APP Processing: A Biochemical Competition Influenced by Exercise-Induced Signalling Mediators

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Abstract

In our aging society neurodegenerative diseases such as Alzheimer's disease (AD) are becoming more prevalent. One specific neuropathological hallmark of this disease is excessive accumulation of amyloid-β (Aβ) peptides, which can aggregate to form the plaques commonly associated with this disease. These plaques are often observed well before symptoms of AD develop. Therefore, it is important to find ways to regulate the pathways involved in the production of these peptides. Evidence indicates that exercise has the capacity to reduce Aβ peptide production in the brain. Exercise promotes the release of many different signalling mediators from various tissues and organs in the body. These exercise-induced signalling mediators could be the driving force behind some of the beneficial effects seen in the brain with exercise. The purpose of this study was to examine if post-exercise serum and the factors it contains can alter neuronal APP processing. Human SH-SY5Y neuronal cells were differentiated with retinoic acid for 5 days and treated with 10% pre- or post-exercise serum for 30 minutes. Cells were collected for analysis of acute (30 minutes; n=6) or adaptive (24 hours post-treatment; n=6) responses. There were no statistical differences in ADAM10 and BACE1 mRNA or protein expression with post-exercise serum treatment at either time point. However, there was an increase in the ratio of sAPPα to sAPPβ protein content (p=0.05) after 30 minutes of post-exercise serum treatment. Additionally, 30 minutes of post-exercise serum treatment increased ADAM10 (p=0.01) and BACE1 (p=0.02) activity. These novel findings suggest that post-exercise serum modulates important enzymes involved in APP processing, potentially pushing the cascade towards the non-amyloidogenic arm.

Acknowledgements

Dr. Rebecca MacPherson

To Rebecca, I am truly grateful for all that you have done for me. I feel as though I have had the best of both worlds, I have been taught how to conduct rigorous scientific research by Dr. MacPherson and learned how to party from Becky. I am especially grateful for the way you guided me when I could not seem to find my way at first, and most importantly thank you for letting me play with Henry! You have taught me so much in our five years working together and I will always cherish my time in the MacPherson Lab. A great deal of my success at Brock University is due in part to you. When I first began my time in your lab, I am not sure I fully believed that I had earned my position, but I respect your opinion so much and have always worked my hardest to make you proud and I hope this has shinned through over the years. As I continue my academic journey it is comforting to know that I have been trained by the best and have gained a colleague and friend. I cannot wait to see what you do next, and I wish you all the best with your future endeavours.

Family and Friends

To my parents, it is hard to imagine accomplishing this seemingly impossible task without you both. You have supported me from day one on everything I have undertaken, big or small, and I owe a large part of my success to you both. You both have always put me first and never ask for anything in return. I am so grateful that I can call you anytime and know that you will drop what you are doing to help me. Thank you for being amazing role models for me throughout the years.

To my grandparents, Oma, and Opa, you have all supported me in so many ways, including financially and most importantly always making sure I was well fed. You have all always made it a point to show me how much I am loved, and words cannot describe how much that means to me.

To Riley, the last three years have been amazing and so much easier with you by my side. We have experienced a lot in the past two years and done a tremendous amount of growing together, including finding a home and subsequently having to hunker down in it to avoid a global pandemic. Thank you for all that you have and will help with, I love you. I cannot wait to begin the next chapter of our journey at McMaster University together.

To Alex (A.K.A "The Snooch"), it has been a pleasure to watch you grow up and I'm so grateful for the time we have gotten to spend together. It is a great feeling to know I always have someone to come home and relax, chat, and play sports with. I wish you all the best as you start your own journey at Brock University. They are lucky to have you.

To my friends, thank you for keeping me humble, always looking out for me, and constantly teaching me new things. Thank you to my hometown friends (The Ildy Boys) for always being there when I come back into town and showing me a good time and never letting me forget where I came from. Thank you to all the new friends I have met along the way at school, you all have been so influential to me and my academic journey. A special thanks to Grant, Jeremy, Brad, Scott, and Kirsten for making my time at Brock and in Cairns very special.

I understand that a simple thank you does not do the amount of gratitude I have for you all justice and a whole other thesis could be written about every single one of you, but I hope you all know how thankful I am to have all of you in my life. You all have shaped me into the person I am today, and I hope I have made you all proud.

Committee Members and Mentors

To my committee members, thank you all for your support and guidance throughout my project, I know my thesis is better off because of it. Under the current circumstances of this global pandemic, it was clear modifications and concessions needed to be made and I am especially grateful for your flexibility and accommodation with this project.

Lab Members and Cairns Researchers

Thank you to my lab members for all the help they have provided me with over the years. Thank you to Nigel for his help and guidance with this projects study design. A special thanks to Mike for his unwavering support and dedication to this project. We have spent a lot of late nights and early mornings working to complete this project and it would not be possible without you. Thank you to the other researchers in Cairns for teaching me how to use different lab equipment and sharing their resources with me. It has been a pleasure to learn alongside and collaborate with each of you.

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List of Abbreviations

Aβ	Amyloid Beta
AD	Alzheimer’s Disease
ADAM10	A Disintegrin and Metalloproteinase 10
AICD	Amyloid Precursor Protein Intracellular Domain
AMPK	AMP-Activated Protein Kinase
APP	Amyloid Precursor Protein
BACE1	Beta-Site Amyloid Precursor Protein Cleaving Enzyme 1
BBB	Blood-Brian-Barrier
BDNF	Brain-Derived Neurotrophic Factor
BSA	Bovine Serum Albumin
CDK5	Cyclin-Dependent Kinase 5
CREB	cAMP-Response Element Binding Protein
CTF	Carboxyterminal Fragment
DMEM	Dulbecco's Modified Eagle's Medium
ETS	Erythroblast Transformation Specific
FGF-2	Fibroblast Growth Factor 2
FGF-21	Fibroblast Growth Factor 21
FNDC5	Fibronectin Type 3 Containing Domain 5
GDF-15	Growth Differentiation Factor 15
GLP-1	Glucagon-like Peptide 1
GLUT4	Glucose Transporter 4
IGF-1	Insulin-like Growth Factor 1

IL-6	Interleukin-6
JNK	c-Jun N-terminal Kinase
MMP-9	Matrix Metalloproteinase-9
MCTs	Monocarboxylate Transporters
NFTs	Neurofibrillary Tangles
PCR	Polymerase Chain Reaction
sAPPα	Soluble Amyloid Precursor Protein Alpha
sAPPβ	Soluble Amyloid Precursor Protein Beta
TBST	Tris-buffered saline/0.1% Tween 20
T2D	Type 2 Diabetes
VEGF	Vascular Endothelial Growth Factor

Chapter 1: Introduction

It is estimated that 35 million individuals are affected by dementia world-wide with the prevalence predicted to double every 20-years [1]. This rate of growth will result in approximately 66 million individuals affected by dementia in 2030 and 115 million by 2050 [1]. Alzheimer's disease (AD) is the most common form of dementia, with epidemiological data highlighting that it accounts for approximately 60-70% of all dementia cases [2, 3]. There are currently two main types of AD, familial and sporadic. Familial AD, also known as early-onset, accounts for about 5% of all AD cases and is the result of autosomal genetic mutations predominantly of the amyloid precursor protein (APP), as well as Presenilin-1 and -2 genes [4-6]. The second type of AD is known as sporadic or late-onset, and largely occurs in individuals 65 years of age and older [7]. Unlike familial AD, sporadic AD accounts for approximately 95% of cases. Aging is the most significant risk factor for the development of sporadic AD, with data indicating that an individual's risk doubles every five years after the age of 65 [2, 3]. Other important risk factors for AD include type 2 diabetes (T2D), obesity, and cardiovascular disease [3]. In addition to these risk factors, it is currently understood that modifiable risk factors such as diet and exercise can have a significant impact not only on the above-mentioned diseases but also on AD risk [4, 5, 7]. Interestingly, sporadic AD shares many characteristics with T2D, such as brain insulin resistance, and decreased glucose uptake and metabolism. Thus, some researchers have dubbed sporadic AD as a type 3 diabetes [8]. However, it is important to note that there is still a small genetic component with individuals carrying the ApoE4 allele being at a higher risk for developing the disease [3, 8-10].

AD is a neurodegenerative brain disorder typically brought on by a loss of both cortical and hippocampal neurons, resulting in a reduction in overall cognitive function [6]. The two

major neuropathological hallmarks of AD are amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs), however the focus of this review will be on the Aβ pathology of the disease [11-13]. Aβ plaques accumulate extracellularly whereas NFTs are found intracellularly, however both proteins have been shown to correlate with the symptoms and progression of AD [11-13]. Recent evidence has suggested that a decrease in brain metabolism contributes to the disease pathology and progression [7]. This hypometabolism, similar to what is seen in patients with T2D, is thought to appear in patients with AD well before clinical symptoms manifest and is characterized by declines in glucose transport, glycolysis, and mitochondrial function [7].

With a large body of knowledge on the cellular characteristics of AD it is troubling that there is no well-established treatment or preventative strategy. Additionally, there has yet to be an effective widely used pharmaceutical developed for AD that goes beyond merely treating the symptoms, despite how much is known about the hallmarks of this disease. This lack of pharmaceutical interventions highlights the importance for the development of alternative approaches for reducing the prevalence and slowing the progression AD, which is growing exponentially every year [1]. One of the most promising preventative and treatment strategies is exercise, which has the capacity to reduce both the risk of AD development and various pathological hallmarks of the disease [14-19]. It is still up for debate as to exactly how exercise can have beneficial effects on the brain, but one of the prevailing theories among researchers is that exercise has the ability to drive the release of various signaling molecules [20-23]. These signaling molecules are released from several metabolic tissues during and post-exercise. The molecules can have local autocrine and paracrine effects but can also travel through the bloodstream to their target tissue where they will elicit a beneficial endocrine response [20-23]. Of particular interest for this review are brain-derived neurotrophic factor (BDNF), cathepsin B,

lactate, and interleukin-6 (IL-6). These factors have been shown to play roles in neuronal cell proliferation, differentiation, and cell survival, as well as promote synaptic formation, activity, and long-term potentiation [20-23]. All of these beneficial effects at the cellular level have the potential to increase learning and memory, which is impaired in individuals suffering from AD [6]. However, the exact mechanisms that drive exercise-mediated improvements in AD pathology currently remain unknown. Therefore, the purpose of this study is to determine if factors present in exercise serum can modulate proteins involved in APP processing.

Chapter 2: Literature Review

2. 1 Aβ Peptides and Alzheimer's Disease

2. 1. 1 Amyloidogenic Cascade and Amyloid-Beta Peptide Formation

APP processing can be divided into two competing pathways (Figure 1). First, is the non-pathological pathway, which is initiated by the cleavage of the type 1 transmembrane bound protein APP by the enzyme α-secretase (also known as ADAM10) at the leucine^{17} site [24]. This cleavage occurs within the Aβ domain, which does not result in Aβ peptide formation or aggregation. From this cleavage reaction an extracellular fragment, known as soluble amyloid precursor protein alpha (sAPPα), and a membrane bound carboxyterminal fragment 83 (CTF83) are formed [25, 26]. Following this reaction, another enzyme called γ-secretase cleaves the membrane bound CTF83 fragment, which results in an extracellular P3 fragment, along with an APP intracellular fragment (AICD) [24-26]. Conversely, the pathological amyloidogenic pathway begins with the cleavage of the membrane bound protein APP by the enzyme beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) at the major glutamate^{11} site or the minor aspartate^{1} site [25, 26]. This discrepancy in cleavage site can be attributed to the imprecise nature of BACE1, which results in variable CTF length formation. After BACE1 cleaves APP an extracellular soluble amyloid precursor protein beta (sAPPβ) fragment and a membrane bound CTF89 or CTF99 fragment are formed [25-28]. The length of this membrane bound protein varies based on what cleavage site BACE1 cleaves APP, with cleavage at the glutamate^{11} site resulting in the CTF89 fragment and cleavage at the aspartate^{1} site resulting in the CTF99 fragment [25-28]. Following this reaction, γ-secretase cleaves the membrane bound CTF89 or CTF99 fragment in the transmembrane domain, which results in an Aβ peptide, and an AICD fragment [24]. Cleavage by γ-secretase is considered somewhat imprecise and as a result

contributes to the production of Aβ peptides in varying length. Normally, peptides produced from this cleavage reaction are between 38 and 43 amino acids in length, with Aβ40 being the predominate length released extracellularly (Figure 2) [29, 30]. That said, the longer peptides, such as Aβ42, are considered to be more pathological as they are highly hydrophobic and fibrillogenic, thus making them more prone to aggregation [29]. When this pathway is hyperactivated it can result in overproduction of Aβ peptides, which can aggregate extracellularly causing neuronal dysfunction, death, and overall reductions in cognition [29, 31]. As BACE1 is the rate limiting enzyme in this cascade, understanding the mechanisms of how this enzyme is regulated is paramount for determining how Aβ peptide production can be altered in order to preserve normal neuronal and brain function. It is also important to understand the role of α-secretase and the balance between the two competing pathways.

