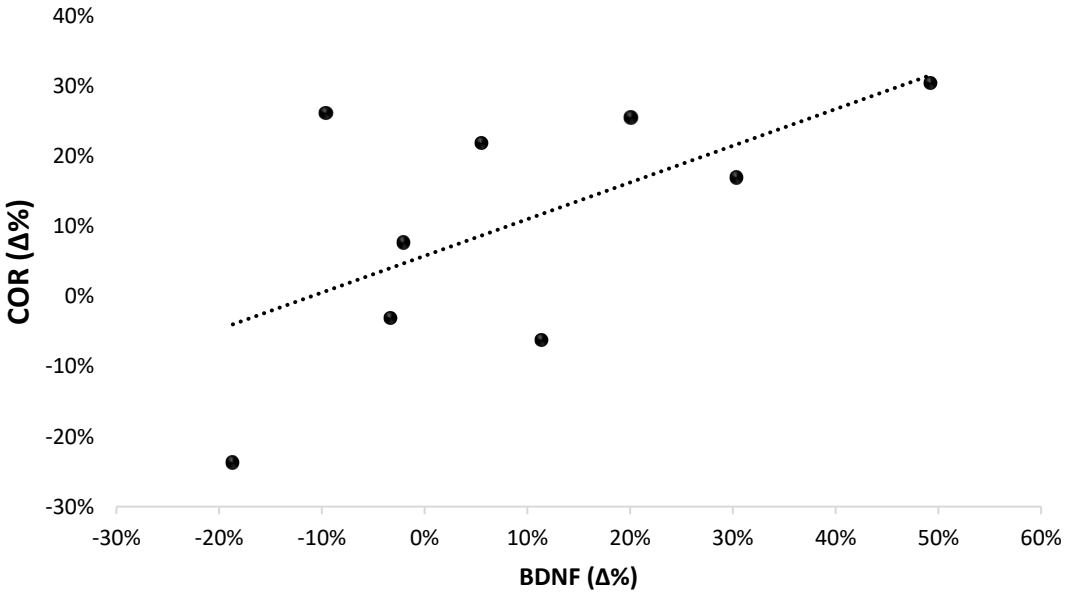

Figure 22. The relative changes in COR and BDNF between PRE and HIGH- measurements on snow. Opposite to the absolute changes, no clear correlation was found even if a trend towards a positive correlation can be seen for some of the subjects.

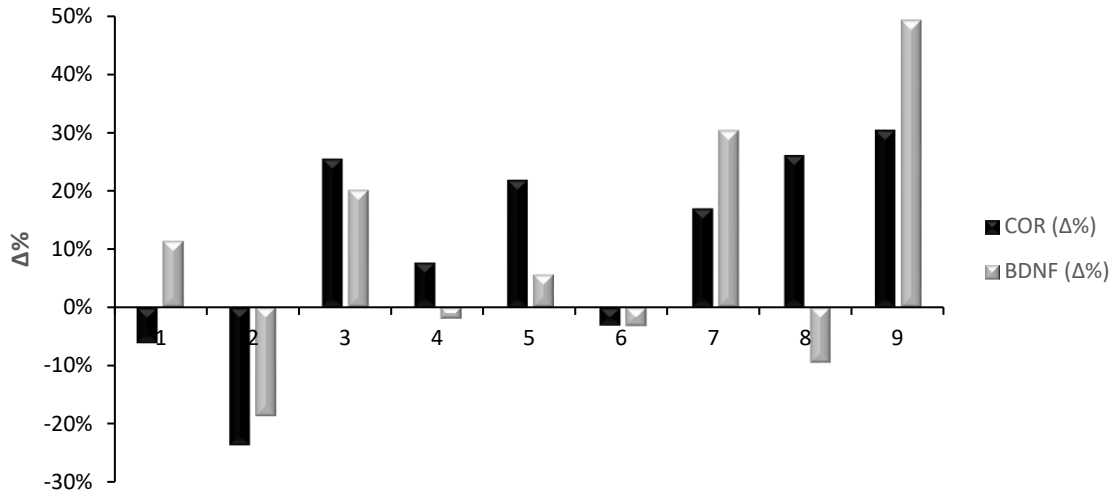

Figure 23. The relative individual changes for COR and BDNF on snow from PRE to HIGH. From the figure it can be seen that there are individual variations in the dynamics of BDNF and COR during the intense intervals between the subjects (1-9). It can be seen, that for most subjects either the both values are upregulated or downregulated in response to intense exercise. Only for three subjects (1, 4 and 8) the relative changes are opposite from each other.

For BDNF and IGF-1, a strong positive correlation was found between BDNF and IGF-1 in the PRE-blood sample on snow ($r=0.81$, $p<0.05$). The same positive correlation between BDNF and IGF-1 was found also in the measurements on normal treadmill in the pre-tests ($r=0.69$, $p<0.05$). The correlations between BDNF and IGF-1 are illustrated in Figure 24. No further correlations were found for BDNF and IGF-1 in any other measurement points.