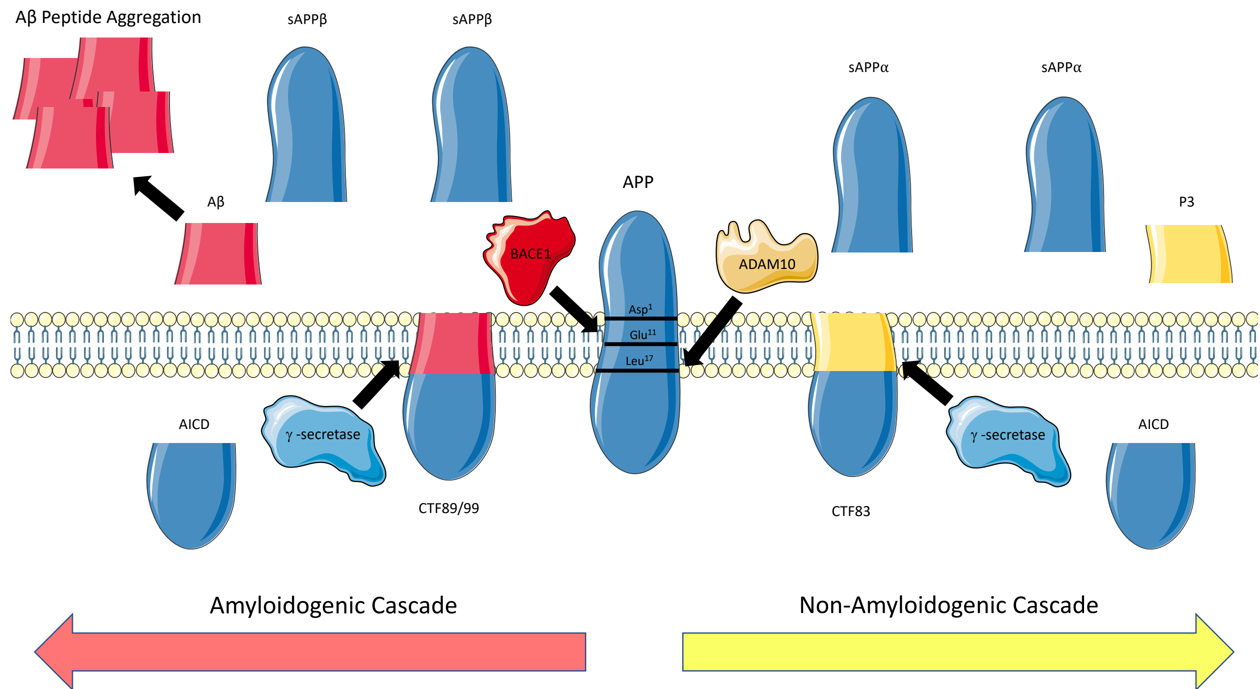


Figure 1: Competing Pathways of APP Processing. *Non-pathological products are produced through the non-amyloidogenic cascade, which is initiated through the cleavage of APP by ADAM10 at the leucine[17] site. Pathological Aβ peptides are formed through the amyloidogenic cascade, which is initiated through the cleavage of APP by BACE1 at either the glutamate[11] or asparate[1] site.*

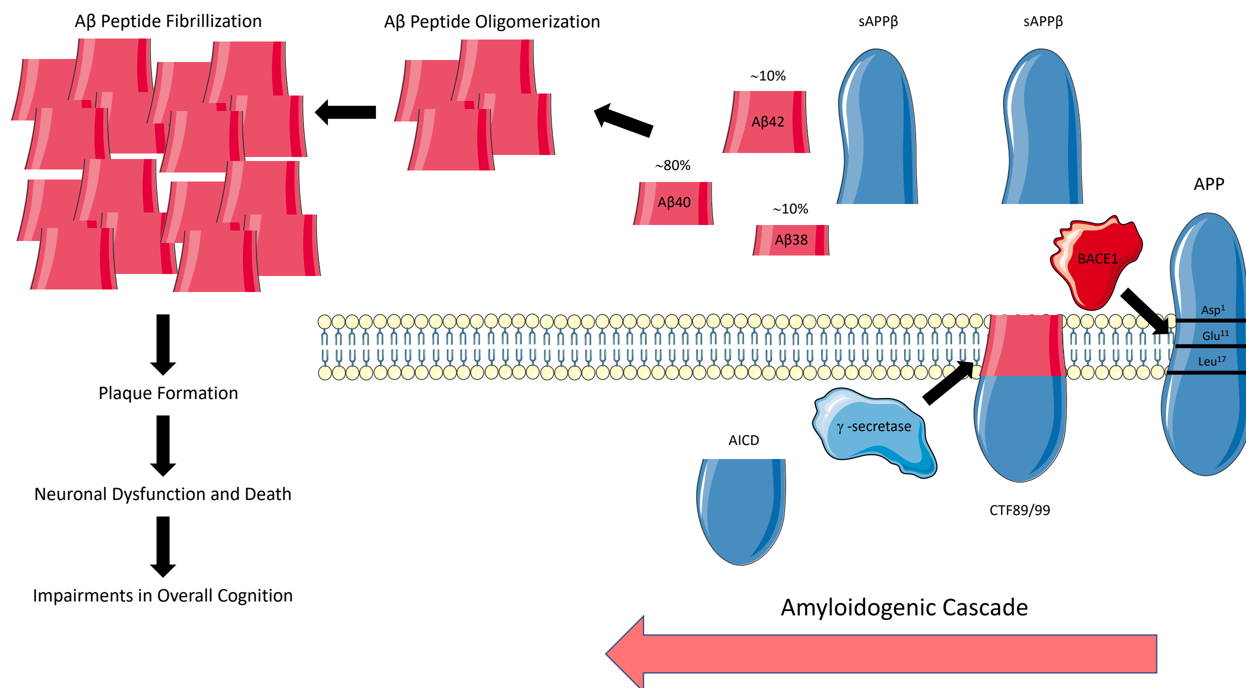


Figure 2: Pathological Role of Aβ in Neuronal Cells. *In the amyloidogenic cascade BACE1 cleaves APP imprecisely to form peptides that vary in length, with Aβ40 being the major length produced. These peptides can aggregate in the extracellular space of neuronal cells to form oligomers, which can form fibrils. These fibrils lead to plaque formation, which subsequently results in neuronal dysfunction and death.*

2. 1. 2 Beta-site Amyloid Precursor Protein Cleaving Enzyme 1

BACE1 is a type 1 transmembrane bound aspartic protease that cleaves proteins, such as APP (Figure1 and 2) [32]. There are two different isoforms of the enzyme, BACE1 and BACE2, however in the brain BACE1 is the predominant form of the enzyme that is expressed. BACE1 functions optimally in acidic pH environments and is often found in endosomes within the cell, which are constantly trafficked to the cell membrane [33]. Furthermore, this enzyme is highly expressed by neurons, specifically in the presynaptic terminals [33-35]. BACE1 is the rate limiting enzyme responsible for the production of Aβ peptides [29, 32]. Due to the high abundance of BACE1 in neuronal cells, this enzyme is thought to play a critical role in the

regulation of synaptic function through Aβ peptide production. Several studies have shown that normal Aβ peptide production is essential for maintaining neuronal function [36-39]. For example, a study conducted by Kamenetz and others proposed a novel negative feedback loop where Aβ peptides would keep neuronal hyperactivity controlled [40]. It was also postulated that if this feedback loop is disrupted and Aβ peptides are overproduced, AD progression may be worsened due to Aβ peptide-induced decreases in synaptic activity, which would lead to overall decreases in cognition [40]. This study used 6-day old organotypic brain slices from the hippocampus of transgenic rats that expressed the Swedish APP mutation and found that significantly increased neuronal activity resulted in increased Aβ peptide production [40]. Moreover, they found that Aβ peptides decreased excitatory synaptic transmission onto neurons, thus concluding that Aβ peptides indeed play a role in the regulation of synaptic activity [40]. Furthermore, several years later a study by Cirrito and colleagues followed up on this line of inquiry and investigated brain slices from the hippocampus of APP overexpressing Tg2576 mice at 3–5 months of age [41]. They found that significantly increased neuronal activity resulted in increased Aβ peptide production, which also reduced excitatory synapse activity [41]. This shows that synaptic activity is capable of triggering the production and release of Aβ peptides from neurons, which in turn down regulates neuronal activity and could prevent excitotoxicity [41]. Therefore, normal Aβ peptide production is vital for maintaining neuronal and synaptic function and should be considered when looking at therapeutic targets for AD. Even though BACE1 is the rate limiting enzyme in the amyloidogenic cascade and is highly involved in Aβ peptide production and AD progression, this evidence highlights that Aβ peptide production should not be eliminated entirely. Consequently, when the amyloidogenic cascade and its constituents are overactivated, as is the case with AD, Aβ peptides are produced in abundance,

which can then aggregate extracellularly causing neuronal dysfunction and death [31]. In response to this evidence, many efforts to examine the effects of removing BACE1 through knockout models have been explored due to the therapeutic potential. However, recent research has shown that animals that have a germline knockout of the *Bace1* gene often experience several undesirable phenotypes, such as reduced cell viability, reduced peripheral and central myelination, abnormal energy metabolism, abnormal neurogenesis, and impaired long-term potentiation, as well as memory [42-45]. Therefore, this evidence suggests that it is not desirable to delete the *Bace1* gene in the early stages of life due to its normal physiological role in producing Aβ peptides, as well as the other detrimental side effects. Evidence from human clinical trials have also yielded less than impressive results, so much so that five major prospective BACE1 inhibitors have been recently discontinued from clinical trials due to the participants developing several undesirable adverse effects, which were not outweighed by the benefits of the pharmaceutical [46, 47]. In light of all this evidence from animal and human studies, future research should be directed at finding alternative ways to regulate this enzyme's function, rather than eliminating it completely. One of the more promising methods for modulating BACE1 is the implementation of regular exercise.

2. 1. 3 Exercise and Beta-site Amyloid Precursor Protein Cleaving Enzyme 1

There have been decades of research to support the idea that physical activity and exercise are integral for maintaining health and fending off disease [48-50]. In the brain specifically, studies show that exercise has the capacity to increase learning, memory, and cognition while helping to prevent the onset of neurodegenerative disorders, such as AD [51-53]. At the cellular level evidence has suggested that exercise training has a neuroprotective effect

against Aβ peptide formation in several transgenic animal models of AD, such as NSE/APP_{sw} mice, 3xTg-AD mice, PS2 mice, and APP/PS1 mice [15, 54-58]. This neuroprotection is likely due to the fact that exercise has the ability to down regulate BACE1 content and activity, thereby reducing the amount of pathological Aβ peptides that are formed [15, 54-58]. A recent study by Xia and colleagues exercised 3-month-old male APP/PS1 expressing transgenic mice for 45 minutes per day, 5 days per week for 3 months [57]. This study found that just 3 months of treadmill exercise was able to significantly reduce Aβ peptide and plaque formation in the hippocampus, which was also accompanied by reduced BACE1 protein content [57]. This effect of long-term regular exercise on markers of AD has been shown in several different animal models of AD [15, 54-58]. Evidence from these studies also shows that exercise is capable of reducing *Bace1* mRNA transcription, upregulating α-secretase content, and downregulating products from the amyloidogenic cascade, such as the C89/99 fragments [15, 54-58]. It should be stated that studies using long-term exercise training protocols often also see improvements in adiposity, glucose homeostasis, insulin sensitivity, and reductions in inflammation, making it difficult to determine whether exercise has a direct effect on brain APP processing, or if the results are secondary to the previously stated benefits of exercise. Acute exercise training studies are a feasible alternative to address this limitation and may help provide a more complete picture looking at the effect of exercise on APP processing in the brain.

There is emerging evidence that an acute bout of exercise is capable of modulating BACE1 content and activity, demonstrating that there is a direct effect of exercise on the brain. A study done by MacPherson and others in 2015 showed that C57BL6/J mice on a high fat diet that were treadmill exercised for 2-hours had significant reductions in BACE1 protein content, as well as significantly reduced BACE1 activity in the prefrontal cortex 2-hours post-exercise [16].

In a follow up study by the same group, they demonstrated BACE1 content was reduced 2- and 8-hours post-exercise in the prefrontal cortex and 8- and 24-hours post-exercise in the hippocampus, highlighting regional differences in the response to exercise [19]. Results from both of these studies highlight that an acute bout of exercise is capable of reducing BACE1 activity and content, and that there seems to be regional differences in response to the exercise stimulus [16, 19]. Overall, there is sufficient evidence to suggest that exercise plays a role in the regulation of BACE1, however, it still remains unclear as to exactly what about the exercise stimulus could be driving these beneficial changes.

2. 1. 4 Alpha-secretase Regulation with Exercise

In the brain, the non-pathological pathway of APP processing is initiated by α-secretase (Figure 1) [24, 59]. There are several different α-secretase enzymes that are responsible for the cleavage of APP. This family of proteases are termed a disintegrin and metalloproteinase (ADAM) and there are several different forms in the brain, including ADAM9, ADAM10, ADAM17, and ADAM19, with ADAM10 being the most active in the processing of APP [60]. There is evidence that α-secretase content and activity is altered in a state of AD. Specifically, decreases in ADAM10 content have been shown in sporadic AD brain samples, as well as reduced ADAM10 activity in cerebral spinal fluid from AD patients [61-64]. Due to the ability of ADAM10 to cleave APP and synthesize non-pathological products, researchers have identified this enzyme as a potential therapeutic target for AD, and thus are trying to find ways to enhance its content and activity. One such way this enzyme could be regulated is through exercise. A study done by Koo and colleagues found that treadmill exercising transgenic mice with the Swedish APP mutation for 30-60 minutes per day, 5 days per week, for 12 months

resulted in significant increases in ADAM10, sAPPα, and CTFα protein expression in the cortex of the brain [15]. This study also found that products from the amyloidogenic cascade were reduced, such as sAPPβ, CTFβ, as well as Aβ40/42 peptides [15]. This evidence suggests that exercise has the ability to drive non-pathological processing of APP, however it still remains unclear as to the mechanism governing this effect. Mattson and his group sought to explain this phenomenon and proposed that BDNF, which is secreted during and post-exercise, would be able to increase α-secretase cleavage of APP [65]. In this study, male transgenic APP/PS1 mice that participated in voluntary wheel running for 3 weeks had significant increases in sAPPα and BDNF content, accompanied by decreases in Aβ40/42 peptide content in the hippocampus compared to controls [65]. In order to test if this decrease in Aβ peptides and increase in sAPPα was due to exercise-induced BDNF, this group used cultured human neuroblastoma SH-SY5Y cells. Cells were treated with 50ng/mL of BDNF, 5μM of batimastat (an α-secretase inhibitor), or a combination of BDNF and batimastat [65]. Importantly, 3 days of BDNF treatment led to decreases in Aβ40/42 peptide formation, along with increases in sAPPα into the medium, which was measured using a multiplex ELISA [65]. However, when cells were treated with both BDNF and batimastat, Aβ40/42 peptide formation remained significantly higher than the control, indicating that BDNF likely works through an α-secretase related mechanism [65]. Furthermore, α-secretase cleavage of APP produces sAPPα, which has a role in the regulation of BACE1. There has been both *in vitro* and *in vivo* evidence that sAPPα can directly inhibit BACE1, and reduce subsequent products of the amyloidogenic cascade, such as sAPPβ, CTFβ, and Aβ40/42 peptides, highlighting the potential existence of an inhibitory feedback mechanism [65, 66]. Exercise and exercise-induced BDNF may be able to promote non-amyloidogenic processing of APP, which would lead to increases in sAPPα that has been shown to inhibit the initiation of the

pathological amyloidogenic cascade [65, 66]. More evidence is required investigating the exact mechanisms that govern α-secretase activity and if BDNF is the only exercise-induced factor capable of modulating its function.