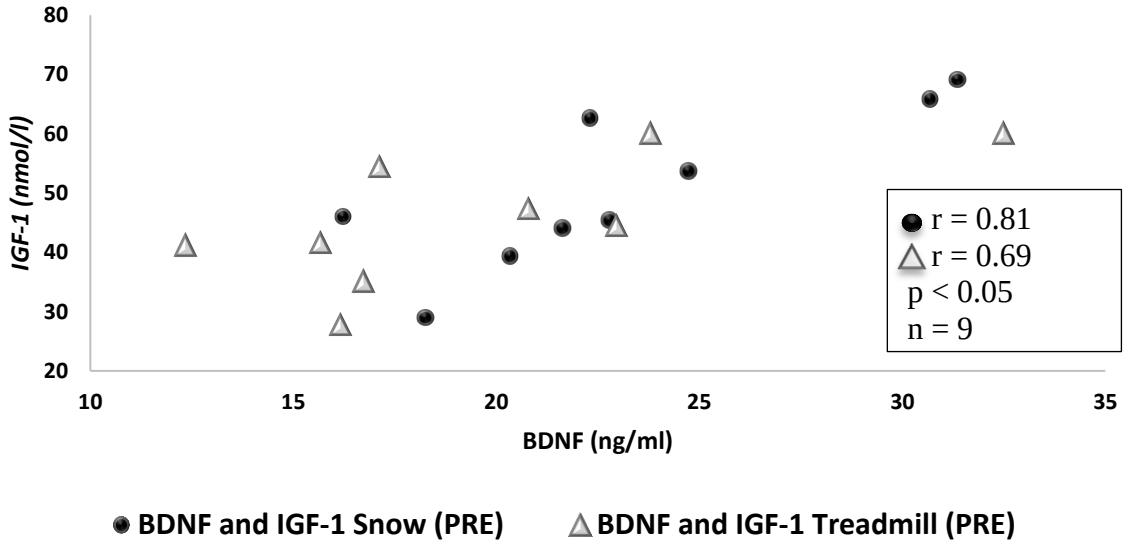

Figure 24. The correlations between absolute BDNF and IGF-1 values. On Snow, there was found a strong positive correlation between absolute BDNF and IGF-1 values in the PRE-measurement (the black dots). The same correlation, though not as strong as on snow, was found also in the normal treadmill measurements (the grey triangles).

Technique changes. In all intervals, a significant difference was found in the number of technique changes when comparing the snow session to both treadmill sessions ($p < 0.05$). The number of technique changes were on snow 25 ± 4 in LOW, 26 ± 4 in HIGH and 25 ± 5 in MAX and on virtual treadmill 11 ± 2 in LOW, 11 ± 2 in HIGH and 11 ± 3 in MAX and on normal treadmill 9 ± 2 in LOW, 8 ± 1 in HIGH and 8 ± 1 in MAX respectively. (Figure 25).

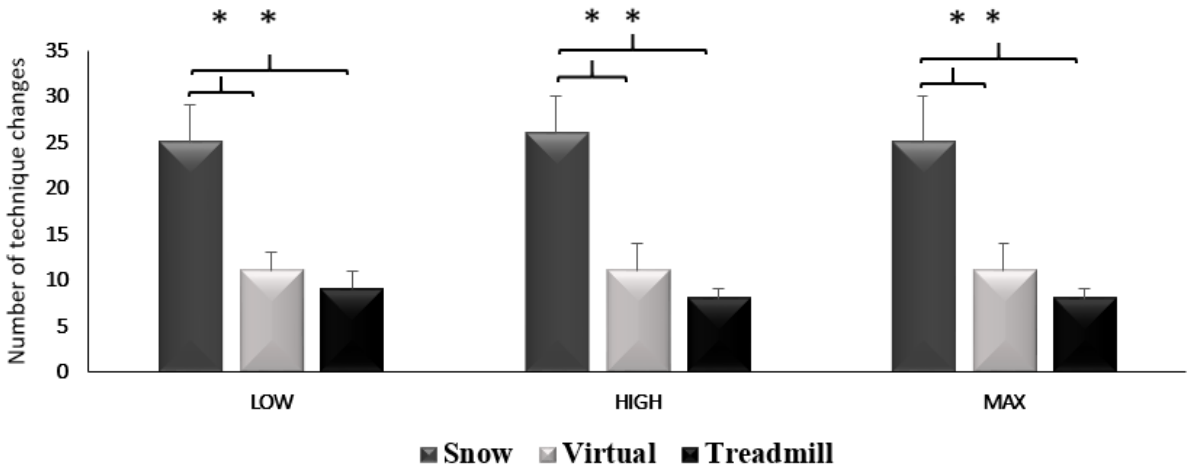

Figure 25. The difference between the number of technique changes in the different environments and intervals. In every interval, there were significantly more technique changes on snow compared with both the treadmill environments.

8 DISCUSSION

In the current study, the aim was to compare the effect of a high intensity interval skiing session carried out in different environments on BDNF, IGF-1 and Cortisol levels and the dynamics of these substances. The aim was also to compare the effect of the different environments and skiing modalities (skiing on snow and roller skiing) on BDNF, on the physiological parameters (HR, LA and RPE) and on skiing techniques used.

The main findings of this study were that BDNF is upregulated by high intensity exercise, but maximal intensity exercise might lead to a drop in the peripheral concentration of BDNF. When it comes to the impact of the exercise environment on BDNF, the normal treadmill environment with its graphs and numerical information on the screen seemed to boost the BDNF production most effectively. On snow and normal treadmill environment, a positive correlation between BDNF and IGF-1 in resting (PRE) values was found. Thus, IGF-1 might possibly be an upregulator of BDNF- production. In the snow environment measurements, a negative correlation with BDNF and cortisol was found in rest (PRE), but the absolute changes of cortisol and BDNF correlated positively between the first and second interval (PRE-HIGH) in the snow measurements during the session. Thus, the impact of cortisol on BDNF seem to be condition dependent. Finally, it showed out that maximal intensity skiing on snow might be physiologically more demanding than roller skiing on treadmill.