2. 2 Various Exercise-Induced Signaling Mediators

2. 2. 1 Exercise Mediated Muscle-Brain Crosstalk

Regular exercise is integral for procuring and maintaining brain health not only in patients with neurodegenerative disorders, such as AD, but also healthy individuals [49, 53, 67]. Recent evidence suggests that the muscle and brain are connected, however it is not entirely clear as to the mechanism or set of mechanisms that govern this connection. One major hypothesis that explains this connection between exercise and brain health is the existence of a muscle-brain endocrine loop, which allows for tissue crosstalk [20-22, 49, 68-74]. During and post-exercise skeletal muscle releases various signaling mediators called myokines into the bloodstream that facilitate inter-tissue communication, such as BDNF, cathepsin B, lactate, and IL-6 [20-22, 49, 68-75]. It should be noted that some of these signaling mediators are also released by other metabolically active organs in the body, such as the brain, adipose tissue, liver, and gut (Figure 3) [20, 74]. Due to this, some researchers have proposed that the sum of these signaling mediators be deemed "exerkines", as they are released by multiple tissues with an exercise stimulus and are the likely candidates that facilitate tissue crosstalk [22, 73, 74, 76]. These exerkines are often packaged in specialized extracellular vesicles called exosomes, along with other various proteins, nucleic acids, peptides, DNA, and RNA, which are carried throughout peripheral circulation to various different targets [74, 76]. In humans, an acute bout of exercise has been shown to increase exosome release into circulation, indicating that these vesicles not

only play a role in mediating inter-tissue communication, but also are released with an exercise stimulus [77]. It remains unclear how many of the known exercise-induced signaling mediators can actively cross the blood-brain-barrier (BBB), and what their role is in the brain if they are able to cross. In the following sections evidence will be presented for likely exercise-induced signaling mediators that could be responsible for driving the changes seen with exercise in the brain.

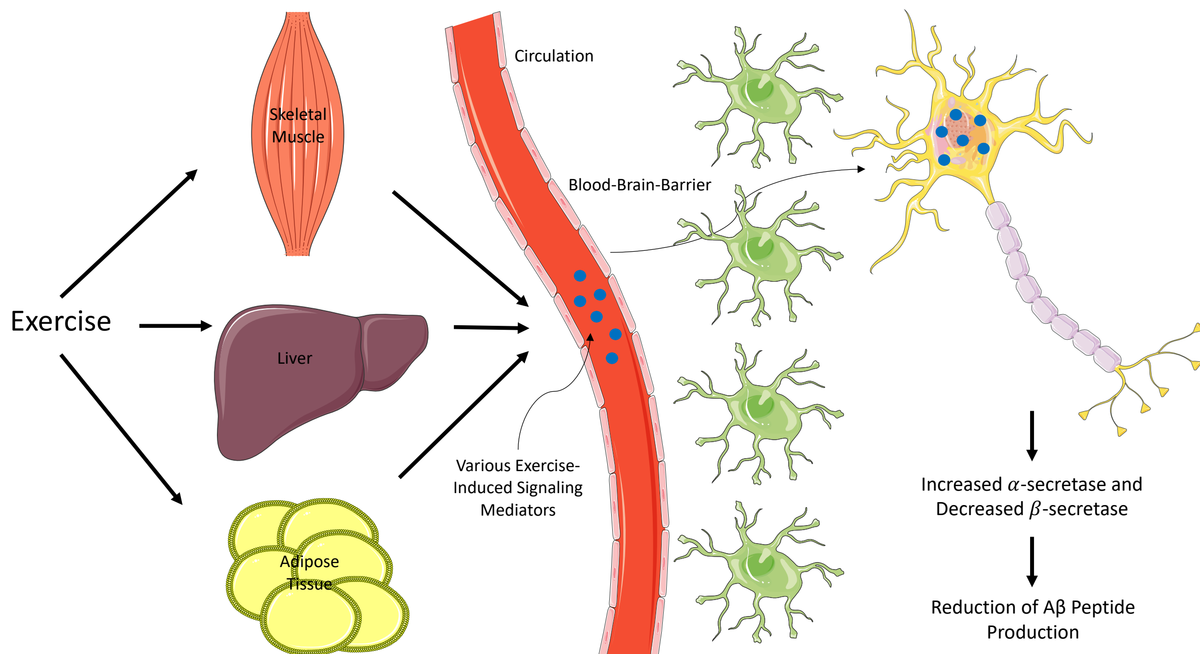


Figure 3: Tissue Release of Various Exercise-Induced Signaling Mediators. *Exercise has the capacity to facilitate the release of various signaling mediators into circulation that can facilitate tissue crosstalk between several metabolically active organs. Certain exercise-induced signaling mediators travel to the brain and evoke changes in various signaling cascades, such as APP processing. This may lead to a shift towards non-pathological processing of APP through increased α-secretase activity.*

2. 2. 2 Brain-Derived Neurotrophic Factor

Neurotrophins are proteins that play an integral role in the development and maintenance of cells throughout the body. BDNF is an important neurotrophin, which was originally

discovered in 1982 in pig brains and identified as a growth factor [78]. However, the function of BDNF does not solely affect the brain. In fact, BDNF can be found in several tissues, such as skeletal and cardiac muscle, lung, bladder, and intestinal tissue, as well as vascular endothelial cells, peripheral blood mononuclear cells, and platelets [79-84]. This evidence suggests that BDNF may be a likely candidate for mediating tissue crosstalk with other organs in the body. In the brain specifically, BDNF is vital for the proliferation, differentiation, and maturation of immature neurons, as well as maintaining synaptic plasticity and normal function in already mature neurons [85-87]. In the areas of the brain that display a high degree of plasticity, namely the prefrontal cortex and hippocampus, BDNF is highly expressed [87, 88]. In cases of neurodegenerative diseases, specifically AD, in neuronal plasticity, leading to decreases in learning, memory, and executive functions [6]. This decline in functional outcomes is accompanied by a decrease in BDNF content, which highlights the importance of finding new methods to maintain and increase BDNF expression throughout the lifespan [89].

Exercise is potent driver of BDNF release. Studies have shown that a wide range of exercise methods can elicit BDNF release in normal healthy individuals, as well as patients with AD [90-92]. This means exercise may be a safe alternative to pharmaceutical treatment of neurodegenerative disorders, such as AD. There is variability in the literature but typically, circulating levels of BDNF can be roughly 100-1,000pg/mL and can increase upwards of 2-fold after exercise [92-95]. There is still a debate as to whether BDNF has the ability to cross the BBB due to the lack of BDNF transporters on the cerebrovascular endothelium, as well as the cationic properties of the protein [96, 97]. However, more recent evidence has suggested that BDNF does cross the BBB in a bi-directional manner through a high-capacity saturable transport system [98-101]. Current research has employed the use of various animal models to measure

BDNF expression, release, signaling, and understand its overall role in the brain, as it is challenging to measure BDNF in the human brain with the methods currently available. Acute bouts of aerobic exercise have been shown in several rodent models to have the capacity to upregulate the transcription of BDNF mRNA, as well as BDNF protein content in the hippocampus [102-105]. This evidence suggests that exercise has beneficial effects on the brain that are likely mediated in part by exercise-induced BDNF. Interestingly, a study by Matthews and colleagues showed that human skeletal muscle contraction increased intramuscular BDNF protein expression but was not released into circulation [102]. To further test this phenomenon, electrical stimulation of C2C12 muscle cells resulted in increased BDNF protein levels within the cells, however no changes in BDNF were detected in the cell media [102]. These results suggest that skeletal muscle derived BDNF acts in an autocrine/paracrine fashion and does not significantly contribute to circulating BDNF levels. However, there is now new evidence to suggest muscle is capable of releasing BDNF. A comprehensive study by Fulgenzi and colleagues showed that after 90 minutes of electrical stimulation, differentiated C2C12 muscle cells released BDNF into the cell media [75]. Additionally, human myocytes were found to release biologically active BDNF into the cell media after differentiation was complete [75]. To test this phenomenon further, muscle specific BDNF KO mice were used. It was observed that BDNF KO mice have lower circulating levels of BDNF as compared to a control, indicating that muscle does release BDNF into the periphery and thus contributing to the total pool of circulating BDNF [75]. Although the source of exercise-induced BDNF that acts on the brain remains controversial, there is new evidence to suggest post-exercise BDNF plays a role in APP processing, further highlighting its potential as a therapeutic target for neurodegenerative diseases, such as AD.

There is now direct evidence that BDNF influences APP processing. A study done by Baranowski and others demonstrated this connection using an explant study [105]. Both the prefrontal cortex and hippocampus from sedentary C57BL/6J male mice were collected and subsequently treated with 100ng/mL of BDNF for 30 minutes. Following treatment, BACE1 activity was measured, and it was revealed that in the prefrontal cortex the activity of this enzyme was reduced by 39.4%. There were no observed changes in BACE1 activity in the hippocampus, however the acute 30 minute treatment may not have been a long enough duration, as another study from this group showed that exercise-induced changes in hippocampal BACE1 take significantly longer than the prefrontal cortex [19]. Findings from this study, along with others cumulatively suggest that exercise-induced BDNF may promote a shift in APP processing towards the more favourable non-pathological pathway by decreasing BACE1 function and increasing ADAM10 function [15, 65, 105]. During exercise, there would be a multitude of other signalling mediators secreted into circulation, which could potentially influence APP processing similar to that of BDNF.

2. 2. 3 Cathepsin B

Cathepsin B is a part of a family of lysosomal cysteine proteases, which are involved in the degradation of various peptides and proteins. Following the discovery of cathepsin B, this protein has gained attention for its role in altering Aβ peptide formation [106-109]. There is now evidence that aerobic exercise stimulates the release of this protein from skeletal muscle to act in an endocrine fashion [107]. In a study by Moon and colleagues both acute 14- and 30-day voluntary wheel running protocols increased muscular expression of the *Ctsb* gene, which encodes cathepsin B, as well as increased protein levels in the gastrocnemius muscle and plasma

of C57BL/6 male mice [107]. Furthermore, a chronic treadmill exercise protocol consisting of 0.25 miles per day, 5 days per week, for 4 months increased peripheral cathepsin B levels in Rhesus monkeys, further highlighting the ability of an exercise stimulus to promote the release of cathepsin B [107]. These findings in animal models were corroborated in a human model as 4 months of moderate to intense treadmill exercise resulted in increased circulating levels of cathepsin B in young healthy adults [107]. This observed increase in exercise-induced cathepsin B levels was also correlated with improvements in hippocampus-dependent task performance, highlighting the potential of this signaling molecule to promote tissue crosstalk with the brain [107]. In order to test this signaling molecules ability to cross the BBB and potentially mediated tissue crosstalk Moon and colleagues injected 50μg recombinant CTSB intravenously into CTSB KO mice [107]. Following injection, there were significant increases in blood and brain CTSB 15 minutes post-injection, further emphasizing the ability of this exercise-induced signaling molecule to mediate inter-tissue communication [107].

This novel exercise-induced signaling mediator has also been thought to have effects on the amyloidogenic cascade, as increases in cathepsin B activity and content in the brain have been shown to be associated with decreased Aβ peptide levels in mice overexpressing human APP [106, 108, 109]. A study by Mueller-Steiner and colleagues showed that in mice lacking cathepsin B and overexpressing human APP there were increases in Aβ peptide formation and plaque deposition [108]. Additionally, a recent study by Embury and others showed that transgenic APP/PS1 mice with adenoviral overexpression of cathepsin B exhibited decreased Aβ40/42 peptide formation in the hippocampus, as well as improved spatial learning and memory as measured with a radial arm water maze task [106]. Taken together, these results show

the beneficial role of cathepsin B on APP processing and highlight the potential importance of exercise-induced cathepsin B for neurodegenerative diseases, such as AD.

These beneficial effects of cathepsin B may be mediated by its the ability to increase hippocampal BDNF expression, which is a key player in synaptic plasticity and neurogenesis [85, 88]. Along with the ability of cathepsin B to be released by skeletal muscle with exercise, it has also been shown to increase *Bdnf* mRNA expression and BDNF protein content in hippocampal adult neural progenitor cells [107]. Cumulatively, this evidence suggests that exercise-induced increases in cathepsin B could have beneficial effects on hippocampal neurogenesis, learning, memory, and BDNF expression, which make it a potential therapeutic target for the prevention and treatment of AD.

2. 2. 4 Lactate

More than 100 years after the initial discovery of lactate, new research is being published highlighting the unique properties of the molecule once thought to be just a metabolic waste product. During and after exercise, lactate is released from skeletal muscle into the blood where it can be subsequently taken up by several tissues, including the liver, heart, kidneys, adipose tissue, lungs, skeletal muscle, and the brain [110-112]. In these tissues, lactate can be taken up and oxidized for energy, as well as releasing lactate into the periphery and contributing to the circulating lactate levels [112]. Under normal conditions, resting human lactate levels are found to be between 0.5-1.5mmol/L, however with exercise, circulating lactate levels can rise to anywhere between 10-25mmol/L into a state of hyperlactatemia [112-114].

In the brain, lactate has been identified to play an important role as an energy source as well as a signaling molecule. Post-exercise lactate released into the periphery by skeletal muscle

and has the ability to reach the brain by crossing the BBB via endothelial monocarboxylate transporters (MCTs), highlighting its ability to promote tissue crosstalk with the brain [115]. In the brain, several MCTs are expressed, including MCT1, 2, and 4 [115]. At the cellular level, MCT2 is predominantly expressed by neurons, while MCT4 seems to be specific to astrocytes [115]. Once in the brain, lactate can be oxidized to pyruvate by the lactate dehydrogenase enzyme and serve as a fuel source for neurons under both basal and hyperlactatemic conditions [111, 116]. Cerebral lactate allows for the promotion of long-term potentiation of neurons, adult hippocampal neurogenesis, synaptic plasticity, and memory formation [117-122]. However, in cases of neurodegeneration, such as with AD, lactate metabolism can be dysregulated and may be an indication of other underlying metabolic disturbances. In a study by Zhang and colleagues, 3-month-old APP/PS1 transgenic mice were found to have decreased quantity of neurons, lactate content, and MCTs, while also exhibiting increases in Aβ plaques and astrocytes [123]. This study highlights reductions in lactate and its transporters in an AD model may propagate the disease progression. There is sufficient evidence that lactate is beneficial in the promotion and maintenance of neuronal function, which emphasizes the importance of exercise-induced lactate.