8.1 The dynamics of BDNF in response to high intensity skiing

When it comes to BDNF, the results were quite well in line with previous research and the hypothesis: There was found an upregulation in serum BDNF- levels in the normal treadmill environment until the second hardest interval (HIGH), but after this during the maximal effort interval, there was seen a significant drop in the peripheral BDNF- values. The same trend for BDNF- dynamics was similar in all the environment: An upregulation for BDNF was seen up to the second hardest interval (HIGH) and a slight drop during the "MAX" interval A significant change was only seen in the normal treadmill environment. The drop in BDNF- levels in the last MAX- interval was somewhat unexpected and opposite to the hypothesis. This is also something

that has not been seen in previous research. Still, in some previous research there are suggestions why peripheral BDNF values drop after acute intense exercise. The drop of BDNF- levels in the periphery might possibly be explained by the clearance- effect, in which the BDNF- molecules are transported to CNS followed by intense exercise or used elsewhere (Knaepen et al 2010), clearance of BDNF by the liver or simple dilution. (Rasmussen et al 2009). Thus, in the high and maximal effort intervals the clearance of BDNF might have happened due to heavy blood flow, and this in turn might have led to dropping peripheral BDNF values already during the exercise. Still, the fact that extremely vigorous exercise also could be negative when talking of BDNF regulation cannot be ruled out and future research is needed when considering the effect of extremely heavy workout on BDNF.

8.2 The effect of different exercise environments on BDNF

When it comes to the effect of the different environments on BDNF, the results are totally opposite to the hypothesis, which stated that the virtual environment would cause the largest upregulation in serum BDNF due to the visual stimuli and cognitive challenge. In previous research, it is shown that more cognitively demanding tasks (Oztasyonar 2017) and visual input (Castrén et al 1996) would enhance the expression of BDNF in the brain. But instead of the virtual environment, the only significant rise in BDNF- levels was found in the "normal treadmill environment" up to the second (HIGH) interval as mentioned earlier. There might exist some explanations to this result, and the first thing discussed could be the actual cognitive demands of the different environments, especially the two treadmill environments. The virtual environment was meant to be the cognitively most demanding environment of the sessions, but actually it might happen that the normal treadmill environment instead was the most challenging one. In the normal treadmill environment, the subjects had graphs and different kind of numbers indicating the speeds, inclines, time and distance in front of them, and this might actually have lead to a response in the brain leading to higher BDNF- upregulation.

Rasmussen et al (2009) showed in their study with mice that BDNF expression is altered differently in the different kind of brain areas in response to exercise. In their study, BNDF was upregulated in hippocampus and cortex, but not in cerebellum. The authors speculated the role of the complexity of the motor task performed and the role of the environment in the upregulation of BDNF in the different brain areas. They speculated that there might exist some kind of

thresholds for the specific tasks and demands of the performed exercise, and depending on these there would be a different kind of upregulation in certain brain areas. The authors further speculated that for instance cerebellum might not be so heavily activated when well known simple tasks are performed in known environments. It can be speculated, that somehow the more unknown "normal treadmill" with its graphs and mathematic models could have caused a higher activation of certain brain areas, like cerebellum, and thus led to a higher upregulation of BDNF in these certain areas when compared with the virtual environment or snow environment.

In addition, Rasmussen et al (2009) further discussed the possibility of feeling of exertion as a possible trigger of BDNF expression in cortex and hippocampus rather than the motor task itself. However, in the current research, there was no correlation between rate of perceived exertion (RPE) and BDNF in any of the environments investigated.

8.3 The dynamics of BDNF, cortisol and IGF-1

In the snow measurements, a positive correlation was found in the change of absolute BDNF levels (ΔBDNF) and change of absolute cortisol levels (ΔCOR) between PRE and HIGH measurements. In other words, the larger the rise in absolute cortisol levels in response to exercise, the larger the rise in absolute BDNF levels as well. The correlation in the absolute changes of BDNF and cortisol was seen only in the snow measurements. On the other hand, in the present study a negative correlation in the pre- snow measurements in the resting (basal) BDNF and cortisol levels was found; ie. the higher the basal cortisol levels, the lower the basal BDNF levels. Thus, according to the results of the present study, there might thus exist a different relationship between BDNF and cortisol depending on the stimulus or situation. It seems to be that high resting levels of cortisol might negatively influence the levels of BDNF, but on the other hand, the physical activity stimulated release of cortisol might actually be a stimuli for BDNF upregulation.

When looking at the previous research and literature, some explanations and support might be found for this binomial effect of cortisol on BDNF regulation found in the current study. In rodent models, a negative relationship between BDNF and cortisol upregulation induced by chronic stress has been shown to exist. In their study with rats, Smith et al (1995) found that repeated immobilization stress caused by taping the limbs of the rats to a metal board for 2 hr/d on 7

consecutive days caused downregulation of BDNF mRNA. The researchers concluded, that “it is likely that basal levels of glucocorticoids are necessary for maximal inhibition of BDNF mRNA by stress.” (Smith et al 1995, 1774). But in the same paper, the researchers also pointed out that corticosterone feedback is not the only factor in the stress response contributing to the decrease in BDNF mRNA. (Smith et al 1995).

However, in contrast with the previous mentioned rodent research, in human exercise studies there exist research results showing that during exercise related acute stress cortisol could actually via multistage signaling pathways be an upregulator of BDNF synthesis. In their study with 8 young athletes, Rojas-Vega et al (2006) found an increase both in BDNF and cortisol levels following an incremental ramp-test to exhaustion, but they did not find any correlation with BDNF and cortisol. Similarly, in their study with eleven well trained young male cyclists, Heyman et al (2011) found a significant upregulation of BDNF, the endocannabinoid anandamide (AEA) and cortisol in response to a 90min high intense cycling session. The researchers found a correlation between BDNF and the endocannabinoid molecule anandamide (AEA) suggesting that AEA would be an upregulator of exercise induced upregulation of BDNF. Since also a positive correlation with plasma AEA and cortisol was found in the study, the researchers speculated that cortisol would be a trigger for the upregulation of AEA, which in turn would upregulate the synthesis of BDNF in the brain even if no direct correlation with cortisol and BDNF was found. (Heyman et al 2011).