Exercise-induced increases in peripheral blood lactate levels are associated with increased circulating BDNF levels, which could be mediating some of the observed neurological benefits [124-127]. A recent study done by El Hayek and colleagues examined this connection between exercise, lactate, and BDNF using C57BL/6 male mice [128]. Mice were injected intraperitoneally with either 117mg/kg or 180mg/kg of lactate in order to mimic the levels of exercise induced lactate [128]. Lactate injection resulted in significantly increased *Bdnf* gene expression and BDNF protein content in the hippocampus, as well as increased protein levels of the synaptic plasticity genes ARC and ZIF268 [128]. Inhibition of MCTs with 50nM of AR-

C155858 resulted in abolition of the lactate induced *Bdnf* expression in the hippocampus, further highlighting the importance of lactate crossing the BBB and entering the brain [128]. To test whether increased lactate concentrations resulted in functional outcomes on learning and memory, mice were injected with lactate and then underwent a Morris Water Maze task [128]. It was found that 4 hours after the lactate injection mice experienced increased memory recall times compared to the controls [128]. While the effects of lactate as a signaling molecule in neurons are still emerging, the current body of literature suggests that lactate plays a role in the induction of BDNF expression, synaptic plasticity, as well as learning and memory formation. This evidence highlights the potential of lactate as one of the exercise-induced molecules that could be beneficial for neurodegenerative diseases, such as AD, however a connection to APP processing has yet to be examined.

2. 2. 5 Interleukin-6

Cytokines are small proteins released by various cells in the human body that have many roles, one of which is to mediate communication between cells [129]. These cytokines can form complex communication networks that are integral for maintaining whole-body metabolic function [68, 70]. Immunomodulatory cytokines can act as messengers within these networks by circulating in low concentrations and exerting effects on major metabolic tissues [68, 70]. One major cytokine is IL-6, which is a pleiotropic cytokine that has both pro- and anti-inflammatory properties [129]. In the past, the role of chronic IL-6 exposure has been prioritized, however there is new evidence emerging that highlights the role acute IL-6 plays in regulating energy homeostasis [70, 71, 130-132]. Exercise is a potent driver of IL-6 release from various tissues. At rest, plasma IL-6 concentrations are typically below 10pg/mL, and with exercise this

concentration can increase by approximately 10-fold with the maximum value usually not exceeding 100pg/mL [133-138]. IL-6 was identified as one of the first cytokines to be released from skeletal muscle in response to exercise and was the inspiration for the term “myokine”, which was introduced by the Swedish scientist Bengt Saltin in 2003 [139]. Exercise induced IL-6 is now known as an important signaling molecule able to promote tissue crosstalk [68, 70, 71]. Research has shown the ability of acute IL-6 to promote glucose uptake and metabolism in tissues such as the liver, skeletal muscle, and the brain, showing the importance of IL-6 as a regulator of glucose homeostasis [131, 132].

In the brain and central nervous system, IL-6 has been shown to influence neuronal differentiation and adult neurogenesis [129, 140, 141]. Under normal physiological conditions IL-6 induces a cholinergic phenotype of sympathetic neurons [142-144]. Furthermore, IL-6 has been shown to promote the survival of several different cell types, including retinal ganglion cells, sympathetic neurons, dorsal root ganglion cells, basal forebrain and septal cholinergic neurons, as well as mesencephalic catecholaminergic neurons [142, 145-148]. There is also some evidence connecting IL-6 and BDNF, as it has been demonstrated that IL-6 influences the synthesis of BDNF [149]. A study done by Murphey and colleagues demonstrated that intrathecal infusion of 250ng/hour of recombinant IL-6 in Sprague-Dawley rats resulted in increased BDNF mRNA in the adult rat lumbar dorsal root ganglia [149]. This evidence suggests that IL-6 may be capable of promoting and increasing BDNF expression, which could have beneficial effects for healthy individuals, as well as patients with neurodegenerative diseases, such as AD.

The brain has been shown to release IL-6 during prolonged exercise, also highlighting the brain as a source of exercise-induced IL-6, and the ability of this cytokine to cross the BBB

[150]. The role of brain derived IL-6 with exercise is not fully understood. Rasmussen and colleagues showed that C57BL6 mice that ran for 2 hours at a pace of 18 meters/minute on a 10% slope had increased hippocampal IL-6 mRNA expression, coupled with decreased glycogen content [151]. This evidence indicates that exercise-induced IL-6 could be playing a role in regulating cerebral energy homeostasis. Expanding upon this knowledge, a recent study from our laboratory demonstrated the effect of acute IL-6 in the promotion of glucose uptake in neurons. Human SH-SY5Y neuroblastoma cells were differentiated into cholinergic neurons using 10μM of retinoic acid and treated with 100nM of human insulin, 10ng/mL human IL-6, or 20ng/mL human IL-6 [132]. It was found that treatment with 20ng/mL of IL-6 resulted in significant phosphorylation of proteins in the classical IL-6 signaling cascade, such as STAT3, as well as proteins in the AMPK signalling pathway responsible for promoting glucose uptake, such as AS160, which were measured using western blot analysis [132]. This increase in protein phosphorylation due to IL-6 treatment also increased glucose uptake and GLUT4 translocation to the neuronal plasma membrane in an insulin independent manner [132]. Glucose uptake was measured using a [^{3}H]-2-deoxy-D-glucose (2DG) uptake assay, and transfected SH-SY5Y cells with fluorescently labelled GLUT4myc-GFP DNA constructs [132]. Overall, acute IL-6 treatment is able to activate proteins involved in GLUT4 translocation and promote glucose uptake in neurons.

New evidence has been published showing that exercise-induced IL-6 may be linked with increases in exercise-induced lactate. A recent study by the Pedersen group showed that IL-6 release during exercise is mediated by a lactate-dependent increase in protease activity [152]. In the study, 13 healthy young men participated in 2 hours of high-intensity interval cycling, where it was found that both circulating lactate and IL-6 levels were increased during the exercise trial

[152]. This effect was also observed in mice, where female NMRI mice that were intramuscularly injected with 20μL of lactate showed significant increases in circulating IL-6 levels [152]. Furthermore, electrical stimulation of myotubes from human vastus lateralis skeletal muscle also increased IL-6 [152]. The authors further investigated the lactate-dependent increase in protease activity as a mechanism behind the observed IL-6 release. In mice performing swimming exercise, it was identified through bioimaging that a host of intramuscular proteases were increased with exercise, specifically matrix metalloproteinase-9 (MMP-9) [152]. However, with the application of the MMP2/9 blocker Marmistat, exercise-induced IL-6 release was blunted during the swim exercise [152]. Additionally, intramuscular injection of the protease hyaluronidase resulted in increased serum IL-6 levels [152]. Altogether, this study indicates that exercise-induced IL-6 released from skeletal muscle during exercise is stimulated by a lactate-dependent increase in protease activity indicating that the release of lactate can drive increases in IL-6, which could potentially have an effect on the brain, specifically APP processing. This further highlights the inter-connectivity of these exercise-induced signaling mediators potentially indicating the importance of not just one factor but a combination of factors to improve health, and specifically brain health.

The link between IL-6 and APP processing in the brain remains understudied. However, with the role of acute exercise-induced IL-6 emerging there is now sufficient evidence to investigate the role of acute exercise-induced IL-6 on APP processing, which could have a beneficial role in neurodegenerative diseases, such as AD [153].

2. 2. 6 High Intensity Interval Training and Exercise-Induced Signaling Mediators

High intensity interval training (HIIT) is defined as a short burst of vigorous physical exercise followed by a relatively short recovery period [154-160]. This vigorous exercise followed by recovery is then repeated in a series of intervals with the total amount of time lasting approximately 15-30 minutes per workout [154-160]. Unlike traditional low/moderate intensity continuous training, HIIT workouts are short in duration due to their high degree of intensity [154-160]. In our current society this exercise regime is attractive for individuals who live busy lives and have limited time to exercise. This style of physical exercise may also prove useful for individuals who are unable to exercise for long durations, due to physical limitations, to still get the benefits of exercise without spending hours working out. Additionally, HIIT has been reported to be more enjoyable than continuous training and seems to have similar health benefits to continuous training in terms of metabolism, cardiorespiratory fitness as well as cerebrovascular, musculoskeletal, and brain health [154-161]. Currently, the effect of HIIT on the brain is limited but some studies indicate that HIIT improves cognitive performance in both young and old individuals, indicating this type of exercise influences the brain [162-165].

One of the reasons HIIT training may be so effective despite the short duration is the potent release of various exercise-induced factors or exerkines due to the intense nature of the exercise. Release of factors such as BDNF, IL-6, and lactate have all been shown to be intensity and duration dependent [92, 93, 152]. These factors all play a role in the maintenance of neuronal health and HIIT seems to be a safe and time efficient modality to increase these factors into circulation, potentially having an effect on modulating APP processing [20-23].

Chapter 3: Purpose and Specific Aims

3. 1 Statement of the Problem

Production and aggregation of Aβ peptides in the brain is detrimental to neuronal cell production, survival, function, and often results in neuronal dysfunction and death [26]. The specific mechanism or set of mechanisms that governs this increased accumulation of Aβ peptides is complex. BACE1 is the main enzyme in the amyloidogenic cascade and is responsible for the cleavage of APP [28, 30]. Overactivation of this enzyme leads to an increase in Aβ peptide formation and subsequent aggregation, which results in neuronal dysfunction and death [28, 30]. On the other hand, ADAM10 also competes for the cleavage of APP and is the main enzyme in the non-amyloidogenic cascade, which produces non-pathological products that do not impair neuronal function [24, 60]. Therefore, understanding the mechanisms of how BACE1 and ADAM10 are regulated is paramount for determining how Aβ peptide production can be altered in order to preserve normal neuronal function. Previous work has shown that exercise is capable of regulating BACE1 activity and content in mice, however it remains unclear as to what about the exercise stimulus is precipitating the changes observed with BACE1 [16, 19]. Recent research has postulated the idea of exercise-induced signaling factors, sometimes called exerkines, that are released into circulation following a bout of exercise [20-22, 49, 68-74]. Specifically, BDNF, cathepsin B, IL-6, and lactate are four very promising exercise intensity induced candidates for signaling mediators that can cross the BBB and influence APP processing. That said, further investigation is required into if these factors, which are present in exercise serum, do indeed influence APP processing, especially in neurons.

3. 2 Purpose

The purpose of this study is to examine whether post-exercise serum from humans can influence APP processing in cultured neurons. Specifically, this study examines whether exercise-induced factors present in post-exercise serum will alter the activity, mRNA, and or protein content of the key players in the amyloidogenic and non-amyloidogenic cascades, namely BACE1 and ADAM10 in human SH-SY5Y neuronal cells.

3. 3 Hypothesis

It is hypothesized that factors present in post-exercise serum will be able to lower amyloidogenic processing of APP. This change in APP processing will be observed in the form of decreases in BACE1 mRNA, content, and activity, as well as decreases in sAPPβ protein content. Serum treatment will also result in a shift towards non-amyloidogenic processing of APP, resulting in increases in ADAM10 mRNA, protein content, and activity, followed by increases in sAPPα protein content.

Chapter 4: Materials and Methods

4.1 Materials

High glucose Dulbecco's Modified Eagle's medium (DMEM) (cat# D6429-500mL), non-essential amino acid solution (cat# M7145-100mL), penicillin/streptomycin solution (cat# P4333-100mL), trypsin EDTA solution (cat# T4049-500mL), fetal bovine serum (FBS) (cat# F1051-500mL), retinoic acid was acquired from Sigma-Aldrich (cat# R2625). SH-SY5Y cells used for cell culture were donated by Dr. Brian Roy from Brock University. Cell culture plates (6-well) were from Fischer (cat# 10062-892). Taqman gene expression assays for *APP* (HS00169098_m1), *BACE1* (HS01121195_m1), *ADAM10* (HS00153853_m1), *BDNF* (HS02718934_m1), and *GAPDH* (HS02786624_m1) were also purchased from Fischer for this study. Taqman Fast Universal PCR Master Mix 2X (cat# 4352042) and MicroAmp Fast 96 Well Reaction Plates (cat# 4346907) were purchased from Applied Biosystems. DNA-Free Kit (cat# AM1906), SuperScript II Reverse Transcriptase (cat# 18064-014), 0.1 mM DTT (cat# Y00147), RNase Out (cat# 100000840), and 5X First Strand Buffer (cat# Y02321) were all purchased from Invitrogen. NP40 Cell Lysis Buffer was purchased from Life Technologies (cat# FNN0021) and supplemented with phenylmethylsulfonyl fluoride and protease inhibitor that was purchased from Sigma-Aldrich (cat# 7626-5G, cat# P274-1BIL). Antibodies against APP (Biolegend, cat# SIG039152), BACE1 (1:1000, Cell Signaling cat# 5606P), sAPPβ (1:1000, BioLegend cat# SIG-39138), ADAM10 (1:1000, Abcam, cat# ab1997), sAPPα (1:1000, Immuno-Biological Laboratories, cat# 11088) were purchased for this study. Horseradish peroxidase-conjugated donkey anti-rabbit (cat# 711-035-152) and goat anti-mouse (cat# 115-035-003) IgG secondary antibodies were from Jackson ImmunoResearch Laboratories (Westgrove,PA). Molecular weight marker, reagents, and nitrocellulose membranes for SDS-PAGE were acquired from Bio-Rad

(Mississauga, ON) and GE Healthcare Life Science (cat# 10600002). Western lightning Plus-ECL was obtained from PerkinElmer (cat# 105001EA).

4. 2 Post-Exercise Serum Collection

This study involves posteriori analysis of blood collected from twenty male participants ages 19-26 years old before and after the completion of HIIT running trials [166]. Blood collection was approved by the Research Ethics Board at Brock University (19-131). Inclusion criteria, anthropometrics, body composition, nutritional intake, and exercise protocols were as previously described [166]. Briefly, participants completed a HIIT running trial, which consisted of eight 1-minute intervals of running with 1-minute recovery period [166]. Maximum speed and incline achieved during the incremental running test was used to determine the workload for the running intervals [166]. Mean heart rate of the eight running trials was recorded and participants' mean heart rates were ≥90% of their maximum throughout the trials [166]. A total of 10mL of blood was collected from the median cubital vein in the antecubital fossa from participants [166]. Blood was taken pre-exercise, and 5 minutes post-exercise [166]. All blood samples were left to clot for at least 30 minutes in vacutainers before being centrifuged at 3000g and 4°C for 15 minutes in a Beckman Coulter benchtop Allegra ZIR centrifuge [166, 167]. Serum from each sample was aliquoted into microcentrifuge tubes and stored at −80°C for future analysis. To achieve a large spike in exercise-induced factors, serum from the 5-minute post-exercise timepoint was used for all cell treatments in this project.

4. 3 Blood and Biochemical Analysis

4. 3. 1 IL-6 ELISA

Following the conclusion of the high-intensity interval running trials, serum levels of inflammatory cytokines, including IL-6, were measured in duplicate with a commercially available multiplex kit (Cat# HSTCMAG-28SK, EMD Millipore). A human high sensitivity T cell magnetic bead panel was used as previously described [166].