Thus, it can be speculated that cortisol might, depending of the nature and duration of the stress, play a role in both down- or upregulation of BDNF. This hypothesis would also be supported by the findings in the recent study when it comes to cortisol and BDNF. But since the results are somewhat conflicting what comes to the relationship between BDNF and cortisol, it is likely, that there are also other systems contributing to the stress response linked BDNF regulation. It is also likely that the nature of the stress, ie if it is physical, mental, acute and/or chronic leads to different kinds of responses in the human or animal body. These multiple sourced stress signals might in turn cause different kind of pathways to get activated leading to different kind of regulation mechanisms of BDNF synthesis.

It is also likely that there exist individual differences in the dynamics of cortisol and BDNF during an intense training session. Even if there was found a correlation between the absolute changes of BDNF and cortisol values between PRE and HIGH on snow, the correlation was not

seen any more when comparing the relative changes in COR and BDNF in the same situation. Thus it is likely that the level of PRE baseline values also affect the response between BDNF and COR during the session. In the current study, it was clearly seen individual differences between the subjects and it is possible that in the small subject group, the significance in relative changes was diminished since some subjects had really divergent values from the rest.

One mechanism that might possibly play a role in the physical exercise mediated stress response and regulation of BDNF levels could be IGF-1 secretion. Carro et al (2000) showed in their experiment with mice that circulating IGF-1 can travel from the periphery to the brain and there stimulate the neuronal expression of BDNF and thus facilitate long-lasting changes in neuronal activity. Since both acute exercise (Schwarz et al 1996, Copeland & Heggie 2008) and longer training periods (Jeon & Ha 2015) have shown to increase the amount of IGF-1 in blood, it can be speculated that the IGF-1- axis could play a role both in acute or chronic exercise stimulated BDNF mRNA expression and regulation.

In the present study, a significant correlation was found between BDNF and IGF-1 in the rest-state both in the snow and normal treadmill environment measurements indicating that higher resting levels of IGF-1 might lead to higher resting BDNF values. At the same time, the cortisol values in turn correlated negatively with the BDNF values in the resting state in the snow-measurements. When looking at these results it is tempting to speculate that during prolonged stress, like during hard training periods, the rising IGF-1- levels might possibly protect the neurons from the possible harmful effects of rising cortisol levels, since cortisol alone might in turn downregulate the BDNF synthesis. These speculations are further supported by the fact that both the BDNF and IGF 1- values tended to be actually higher during the snow measurements compared with the treadmill- measurements despite the higher cortisol- levels during the snow measurements.

The fact that the relationship between cortisol and BDNF was only seen in the snow measurements might be a bit confusing, but when looking at the changes of cortisol levels, it can be seen that there only occurred a significant rise in the cortisol levels in the snow- measurements from pre to low in the snow – interval session. This might partly explain why the relationship between BDNF and cortisol only was found in the snow measurements and not in the other environments, where no significant changes occurred in cortisol levels during the sessions. It is

also likely that there are individual differences in the responses and these differences had a strong influence on the mean values in the small subject group.

8.4 Comparison of skiing on snow and roller skiing on treadmill

In the current study, also the physiological responses and number of technique changes of skiing on snow and roller skiing on a treadmill with the same intensity and on the same track profile were compared. The major finding was that with lower intensities skiing and roller skiing seemed to be physiologically very similar as expected. With maximal intensity skiing on snow, however, seemed to be physiologically significantly more demanding than maximal effort roller skiing on treadmill when considering heart rate and lactate. The skiing techniques were as well changed more frequently when skiing on snow compared to skiing on treadmill throughout all intensities. The higher physiologic demand of the last interval on snow is somewhat surprising, since previous research has not found major differences in the kinetics of skiing and roller skiing (Suchý & Kračmar 2008) and also in the current study, the difference was seen only in the last maximum effort interval, but not in the first two less intense intervals

The difference in the physiological demand in the maximum speed interval might partly be due to the different techniques chosen when skiing on snow and on treadmill (in both environments). Previous research has shown a difference on aerobic energy cost when skiing on snow between V1 (Gear 2) “offset”, V2A (Gear 4) “two skate”, V2 (Gear 3) “one skate” and skiing without poles- techniques (Millet et al 2003). In their study, Millet et al (2003) showed that V1 was more efficient when comparing to V2- technique and the aerobic energy cost of skiing without poles was 5-9% higher than the energy cost of the other techniques. When it comes to the differences in energy cost between techniques during uphill roller skiing on treadmill, it is shown that the energy cost of the V1 and V2 techniques depends on the incline of the slope V2 technique becoming increasingly costly compared to V1 as slope increases (Kvamme et al 2005). In the present study, the subjects changed the technique significantly more often when skiing on snow and the changes were particularly frequent during the uphill-sections on snow compared to roller skiing. It might happen that this more frequent change of techniques became inefficient and costed more energy when skiing on snow compared to treadmill skiing. Still, the difference in

the amount of technique changes was seen in every interval, but only the last interval showed significant difference in heart rate and lactate values. Thus, this difference in the physiological responses cannot be explained only by the technique changes.

Therefore it can be discussed that the difference in the physiologic responses might to some extend have been due to the hard weather conditions during the on snow session. The weather was humid and warm (0-3 degrees) and the snow conditions were wet. Since the subjects used their own skis during the session, the glide of the skis was not the same for every subject. In the treadmill session in turn, every subject used the same roller skis with standard wheels. Therefore, it might happen that the friction and glide of the skis during the on snow session got worse throughout the session and caused high friction particularly in the last interval. In previous research, it has been shown that higher friction requires more upper body force during skiing (Ohtonen 2010). In the treadmill session, the conditions and friction in turn were constant throughout the whole session. The possibly higher friction particularly during the last interval in the on snow session might have led to higher demand of force and energy and thus also to a higher heart rate and lactate accumulation. One final possible cause for the lower physiological demand during treadmill roller skiing could be the lower air resistance when skiing inside.

8.5 Limitations of the study

This study has several limitations. One important thing to be discussed is also the reliability, set up and timing of the measurements that might have somewhat affected the results. Firstly, all the subjects were informed how to prepare for the tests and to eat similarly on the morning before the sessions. Still, it cannot be ruled out that some of the subjects did not follow the instructions and the different preparations before the sessions might have affected the values of the measured substances. It has been shown that energy intake and fasting can affect the BDNF values somewhat (Rothman et al 2012), and since the subjects were able to decide what and when to eat before the sessions the effect of the nutrition cannot be ruled out when interpreting the results. Testing time and time of day could affect the concentrations of the measured blood markers, but since the researchers were able to design the timetables without major needs to changes initiated from the subjects' side, all the subjects were able to perform the sessions on the same point of day minimizing the effect of diurnal variation on the measured substances in the blood.

The time of the measurements was chosen mainly based on how it suited the subjects, the researchers and conditions of the snow track. This led to the fact that the snow measurements were made during end season, when some of the subjects still were competing and some of them not. The treadmill measurements instead were made when all the subjects had already finished their competition seasons. This might have led to physical condition- related changes in the measured blood values, but at the same time it further gave a topic for discussion since it showed out, that even if the cortisol levels were higher during competitive season, also both BDNF and IGF-1 were higher on this time compared with after season. This could indicate that high IGF-1 levels might protect the brain and BDNF from the harmful effect of chronic physical stress.
Finally, one important thing to be pointed out when interpreting and analyzing the results is the small amount of subjects in the current study. The fact is, that it is very demanding to do statistical analysis with only values from nine subjects and already one or two abnormal results within the subject group might affect the mean values and significances.

8.6 Conclusions

In conclusion, exercise related upregulation of BDNF seem to be a very complicated route of signaling pathways and different substances, which together depending on the magnitude of the physical stress or exercise seems to have the power to regulate the BDNF synthesis. However, it seems to be quite clear that both cortisol and IGF-1 can be some of the key substances regulating these signal pathways. In the current study it was shown that BDNF is upregulated by high intensity exercise. What comes to vigorous maximal intensity exercise in contrast might however lead either to brain re-uptake or drop in the amount of circulating BDNF levels. Therefore, further research is needed to examine what are the exact mechanisms leading to a possible drop in peripheral BDNF levels in maximal intensity exercise.

An environment with cognitive challenges like graphs and numerical information might further stimulate the exercise driven upregulation of BDNF. Therefore, it could be smart to use these kind of environments in combination with exercise. Cognitively challenging exercise environments could be used also in combination with virtual environments, since the virtual environment seemed to be an enjoyable and motivating supplement to the normal exercise

environment. Cognitive challenging virtual environments could be implemented both in recreational activities like active video games, but maybe also for example in rehabilitation.

Cortisol seem to play a role in BDNF regulation, but its effect on BDNF seem to depend on the situation and stimulus leading to the release of the substance. Chronic stress- or training based upregulation of cortisol might have a negative impact to BDNF- levels, but physical exercise related acute upregulation of cortisol might instead positively affect BDNF regulation. It is also likely that individual differences do exist in the response and dynamics of BDNF and cortisol. What comes to IGF-1 it seems that this substance is a positive upregulator of BDNF and is also upregulated in response to hard training periods and intense training session. In the hectic world and lifestyles of today, chronic stress is a norm for many people. Sometimes people even accumulate their stress by doing vigorous exercise in combination with a hectic lifestyle. In the viewpoint of neural health and wellbeing, it could be smart to try to avoid the chronic stress and keeping up the regular, but not necessarily too vigorous exercise regimen to support the wellbeing of the body and mind.

Finally, when it comes to differences between the two modalities skiing snow and roller skiing on treadmill, it looks like that these modalities are very similar to each other in the perspective of the physiological responses in below maximal intensity skiing. During maximum effort in wet conditions though, skiing on snow might be more demanding in the physiological aspect. The higher demand of skiing outside on snow might also partly be due to the higher air resistance outside compared with roller skiing inside on treadmill. This possible higher load is particularly important to take into account, both for coaches and athletes, in wet, high friction skiing conditions, since it might also affect the technique negatively and set an extreme high load on the muscles as well.

8.7 Practical applications

On the basis of the results of the current study there can be given some practical guidelines. For skiers and skiing coaches, it is of great importance to understand and take into account that particularly during wet weather conditions and when skiing outside on artificial snow tracks, the demand of an intense skiing session can become much higher than intended. In the current study, it showed out that the physiological demands were much higher when skiing on maximal

speeds on snow when compared to treadmill. Since skiing with extremely high lactate values can possibly negatively affect for example the technique, in bad outdoor conditions it could be wise to choose a treadmill skiing session instead of skiing on snow. Particularly, if the aim is to do a high intensity session without the aim to gain as high lactate values as possible. The treadmill skiing might in these kind of situations possibly better enhance the speed abilities and the ability to maintain a better technique during fast skiing, since the lactate values seem not to rise as high as outside on snow in maximal speeds.