4. 3. 2 BDNF ELISA

BDNF in the serum samples was measured using a commercially available ELISA (Cat# CYT306, EMD Millipore), which uses rabbit polyclonal antibodies generated against human BDNF [92]. These antibodies are coated onto a microplate and are used to capture BDNF from the post-exercise serum. Monoclonal mouse antibodies conjugated to biotin detect the captured BDNF. A 30-fold dilution was used for this ELISA and a total of 10μL was loaded into each well to detect total BDNF content. BDNF levels present in the post-exercise serum were determined after addition of streptavidin-enzyme, as well as substrate and stop solution, which was measured at 450nm using the SpectraMax M2 reading spectrometer from Molecular Devices.

4. 4 SH-SY5Y Human Neuroblastoma Cell Culture

Undifferentiated SH-SY5Y cells at passage 14 were cultured in DMEM containing 10% FBS, 2% non-essential amino acids (NEAA), and 1% penicillin/streptomycin. Use of this cell line was approved by the Research Ethics Board at Brock University (#17-397). Cells were left to grow until 80–90% confluent in a flask. Cells were seeded into 6-well plates at a density of

100,000cells/mL and cultured in DMEM supplemented with 1% FBS, 2% NEAA, and 1% penicillin/streptomycin, along with 10µM of retinoic acid to initiate the differentiation process [132, 168]. Differentiation was done for 5 days with the media and retinoic acid being changed every two days. Media was changed to fresh DMEM prior to all experiments. All serum samples were heat-inactivated prior to all cell treatments at 56°C for 30 minutes. Serum samples with increased levels of BDNF and IL-6 were pooled together and used for all cell treatments. For acute experiments cells were treated with 10% pre- or post-exercise serum for 30 minutes and collected immediately after [169-177]. For chronic experiments cells were again treated with 10% pre- or post-exercise serum for 30 minutes and collected 24 hours later [178]. Following treatment, cells were lysed using 100µL of NP40 Cell Lysis Buffer supplemented with 34µL phenylmethylsulfonyl fluoride and 50µL protease inhibitor cocktail. Lastly, the cells were scraped and collected in tubes where they were sonicated for two 20 second bouts on ice and immediately stored in a −80°C freezer for future analysis.

4. 5 RT-qPCR

Changes in mRNA expression was determined using real-time quantitative polymerase chain reaction (PCR) as described previously [16, 105]. RNA was isolated from serum treated cells after being scraped off the culture plate. A RNeasy kit was used according to the manufacturer's instructions (Qiagen, cat# 74106). DNA-free treatment was used to purify the samples before use. A 10% volume of 10X DNAse buffer was added to each mRNA sample, followed by 1µL of rDNAase buffer. Samples were then incubated at 37°C for 20 minutes. A 10% volume of 10X DNAse inhibitor was added to each RNA sample and the samples were subsequently centrifuged at 10,000g for 2 minutes. Supernatant was removed from each sample

and yield and purity of the isolated mRNA was determined using the NanoVue plus Nano-drop system from GE healthcare. mRNA samples were prepared at 1µg/µL using RNase free water. mRNA from serum treated cells was extracted and reversed transcribed into cDNA. Synthesis of cDNA was done with primers and dNTPs at a 1:1 ratio, as well as a master mix containing 5x FSB, DTT, RNase out and SuperScript II Reverse Transcriptase. Reverse transcription PCR was performed with a 7500 Fast Real-Time PCR system from Applied Biosystems. Each sample was loaded in duplicate and contained 10µL of PCR master mix, 4µL of RNase free water, 1µL of gene expression assay and 5µL of cDNA, which was diluted with 80µL of RNase free water. Gene expression was measured for *APP*, *BACE1*, *ADAM10*, *BDNF*, as well as *GAPDH*, which was used as a housekeeping gene. Relative differences in mRNA expression were determined using the $2^{-\Delta\Delta CT}$ method [179].

4. 6 Western Blotting

Protein concentration in the cell lysates was determined using a bicinchroninic acid (BCA) quantification assay [180]. Equal amount of protein was loaded on polyacrylamide gels and separated by electrophoresis at 120V for 90 minutes. Proteins from the gel were wet transferred onto nitrocellulose membranes for 1 hour at 100V on ice. Membranes were subsequently placed in a 5% dry milk-Tris-buffered saline/0.1% Tween 20 (TBST) blocking buffer for 1 hour. Primary antibodies were prepared at a ratio of 1:1000 in a 5% bovine serum albumin (BSA) and TBST solution, and membranes were incubated overnight on a rocker at 4°C. Following the overnight incubation, membranes were washed for 5 minutes with TBST and repeated 4 times. The specific horseradish peroxidase secondary antibody was prepared in a ratio of 1:2000 1% milk solution before being added to the membranes, which was incubated at room

temperature for 1 hour. Signals were detected using enhanced chemiluminescence and were subsequently quantified by densitometry using a Bio-Rad ChemiDoc imaging system and analyzed using AlphaView software. Representative ponceau stains were analyzed for each membrane to ensure equal loading (≤10% variability across the membrane) [181]. Western blot analysis was used to examine protein content of proteins involved in APP processing, namely APP, BACE1, sAPPβ, ADAM10, and sAPPα.

4. 7 BACE1 Activity Assay

BACE1 activity was determined using a commercially available BACE1 activity assay kit (Abcam, cat# ab65357) as previously described [16, 19]. All samples were prepared at 0.75μg/μL and a total of 50μL of prepared sample was added to each well in duplicate, followed by 50μL of 2X reaction buffer and 2μL of β-secretase substrate. The plate was left to incubate in the dark covered with aluminum foil at 37°C for 60 minutes and end-point fluorescence was read using the SpectraMax M2 reading spectrometer from Molecular Devices at excitation and emission wavelengths of 335 and 495nm, respectively. BACE1 activity in the cell lysates is tested through the addition of a secretase-specific peptide that is conjugated to two reporter molecules, EDANS and DABCYL. In the un-cleaved form, fluorescent emissions from EDANS are quenched by DABCYL due to their proximity to one another. BACE1 cleaves this peptide, thus physically separating EDANS and DABCYL, which allows the release of a fluorescent signal.

4. 8 ADAM10 Activity Assay

ADAM10 activity was determined using a commercially available α-secretase activity assay kit (SensoLyte 520, cat# AS-72226). All samples were prepared at 0.75μg/μL and a total of 50μL of prepared sample was added to each well in duplicate, followed by 50μL of assay buffer with 0.1mg/mL of recombinant ADAM10. The plate was left to incubate in the dark covered with aluminum foil at 37°C for 60 minutes and end-point fluorescence was read using the SpectraMax M2 reading spectrometer from Molecular Devices. When active ADAM10 cleaves the FRET substrate, it results in an increase of 5-FAM fluorescence monitored, which can be detected at excitation and emission wavelengths of 490 and 520nm respectively.

4. 9 Statistical Analysis

Statistical significance was determined using student T-tests for PCR, western blot, and activity data. Cell viability was determined using a Two-way ANOVA followed by Tukey post-hoc analysis. To test for normality, a Shapiro-Wilk test was conducted. Data is expressed as means ± SEM with significance set at $p \leq 0.05$. Data was analyzed and graphs were made using GraphPad Prism 8 statistical software.

Chapter 5: Results

5. 1 IL-6 and BDNF Serum Analysis

Both IL-6 (p=0.007) and BDNF (p=0.0008) levels were increased in the post-exercise serum compared to the pre-exercise serum (Figure 4).

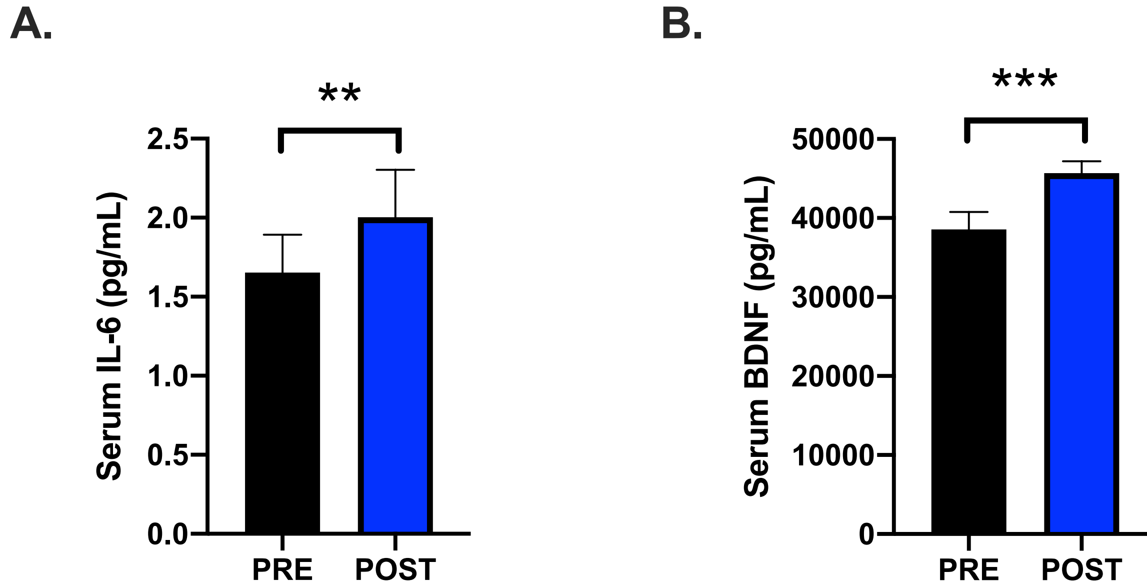


Figure 4: Changes in IL-6 and BDNF Levels in Pre- and Post-Exercise Serum. *Serum was collected 5-minutes post-exercise following the competition of a HIIT running trial. A) IL-6 present in the serum increased after exercise compared to the pre-exercise group (n=19). B) BDNF present in the serum was also significantly increased after exercise compared to the pre-exercise group (n=14). Data are presented as means ± SEM. *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$ as determined using T-test analysis.*

5. 2 Cell Viability with Serum Treatment

Differentiated SH-SY5Y cells were examined with pre- and post-serum treatment. After serum treatment the cells appeared to become slightly more confluent after 24 hours as compared to the 30-minute time point (Figure 5). To examine the viability of the cells after serum treatment, trypan blue assays were performed. After 30 minutes of direct pre- and post-exercise

serum treatment the cells showed ~80% cell viability. After 24 hours, the viability of the cells was higher compared to that at 30 minutes at ~90% with both pre- (p=0.04) and post-exercise (p=0.002) serum (Figure 6).

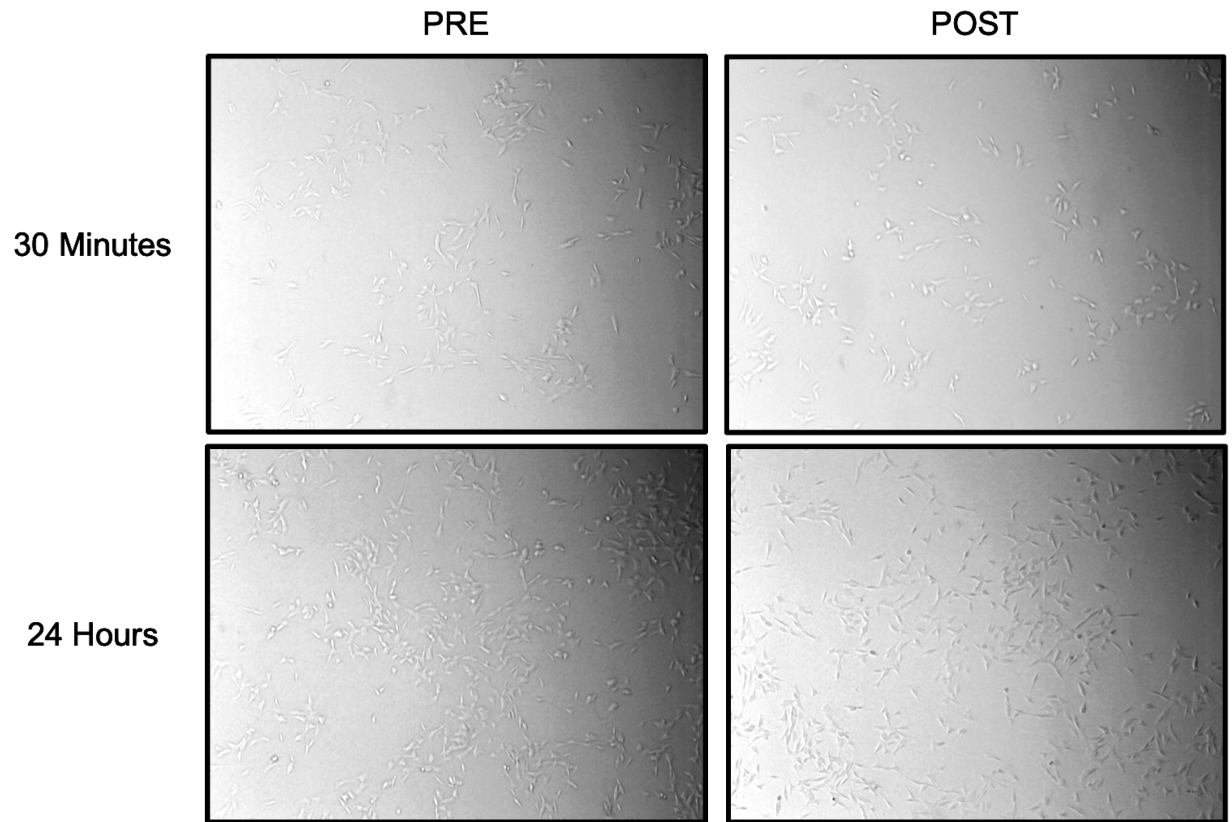


Figure 5: Differentiated SH-SY5Y Cells with Pre- and Post-Exercise Serum Treatment. *Cells were treated with 10% pre- or post-exercise serum. Representative images were taken at both 30 minutes and 24 hours after treatment.*

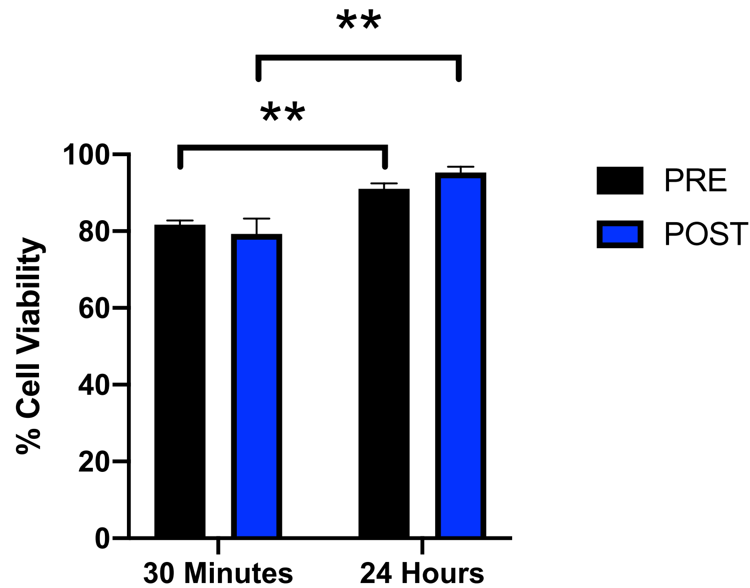


Figure 6: Cell Viability with Pre- and Post-Exercise Serum Treatment. *Pre- and post-exercise serum treatment initially decreased cell viability to ~80% after 30 minutes (n=3). This change was recovered after 24 hours, and cell viability was increased to ~90% (n=3). Data are presented as means ± SEM. A.U., arbitrary units. *p≤0.05, **p≤0.01, and ***p≤0.001 as determined using a Two-way ANOVA and Tukey post-hoc analysis.*

5. 3 RT-qPCR Analysis

In order to detect acute and adaptive changes in human neurons following serum treatment, mRNA expression of human *APP*, *ADAM10*, *BACE1*, and *BDNF* were measured. There were no statistically significant differences in any of the genes measured after either the 30-minute or 24-hour treatment (Figure 7). That said, there was a potential trend in *BDNF* increase with post-exercise treatment after 30 minutes (p=0.09) (Figure 7).

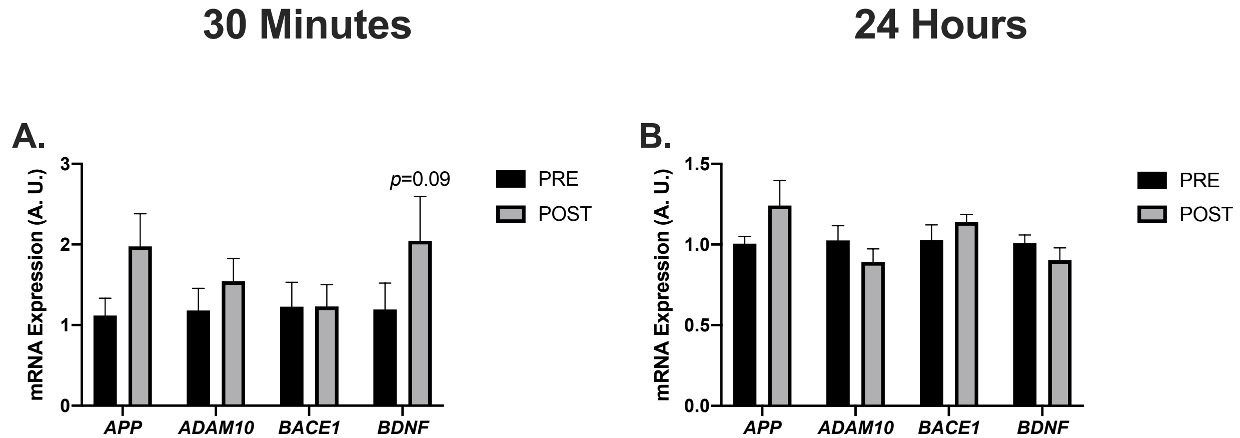
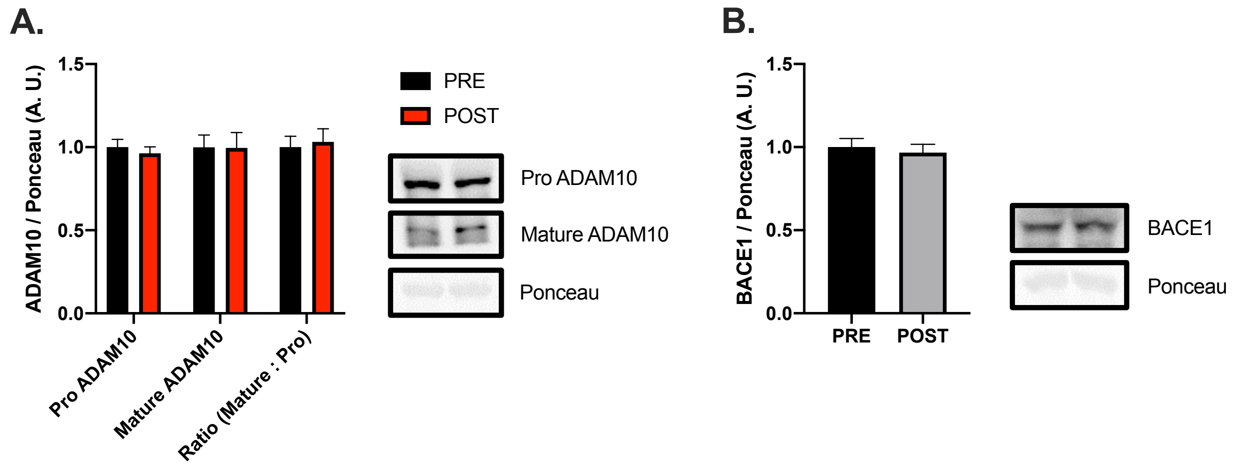


Figure 7: Gene Expression with Pre- and Post-Exercise Serum Treatment. *A) No statistical changes were detected in APP (pre, n=6; post, n=5), ADAM10 (pre, n=6; post, n=6), BACE1 (pre, n=6; post, n=6), or BDNF (pre, n=5; post, n=6) after 30 minutes of serum treatment. B) No statistical changes were detected in APP (pre, n=6; post, n=6), ADAM10 (pre, n=6; post, n=6), BACE1 (pre, n=6; post, n=6), or BDNF (pre, n=6; post, n=6) after 24 hours of serum treatment. Data are presented as means ± SEM. A.U., arbitrary units. *$p \leq 0.05$ as determined using T-test analysis.*

5. 4 Western Blot Analysis

To detect changes in protein expression of various components of the amyloidogenic and non-amyloidogenic cascades were measured. No statistical differences were detected in either ADAM10, BACE1, or APP protein content (Figure 8 and Figure 9). However, after 30 minutes of post-exercise serum treatment sAPPα protein content was higher than pre-exercise serum and was trending significance (p=0.06). This higher sAPPα was accompanied with a lower sAPPβ protein content that was also trending significance (p=0.07). The ratio of sAPPα to sAPPβ was increased with post-exercise serum treatment (p=0.05) (Figure 9). After 24 hours, there were no significant differences in protein content of APP, sAPPα, or sAPPβ. There was a trend in sAPPβ protein content to be lower with post-exercise treatment after 24 hours (p=0.09) (Figure 9).



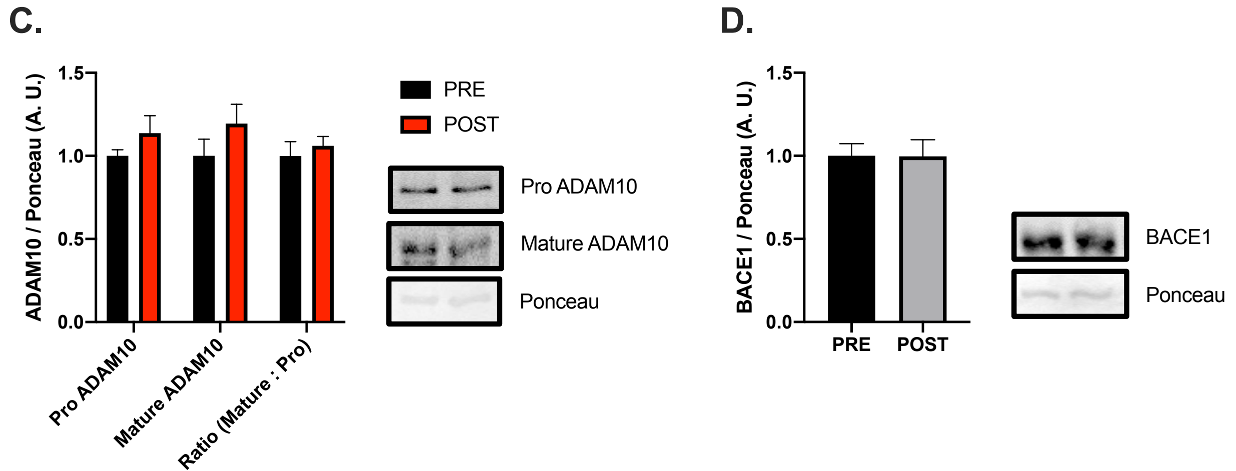
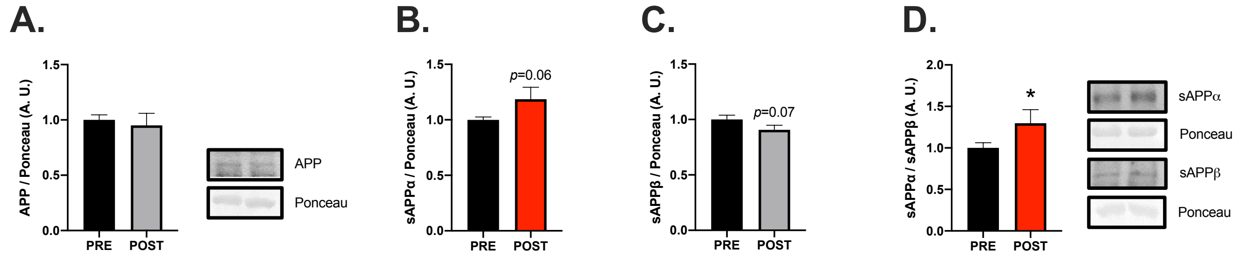


Figure 8: ADAM10 and BACE1 Protein Content After Pre- and Post-Exercise Serum Treatment. *A) No statistical changes were detected in pro, or mature ADAM10 (pre, n=6; post, n=6) after 30 minutes of serum treatment. B) No statistical changes were detected in BACE1 (pre, n=6; post, n=6) after 30 minutes of serum treatment. C) No statistical changes were detected in pro, or mature ADAM10 (pre, n=6; post, n=6) after 24 hours of serum treatment. D) No statistical changes were detected in BACE1 (pre, n=6; post, n=6) after 24 hours of serum treatment. Data are presented as means ± SEM. A.U., arbitrary units. *$p \leq 0.05$ as determined using T-test analysis.*

30 Minutes



24 Hours

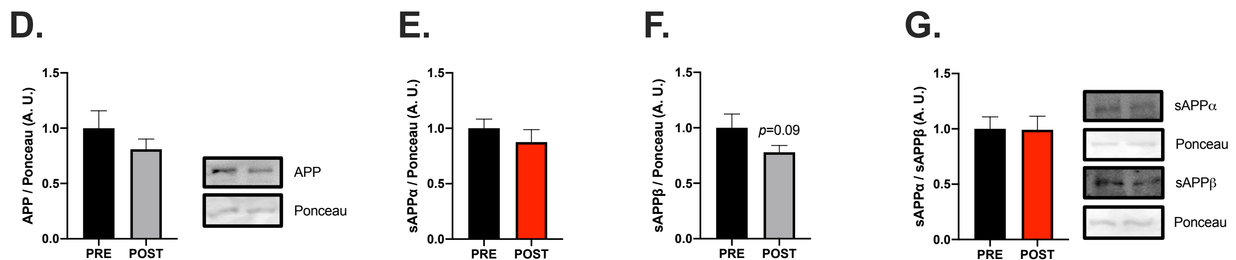
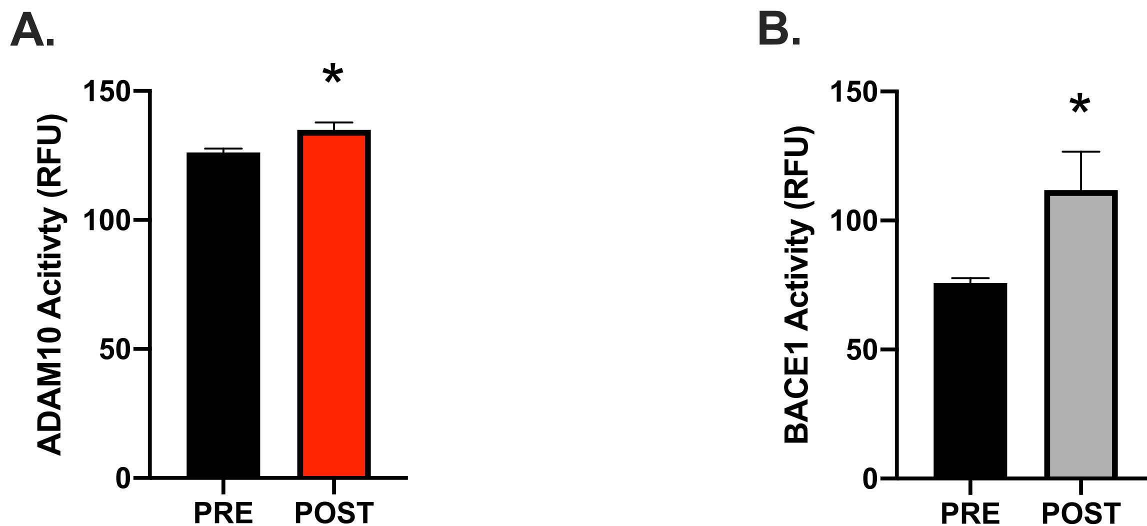


Figure 9: sAPPα and sAPPβ Protein Content Changes with Pre- and Post-Exercise Serum Treatment. *A) No statistical changes were detected in APP (pre, n=6; post, n=6) after 30 minutes of serum treatment. B) No statistically significant changes were detected in sAPPα (pre, n=6; post, n=6) after 30 minutes of serum treatment. C) No statistically significant changes were detected in sAPPβ (pre, n=6; post, n=6) after 30 minutes of serum treatment. D) sAPPα was significantly higher than sAPPβ after post-exercise serum treatment (pre, n=6; post, n=6). D) No statistical changes were detected in APP (pre, n=6; post, n=6) after 24 hours of serum treatment. B) No statistically significant changes were detected in sAPPα (pre, n=6; post, n=6) after 24 hours of serum treatment. C) No statistically significant changes were detected in sAPPβ (pre, n=6; post, n=6) after 24 hours of serum treatment. D) There were no statistically significant differences in sAPPα as compared to sAPPβ after post-exercise serum treatment (pre, n=6; post, n=6). Data are presented as means ± SEM. A.U., arbitrary units. *$p \leq 0.05$ as determined using T-test analysis.*

5. 5 Enzyme Activity Analysis

In order to detect acute and adaptive changes in the main enzymes in the amyloidogenic and non-amyloidogenic cascades after serum treatment, ADAM10 and BACE1 activity was measured. ADAM10 activity was increased after 30 minutes of post-exercise serum treatment (p=0.01). Additionally, BACE1 activity was also increased after 30 minutes of post-exercise serum treatment (p=0.02) (Figure 10). After 24 hours however, there were no statistically significant differences in ADAM10 or BACE1 activity following post-exercise serum treatment.