Virtual environments can strengthen the motivation and add interest and joy to indoor training sessions. Such kind of environments can benefit both elite athletes as well as recreational athletes. Via virtual environments, elite athletes can for instance get familiar to important race environments. For recreational athletes, these kind of environments are a great add-on to an indoor training environment. When cognitive challenges, like for example numeric or graphical information, is added to virtual training environments, these kind of environments can possibly support also the brain health and BDNF production. Therefore it could be good, to develop virtual exercise environments with such kind of properties. These kind of virtual exercise environments could also be used in for example active videogames or rehabilitation.

Finally, on basis of the results obtained in the current study, some advice could be given on how to maximize and enhance the BDNF production in everyday life and through physical activity and exercise. It seems that avoiding too high levels of cortisol and supporting the production of IGF-1 could cause positive effects on BDNF. The avoidance of high cortisol levels is easier said than done in the hectic world of today, but consciously trying to manage and develop strategies to avoid excessive stress might be a good idea in the perspective of BDNF production. Also a lifestyle that boosts IGF-1 production with regular exercise, healthy food habits and enough sleep is recommended to maintain brain health and upregulate the BDNF- levels. Cognitively demanding exercise challenges could be good to implement in the exercise routine, and it could also be a good idea to sometimes do even intense training sessions. The intense sessions do not need to be maximal sessions, but rather submaximal intense interval sessions.

9 REFERENCES

Barbacid, M. 1994. The Trk family of neurotrophin receptors. J.Neurobiol. 25, 1386–1403.

Barde, Y. A., Edgar, D., Thoenen, H. 1982. Purification of a new neurotrophic factor from the mammalian brain. EMBO J. 1, 5, 549–553.

Blinder, D. K., Croll, S. D., Gall, C. M., Scharfma, H. E. 2001. BDNF and epilepsy: too much of a good thing? Trends in Neurosciences, 24, 1.

Carro, E., Trejo, J. L., Busiguina, S., Torres-Aleman, I. 2001. Circulating Insulin-Like Growth Factor I Mediates the Protective Effects of Physical Exercise against Brain Insults of Different Etiology and Anatomy. The Journal of Neuroscience, 21(15):5678–5684.

Castrén, E., Zafra, F., Thoenen, H., Lindholm, D. 1992. Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc. Nad. Acad. Sci. USA, 89, 9444-9448.

Chao, M. V. 2003. Neurotrophins and their receptors: A convergence point for many signalling pathways. Nature Reviews Neuroscience, 4, 299 – 309.

Copeland, J.L, Heggie, L. 2008. IGF-I and IGFBP-3 during Continuous and Interval Exercise. Int J Sports Med, 29,182–187.

Egan, M. F., Kojima, M., Callicott, J.H., Goldberg, T. E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D. R. 2003. The BDNF val66met Polymorphism Affects Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function, Cell, 112, 257–269.

Fiuza-Luces, C., Garatachea, N., Berger, N. A., Lucia A. 2013. Exercise is the real polypill. Physiology, 28, 5, 330-358.

Guyton, A. C. & Hall, J. E. 2000. Textbook of medical physiology. 10. copy. Philadelphia, PA: W.B. Saunders

Hall, J., Thomas, K. L., Everitt, B. J. 2000. Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. Nature neuroscience, 3, 6, 533 – 535.

Halonen J., Ohtonen O., Lemmettylä T., Lindinger S., Rapp W., Häkkinen K., Linnamo V. 2015. Biomechanics of double poling when skiing on snow and using an ergometer. In Book: Science and Skiing VI. Eds. Müller E., Kröll J.,Lindinger S., Pfusterschmied J., Stöggl T. ISBN 978-1-78255-066-2, p. 387-395.

Hébert-Losier K., Zinner, C., Platt, S., Stöggl, T., Holmberg, H.C. 2016. Factors that Influence the Performance of Elite Sprint Cross-Country Skiers. Med Sports,

Heuser, I. J. E., Wark, H-J., Keul, J., Holsboer, F. 1991. Hypothalamic-Pituitary-Adrenal Axis Function in Elderly Endurance Athletes. *J Clin Endocrinol Metab*, 73, 3, 485-488.

Heyman, E., Gamelin, F-X., Goekint, M., Piscitelli, F., Roelands, B., Leclair, E. Di Marzo, V., Meeusen, R. 2012. Intense exercise increases circulating endocannabinoid and BDNF levels in humans – possible implications for reward and depression. Psychoneuroendocrinoogy, 37, 844 – 851.

Huang, T, Larsen, K.T., Ried-Larsen, M., Møller, N. C., Andersen, L.B. 2014. The effects of physical activity and exercise on brain-derived neurotrophic factor in healthy humans: A review. Scand J Med Sci Sports, 24: 1–10.

Jeon, Y. K. Y., Ha, C. H. 2015. Expression of brain-derived neurotrophic factor, IGF-1 and cortisol elicited by regular aerobic exercise in adolescents. J. Phys. Ther. Sci., 27, 3, 737-741.

Knaepen, K., Goekint, M., Heyman, E. M., Meeusen, R. 2010. Neuroplasticity – Exercise-Induced Response of Peripheral Brain-Derived Neurotrophic Factor A Systematic Review of Experimental Studies in Human Subjects. Sports Med, 40, 9, 765 – 801.