30 Minutes



24 Hours

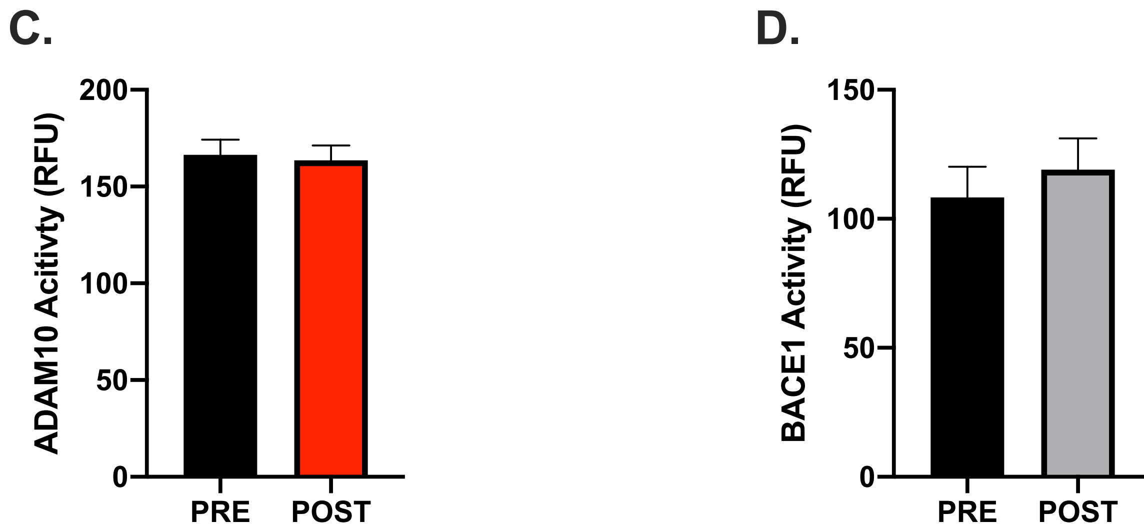


Figure 10: ADAM10 and BACE1 Activity Changes with Pre- and Post-Exercise Serum Treatment. *A) ADAM10 activity was significantly increased (pre, n=6; post, n=6) after 30 minutes of post-exercise serum treatment. B) BACE1 activity was significantly increased (pre, n=6; post, n=6) after 30 minutes of post-exercise serum treatment. C) No statistical changes were detected in ADAM10 activity (pre, n=6; post, n=6) after 24 hours of post-exercise serum treatment. D) No statistical changes were detected in BACE1 activity (pre, n=6; post, n=6) after 24 hours of post-exercise serum treatment. Data are presented as means ± SEM. RFU, relative fluorescent units. *$p \leq 0.05$ as determined using T-test analysis.*

Chapter 6: Discussion

6. 1 Summary of Main Findings

This study aimed to investigate whether post-exercise serum from humans can influence APP processing in differentiated SH-SY5Y neuronal cells. It was found that post-exercise serum treatment alters ADAM10 and BACE1 activity, as well as downstream markers in both the amyloidogenic and non-amyloidogenic cascades. Specifically, acute treatment with post-exercise serum increases the ratio of sAPPα to sAPPβ. This study demonstrates that post-exercise serum and the factors it contains have the power to influence APP processing and potentially push the cascade towards the non-amyloidogenic arm. Importantly, the amyloidogenic and non-amyloidogenic cascades are two pathways that are in constant competition and finding ways to favour the non-amyloidogenic cascade may prove advantageous for brain health, particularly for people who are at risk of developing AD.

6. 2 BDNF and IL-6 Serum Levels in Post-Exercise Serum

BDNF and IL-6 have both been shown to increase with exercise and cross the BBB. Once in the brain these proteins are integral for neuronal proliferation and differentiation [85-87, 129, 140, 141]. Throughout this study we have maintained a unique interest in BDNF and IL-6 and therefore measured the content of these proteins in the pre- and post-exercise serum samples prior to experimentation. Both BDNF and IL-6 were higher in post-exercise serum compared to the pre-exercise serum. Serum BDNF levels in the post-exercise serum samples were on average about half (~4,500pg/mL) of what has been previously reported in the literature, which can be as high as ~10,000pg/mL after HIIT [92, 93]. This could be due to variability in areas such as exercise protocols (running vs. cycling) and the participant's anthropometrics. Although there

are many aspects to consider when trying to get a maximal exercise-induced BDNF response we observed a significant increase in BDNF levels in the post-exercise serum compared to the pre-exercise serum in this study.

Our results indicated that serum IL-6 was increased by ~20%, from ~1.6pg/mL in the pre-exercise serum to ~2.0pg/mL in the post-exercise serum. Previous studies have reported a maximum increase of 100-fold from the resting level of 1pg/mL, making the total concentration 100pg/mL [133, 136, 182-184]. Based on previous reports from the Pedersen group, who are experts in this field, IL-6 levels can appear to increase 2-fold after just 6 minutes of intense exercise and are capable of increasing up to 100-fold after long bouts of continuous exercise greater than 2.5 hours, such as marathon running [133, 136, 182-184]. They have also found consistently that when IL-6 levels are measured after running trials, and not during the exercise trials, that IL-6 levels are the highest right after exercise [133, 136, 182-184]. These studies all measured IL-6 in the plasma, which has been shown to have higher amounts of IL-6 post-exercise compared to serum [134]. However, pilot testing from our laboratory indicated that treating cells directly with plasma drastically decreased cell viability and ultimately caused to much cell death, and therefore serum was the best alternative. There is a large body of studies that have treated a variety of different cell types with exercise serum with minimal technical difficulties, proving that serum treatment of cells is a viable and repeatable method [169-177]. Regardless, the post-exercise serum treatment resulted in alterations in APP processing. The observed increases in BDNF and IL-6 may play a role in these changes and had there been more robust changes in these exercise-induced factors we may have observed a more profound effect on APP processing in this model.

6. 3 Post-Exercise Serum Alters APP Processing

We show for the first time that post-exercise serum results in increased sAPPα to sAPPβ ratio, demonstrating that post-exercise serum, and the factors present within it, are capable of influencing APP processing and appear to shift the cascade away from the amyloidogenic arm towards the non-amyloidogenic arm. In support of these findings ADAM10 activity was increased after 30 minutes of post-exercise serum treatment. Upregulation of ADAM10 protein content has been previously reported with exercise in a rodent models, however most studies to date have examined APP processing with exercise training [15]. With exercise training there are several changes that occur, including increased muscle mass and decreased adipose mass, making it difficult to determine whether exercise had a direct effect on APP processing, or if the results are secondary to other changes seen with exercise training. Studies that include an acute exercise treatment or bout should be included in the future to address this limitation and may help provide a more complete picture of the effect of exercise on APP processing.

A study by the Mattson group found that treatment of SH-SY5Y cells with BDNF lead to significant increases in sAPPα protein content in the cell lysates, as well as a redistribution of active ADAM10 away from the cell surface and more intracellular compared to the control cells [65]. It should be noted that this study treated cells with 50ng/mL of BDNF, which is similar to the amount of BDNF present in the post-exercise serum used for our study [65, 185]. This BDNF mediated increase in sAPPα and change in ADAM10 is similar to what was observed in the present study using post-exercise serum, highlighting the role BDNF may be playing in the serum.

In addition to the potential for exercise-induced BDNF to alter APP processing, our study provides novel insight into a potential role for exercise induced IL-6 in APP processing. In

support of this, pilot data from our laboratory has demonstrated that acute injection of 3ng/g body weight IL-6 can alter APP processing [153]. This dose of IL-6 has been shown to increase circulating IL-6 to a similar extent as what is observed with exercise [186-188]. Our work published and presented at the Experimental Biology Conference in 2021 highlighted that acute IL-6 injection in male C57BL6J mice was able to increase ADAM10 activity while simultaneously decreasing BACE1 activity in the prefrontal cortex and hippocampus of the brain after just 15 minutes post-injection [153]. These changes in activity were accompanied by changes in protein content of both enzymes and downstream markers of both cascades. Specifically, acute IL-6 injection increased ADAM10 and sAPPα protein content in the cortex of brain and decreased BACE1 and sAPPβ protein content in the hippocampus of the brain, highlighting potential regional differences in the response to IL-6 [153]. These results from our laboratory demonstrate that acute IL-6 modulates the key enzymes involved in APP processing, influencing the cascade towards the non-amyloidogenic arm. Future work will examine the contribution of IL-6 to exercise-induced alterations in APP processing by utilizing IL-6 neutralizing antibodies as well as IL-6 receptor blockers in both cell and animal models.

BACE1 activity was significantly increased after 30 minutes of post-exercise serum treatment. This increased activity was an unexpected outcome and based on previous literature we expected a reduction in BACE1 activity with post-exercise serum treatment [15, 54-58]. Previous work has found that both acute and chronic bouts of exercise can decrease BACE1 activity and protein content [15, 54-58]. We postulate that this increase in BACE1 activity could be a compensatory mechanism in response to the serum treatment. During exercise, neuronal and synaptic activity increases to handle the increased neural stimulus (for review see [189]). In order to regulate a sharp rise in neuronal activity seen with exercise, BACE1 may become more

active to process APP and create Aβ peptides, which are known to depress synaptic activity and regulate normal neuronal function [36-41]. With this in mind, the observed acute increase in BACE1 activity in this study may not be detrimental, especially considering this increase in activity was recovered after 24 hours.

During this study it was found that the protein content of the soluble fragments of APP were altered after 30 minutes of post-exercise serum treatment, however there were no observed changes in enzyme protein content. This may be indicative of enzyme protein content being altered earlier or later than 30 minutes and being recovered to basal levels shortly after. That said, there were changes in enzyme activity indicating that the increased activity of these enzymes, especially ADAM10 could be the reason for the increased sAPPα and sAPPβ ratio. Nevertheless, alterations in sAPPα and sAPPβ protein content are indicative of changes in neuronal APP processing, highlighting the need to examine a time-course effect of post-exercise serum on neuronal APP processing. Recently it has been discovered that sAPPα can directly inhibit BACE1 through a potential inhibitory feedback mechanism, thus resulting in reduced products of the amyloidogenic cascade, such as sAPPβ and Aβ40/42 [65, 66]. Changes in the ratio of sAPPα and sAPPβ protein content in this study provide evidence that a factor or combination of factors within exercise serum can increase ADAM10 activity and sAPPα protein content, which has been shown to inhibit the initiation of the pathological amyloidogenic cascade [65, 66].

6. 4 Post-Translational Modifications of ADAM10 and BACE1

Our results demonstrate changes in ADAM10 and BACE1 activity, however it is unclear as to exactly how these enzyme activities are being regulated. There are several post-translational

modifications that can occur to ADAM10 and BACE1, including phosphorylation, nitrosylation, glycosylation, and acetylation [190, 191]. Phosphorylation is the most prominent modification that occurs to these enzymes and there are multiple phosphorylation sites for both ADAM10 and BACE1 [190, 191]. That said, it is conceivable that changes in enzyme activity observed in the present study were due to phosphorylation by different kinases. Both BDNF and IL-6 have been shown to activate AMP-activated protein kinase (AMPK), which can activate cAMP-response element binding protein (CREB), a transcription factor responsible for the regulation of genes involved in neuronal survival and synaptic plasticity [192, 193]. Interestingly, CREB has also been shown to increase the expression of BDNF, which could be why we observed a trend pointing towards an increase in *BDNF* mRNA expression with post-exercise serum treatment [194, 195]. There are other candidate kinases that could be regulating BACE1 and ADAM10 activity as well, including c-Jun N-terminal kinase (JNK) or cyclin-dependent kinase 5 (CDK5). In the brain, JNK is involved in neuronal plasticity and regeneration [196, 197]. Specifically related to APP processing, JNK is implicated in promoting non-amyloidogenic processing of APP and promoting APP degradation, and thus lowering sAPPβ and Aβ peptides [198-201]. There is evidence to support that IL-6 can activate JNK and based on the role of JNK in APP processing this could be why we observed lower sAPPβ protein levels with post-exercise serum treatment [202, 203]. Additionally, CDK5 is a crucial neuronal regulator as it is responsible for normal development of synaptic plasticity and neuronal survival as well as learning and memory formation [204]. There is evidence that CDK5 increases the secretion of both sAPPα and sAPPβ as well as Aβ peptides, while also enhancing endosomal APP content, potentially indicating higher BACE1 activity and cleavage of APP [201, 205, 206]. This could be one reason as to why we observed increases in BACE1 activity with post-exercise serum treatment. Future

mechanistic studies should aim to identify if these kinases are involved in post-exercise alterations in APP processing.

In addition to the above mentioned post-translational modifications, the subcellular location of these enzymes is also important. BACE1 functions optimally in acidic pH environments and is commonly found in endosomes within the cell, which are constantly being trafficked to the cell membrane [201]. ADAM10 on the other hand resides anchored in the plasma membrane of the cell [201]. There is limited information on if exercise-induced factors can affect the location of these enzymes within the cell and future studies should aim to investigate the differences in location with exercise-induced factor treatment. It is important to understand how this treatment effects the function of both ADAM10 and BACE1 and where in the cell this may be occurring.

6. 5 Strengths, Limitations, and Future Directions

This study was the first to attempt an *in vitro* examination of APP processing with exercise serum treatment on a neuronal cell line. We hope that this study this study lays the foundation for future investigations into the effect of exercise and specific exerkines on APP processing in human neuronal cells. That said, there were several strengths and limitations of this protocol that should be addressed with future work. This model used a human cell line differentiated into cholinergic neurons, which are majorly affected by AD, therefore making this model suitable when translating results and a good model to uncover the underlying mechanisms of particular treatments [207]. Conversely, this model is an oversimplification of what occurs *in vivo* given that there is no BBB or interaction with other cell types, such as astrocytes or microglia. Including a BBB-like co-culture system with the interaction of various cell types into

this design would greatly strengthen this model, however recent research has showed that past *in vitro* BBB models may be flawed. A recent study by Lu and colleagues highlighted that human pluripotent stem cells that are differentiated into endothelial cells, commonly used for their BBB like properties, lack functional attributes of endothelial cells [208]. These cells are deficient in various genes pertaining to the vascular lineage as well as erythroblast transformation specific (ETS) transcription factors that are present in true endothelial cells [208]. This study found that by overexpressing various ETS transcription factors that these cells normally lack caused a change in their transcriptome to reflect a true endothelium that would be observed in the BBB. By overexpressing these ETS transcription factors these new cells are suitable for future studies that require an *in vitro* BBB [208]. Another way to improve this model for future studies is to implement another neuronal cell line with different cell types to mimic *in vivo* physiology more closely, such as human neural progenitor ReN cells, which are a combination of glial cells and neurons [209-213]. Additionally, invoking the use of a human primary neuronal culture may also improve the current model as they may be a more accurate representation of human neuronal physiology [214-216]. Although human primary cultures are generally harder to obtain than cell lines as well as more sensitive and difficult to culture, the use of a primary neuronal culture in combination with a BBB co-culture system may be a more accurate representation of human neuronal physiology [215, 216]. Even though the SH-SY5Y cell system used for this study has flaws it is an appropriate *in vitro* model to answer basic mechanistic questions.