Koppel, I., Aid-Pavlidis, T., Jaanson, K., Sepp, M., Pruunsild, P., Palm, K., Timmusk, T. 2009. Tissue-specific and neural activity- regulated expression of human BDNF gene in BAC transgenic mice. BMC Neuroscience, 10, 68.

Kvamme, B., Jakobsen, V., Hetland, S. and Smith, G. 2005. Ski skating technique and physiological responses across slopes and speeds. Eur J Appl Physiol, 95, 205–212

Larson, A. 2006. Variations in heart rate at blood lactate threshold due to exercise mode in elite cross-country skiers. Journal of Strength and Conditioning Research, 20, 4, 855-860.

Laske, C, Banschbach, S., Stransky, E., Bosch, S., Straten, G., Machann, J., Fritsche, A., Hipp, A., Niess, A., Eschweiler, G. W. 2010. Exercise-induced normalization of decreased BDNF serum concentration in elderly women with remitted major depression Int J Neuropsychopharmacol 13,5, 595-602.

Lebrun, B., Bariohay, E., Moyse, E., Jean, A. 2006. Brain- derived neurothropic factor (BDNF) and food intake regulation. A minireview. Autonomic Neuroscience: Basic and Clinical, 126-127, 30-38.

Liu, Q-R., Walther, D.,Drgon,T., Polesskaya, O., Lesnick, T. G., Strain, K. J., de Andrade, M., Bower, J.H., Maraganore, D. M., Uhl, G. R. 2005. Human Brain Derived

Neurotrophic Factor (BDNF) Genes, Splicing Patterns, and Assessments of Associations with Substance Abuse and Parkinson's Disease. American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 134, B, 93–103.

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S.H., Wihler, C., Koliatsos, W.E., Tessarollo, L. 1999. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. PNAS, 26, 96, 15239–15244.

McArdel, W. D., Katch, F. I., Katch V. L. Exercise Physiology Nutrition Energy and Human Performance. 2015. Wolters Kluwer Health, Baltimore.

Mang, C. S., Campbell, K. L., Ross, C. J. D., Boyd, L. A. 2013. Promoting Neuroplasticity for Motor Rehabilitation After Stroke: Considering the Effects of Aerobic Exercise and Genetic Variation on Brain-Derived Neurotrophic Factor. Physical Therapy, 12, 93, 1707-1716.

Marosi, K., Mattson, M. P. 2014. BDNF mediates adaptive brain and body responses to energetic challenges. Trends in Endocrinology and Metabolism, 25, 2, 89 – 98.

Marquez, C.M.S., Vanaudenaerde, B., Troosters, T., Wenderoth, N. 2015. High-intensity interval training evokes larger serum BDNF levels compared with intense continuous exercise. J Appl Physiol 119: 1363–1373.

Matthews, W. B., Åström. M-B., Chan, M.H.S., Bruce, C.R., Krabbe, K.S., Prelovsek, O., Åkerström, T., Yfanti, C., Broholm, C., Mortensen, O.H., Penkowa, M., Hojman, P., Zankari, A., Watt, M.J., Bruunsgaard, H., Pedersen, B.K., Febbraio, M.A. 2009. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. Diabetologia, 52, 1409–1418.

McArdle, W. D., Katch, F. I., Katch, V. L. 2015. Exercise Physiology Nutrition, Energy and Human Performance. Eighth edition, Wolters Kluwer Health, Baltimore.

Metsis, M., Timmusk, T., Arenas, E., Persson, H. 1993. Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. Proc Natl Acad Sci U S A, 90 (19): 8802–880

Millet, G., Boissiere, D., Candau, R. 2003. Energy cost of different skating techniques in cross country skiing. Journal of Sports Sciences, 21, 3-11.

Mooren, F. C., Völker, K. 2005. Molecular and cellular exercise physiology, Human Kinetics, United States of America.

Nakagawa, T., Ono-Kishino, M., Sugaru, E., Yamanaka, M., Taiji, M., Noguchi, H. 2002. Brain-derived neurotrophic factor (BDNF) regulates glucose and energy metabolism in diabetic mice. Diabetes Metab Res Rev, 18, 185–191.

Nassib, S., Moalla, W., Hammoudi-Nassib, S., Chtara, M., Hachana, Y., Tabka, Z., Chamari, K., Elloumi, M. 2016. The IGF-1/cortisol ratio as a useful marker for monitoring training in young boxers. Biol. Sport, 33, 15-22.

Ohtonen, O. 2010. The effect of ski gliding properties on the force production of V2-technique. Master's thesis in Biomechanics, Department of Biology of Physical Activity University of Jyväskylä, Finland.

Oztasyonar, Y. 2017. Interaction between different sports branches such as taekwondo, box, athletes and serum brain derived neurotrophic factor levels. The journal of sports medicine and physical fitness. 57 (4), 457-60.

Pan, W., Banks, W. A., Fasold, M. B., Bluth, J., Kastin, A. J. 1998. Transport of brain-derived neurotrophic factor across the blood–brain barrier. Neuropharmacology, 37, 1553–1561.

Pedersen, B.K, Pedersen, M., Krabbe, K.S, Bruunsgaard, H., Matthews, V.B., Febbraio, M.A. 2009. Role of exercise-induced brain-derived neurotrophic factor production in the regulation of energy homeostasis in mammals. Exp Physiol 94.12 pp 1153–1160.

Poo, M-m. 2001. Neurotrophins as synaptic modulators. Nature Reviews Neuroscience, 2, 24-32.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M.V., Leick, L., Hart, E., Secher, N.H., Pedersen, B.K., Pilegaard, H. 2009. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. Experimental Physiology 94, 1062–1069.

Rojas-Vega, S., Struder, H.K,. Wahrmann, B.V., Schmidt, A., Bloch, W., Hollman, W. 2006. Acute BDNF and cortisol response to low intensity exercise and following ramp incremental test to exhaustion in humans. Brain research, 1121, 59 -65.

Rothman, S. M., Griffioen, K. J., Wan, R., Mattson, M. P. 2012. Brain-derived neurotrophic factor as a regulator of systemic and brain energy metabolism and cardiovascular health. Ann. N.Y. Acad. Sci, 1264, 49–63.

Rosso, V., Gastaldi, L., Rapp, W., Lindinger, S., Vanlandewijck, Y., Linnamo, V. 2016. Biomechanics of simulated versus natural cross-country sit skiing. Biomechanics of

simulated versus natural cross-country sit skiing, Journal of Electromyography and Kinesiology.

Rusko, H. 2003. Physiology of Cross Country Skiing, in Handbook of Sports Medicine and Science: Cross Country Skiing (ed H. Rusko), Blackwell Science Ltd, Oxford, UK.

Russo-Neustadt, A.A., Beard, R.C., Huang, Y.M., Cotman, C.W. 2000. Physical activity and antidepressant treatment potentiate expression of specific brain- derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience, 101, 2, 305-312.

Sandbakk, Ø., Ettema, G., Leirdal, S., Jakobsen, V. Holmberg, H-C. 2011. Analysis of a sprint ski race and associated laboratory determinants of world-class performance. Eur J Appl Physiol (2011) 111:947–957.

Sandbakk, Ø., Ettema, G., Leirdal, S. 2015. The physiological and biomechanical differences between double poling and G3 skating in world class cross-country skiers. Eur J Appl Physiol (2015) 115:483–487.

Seiler, K, S., Kjerland, G, Ø. 2006. Quantifying training intensity distribution in elite endurance athletes: is there evidence for an ‘‘optimal’’ distribution? Scand J Med Sci Sports, 16, 49–56.

Schiffer, T., Schulte, S., Sperlich, B., Achtzehn, S., Fricke, H., Struder, H.K. Lactate infusion at rest increases BDNF concentration in humans. 2011. Neurosciense Letters, 488, 234-237.

Schwarz, A. J., Brasel, J. A., Hintz, R. L., Mohan, S., Cooper, D. M. 1996. Acute effect of brief low- and high-intensity exercise on circulating insulin-like growth factor (IGF) I, II, and IGF-binding protein-3 and its proteolysis in young healthy men. J Clin Endocrinol Metab., 81, 3492–3497.

Smith, M. A., Makino, S., Kvetnansky, R., Post, R. M. 1995. Stress and Glucocorticoids Affect the Expression of Brain-Derived Neurotrophic Factor and Neurotrophin-3 mRNAs in the Hippocampus. The Journal of Neuroscience, 15, 3, 1766-1777.

Stöggl, T., Schwarzl, C., Müller, E.E., Nagasaki, M., Stöggl, J., Scheiber, P., Schönfelder, M., Niebauer, J. 2016. A Comparison between Alpine Skiing, Cross-Country Skiing and Indoor Cycling on Cardiorespiratory and Metabolic Response. Journal of Sports Science and Medicine, 15, 184-195.

Suchý, J. & Kračmar, B. 2008. Analysis of the kinesiology of skate skiing and roller skiing. Ugdymas. Kūno kultūra. Sportas (Baltic Journal of Sport and Health Sciences) 3, (70), 81—87.

Tokuyama, W., Okuno, H., Hashimoto, T., Xin Li, Y., Miyashita, Y. 2000. BDNF upregulation during declarative memory formation in monkey inferior temporal cortex. Nature neuroscience, 3, 11, 1134 – 1142.

Tonoli, C., Heyman, E., Buyse, L., Roelands, B., Piacentini, M. F., Bailey, S., Pattyn, N., Berthoin, S., Meeusen, R. 2014. Neurotrophins and cognitive functions in T1D compared with healthy controls: effects of a high-intensity exercise. Appl. Physiol. Nutr. Metab. 40: 20–27.

Ulucan, K. 2016. Brain-Derived Neurotrophic Factor Factor and Exercise, Can It Be a New Biomarker for Athletic Performance? The Journal Of Neurobehavioral Sciences, 3, 1.

Vaynman, S., Ying, Z., Gomez-Pinilla, F. 2003. Inter.play between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. Neuroscience 122, 647–657.

Vergès, S., Flore, P.,Laplaud, D., Guinot, M., Favre-Juvin, A. 2006. Laboratory Running Test vs. Field Roller Skiing Test in Cross-Country Skiers: A Longitudinal Study. Int J SportsMed 2006; 27: 307–313.

Wackerhage, S. Molecular Exercise Physiology. 2014. Routledge, New York

West, A. E., Chen, W. G., Dalva, M. B., Dolmetsch, R. E., Kornhauser, J. M., Shaywitz, A. J., Takasu, M. A., Tao, X., Greenberg, M. E. 2001. Calcium regulation of neuronal gene expression. PNAS, 98, 20, 11024 – 11031.

Wrann, C.D., White, J.P., Salogiannnis, J., Laznik-Bogoslavski, D., Wu, J., Ma, D., Lin, J.D., Greenberg, M.E., Spiegelman, B.M. 2013. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway, Cell Metab., 18, 5, 649-659.

Xu, B., Goulding, E. H., Zang, K., Cepoi, D., Cone, R. D., Jones, K. R., Tecott, L.H., Reichard, L. F. 2003. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci. 6,7, 736–742.