In our study we chose to examine only two time points, 30-minutes and 24-hours, to examine the acute and adaptive changes that exercise serum might have on APP processing. Due to some of the trends observed in mRNA and protein expression in the current study, a future

direction of this work should implement a time-course study to determine the best timing to detect changes in mRNA and protein expression with serum treatment.

Exercise stimulates a plethora of different exercise-induced factors into peripheral circulation, such as BDNF, cathepsin B, lactate, and IL-6 [20-22, 49, 68-74]. However, it is important to note that there are other factors that could have an effect on APP processing, notably fibronectin type 3 domain containing 5 (FNDC5) or irisin, insulin-like growth factor 1 (IGF-1), glucagon-like peptide 1 (GLP-1), fibroblast growth factor 2 and 21 (FGF-2/FGF-21), vascular endothelial growth factor (VEGF), ketones (β-hydroxybutyrate), growth differentiation factor 15 (GDF15), and adiponectin just to name a few [20-22, 49, 68-74]. Given the wide array of affects these factors have throughout the body future work should aim to determine if any of these specific factors listed above can cross the BBB and affect APP processing. This could be done by implementing an updated co-culture system as described above and treating cells with each individual factor to see if it can first cross the BBB and secondarily affect APP processing. Beyond this cell culture design, animal models could also be implemented, where these individual factors could be administered into circulation via intraperitoneal injection or injected directly into the brain via intracerebroventricular injection. While it is likely that a combination of exercise-induced factors are responsible for changes brain health, both of the above mentioned methods could provide insight into the effect that individual factors have on APP processing.

6. 6 Conclusions

Finding ways to shift APP processing away from the pathological amyloidogenic pathway and push more towards the non-amyloidogenic cascade is of growing interest to those who study AD. However, it is first important to understand how exercise influences this pathway

before studying the effect individual exercise-induced factors may have on this cascade. This study provided novel evidence that post-exercise serum alters APP processing and increases and decreases in sAPPα and sAPPβ respectively. Our data offers foundational knowledge into how exercise regulates APP processing and hope to spark a new line of studies examining the effect of exercise-induced factors or exerkines on APP processing both *in vitro* and *in vivo* models. This line of work is especially important for those who are at risk of developing AD.

Chapter 7: Appendix

PANEL ON
RESEARCH ETHICS
Navigating the ethics of human research

TCPS 2: CORE

Certificate of Completion

This document certifies that

Danny Marko

has completed the Tri-Council Policy Statement:
Ethical Conduct for Research Involving Humans
Course on Research Ethics (TCPS 2: CORE)

Date of Issue: **14 May, 2018**

Figure 11: TCPS Certificate for Human Research Ethics. *Completion of this course was achieved to work with the human neuroblastoma SH-SY5Y cells used for this project.*

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	7/3/2018
PRINCIPAL INVESTIGATOR:	MACPHERSON, Rebecca - Health Sciences
CO-INVESTIGATOR(S):	Bradley Baranowski (bb12qf@brocku.ca); Danny Marko (dm14vw@brocku.ca)
FILE:	17-397 - MACPHERSON
TYPE:	Masters Thesis/Project STUDENT: Grant Hayward SUPERVISOR: Rebecca MacPherson

TITLE: Examination of neuron health using SH-SY5Y cells

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 7/1/2019

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **7/3/2018** to **7/1/2019**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 7/1/2019. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Craig Tokuno, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Figure 12: Brock University Ethics Approval for Use of SH-SY5Y Cells. *Ethics approval was acquired to work with the human neuroblastoma SH-SY5Y cells used for this project.*

Brock University
Office of Research Ethics
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	December 18, 2019		
PRINCIPAL INVESTIGATOR: CO-INVESTIGATOR:	KLENTROU, Nota - Kinesiology Rebecca McPherson		
FILE:	19-131 - KLENTROU		
TYPE:	Faculty Research	STUDENT:	Nigel and Danny Marko
		SUPERVISOR	Nota Klentrou

TITLE: Acute effects of high intensity interval exercise (cycling and running) on markers of bone metabolism, inflammation and oxidative stress in young adults

ETHICS CLEARANCE GRANTED

Type of Clearance: MODIFICATION Expiry Date: 11/1/2020

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

Modification(s): Change to study personnel and minor editing of consent form

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **11/1/2020**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Office of Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Craig Tokuno, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable. If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Figure 13: Brock University Ethics Approval for Use of Human Serum. *Ethics approval was acquired to work with the human serum used for this project.*

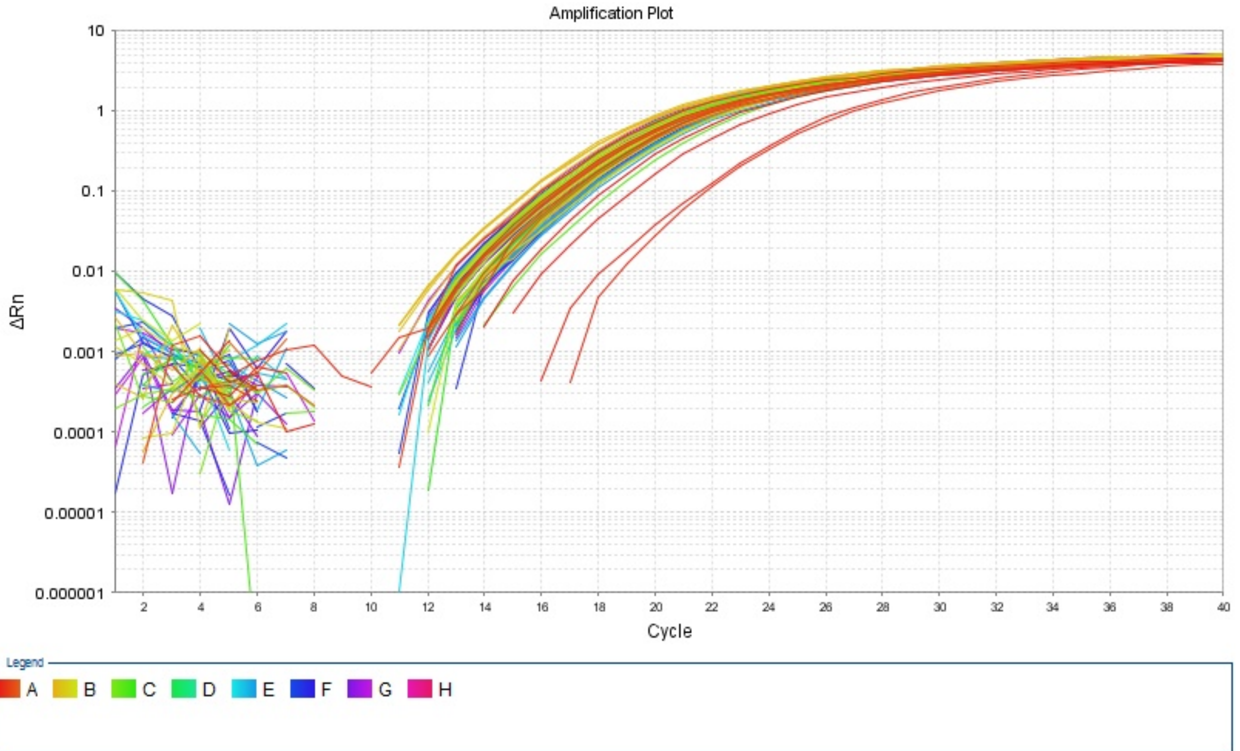


Figure 14: GAPDH Gene Expression Curve. *Representative PCR curve of the human housekeeping gene GAPDH.*

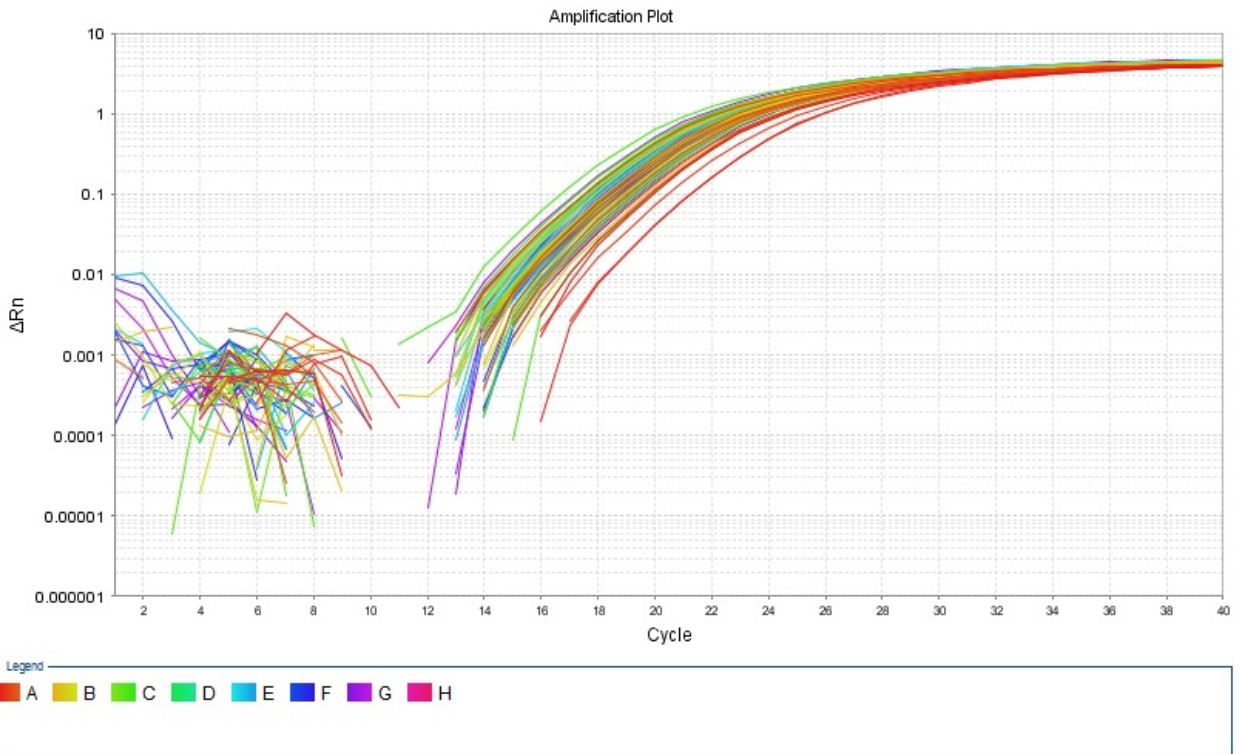


Figure 15: APP Gene Expression Curve. *Representative PCR curve of the human gene APP.*

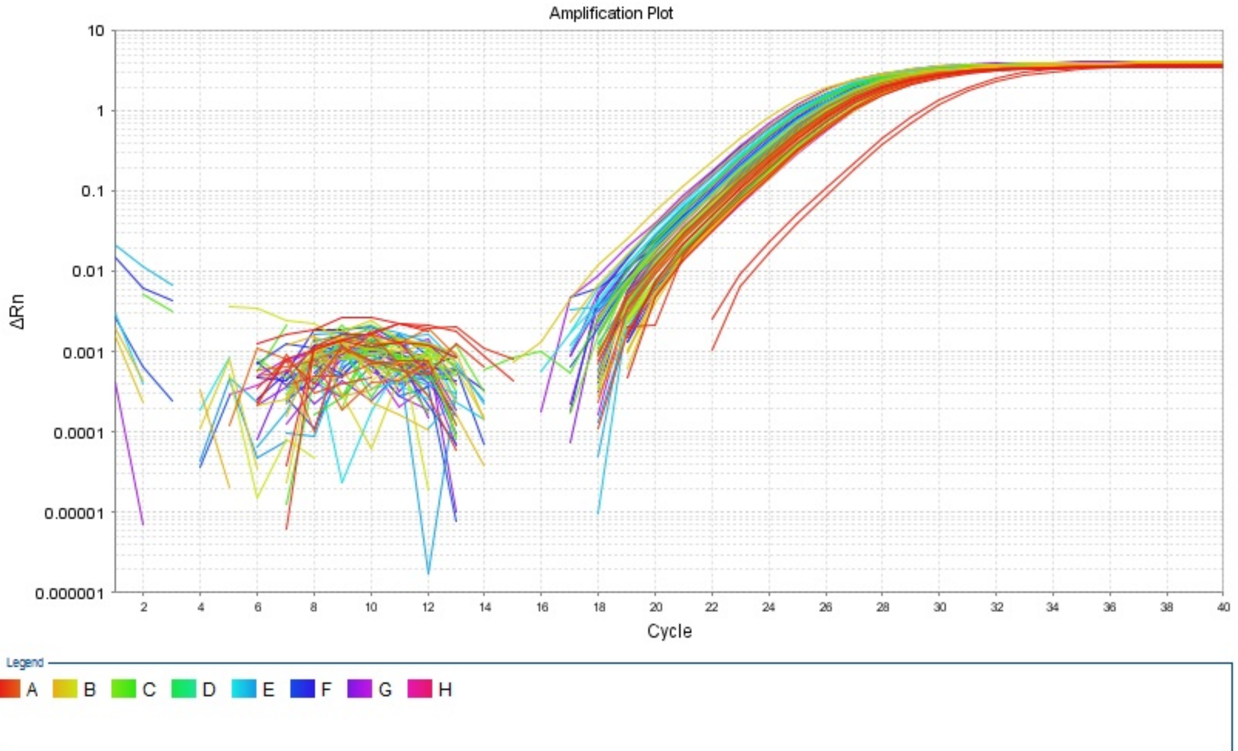


Figure 16: ADAM10 Gene Expression Curve. *Representative PCR curve of the human gene ADAM10.*

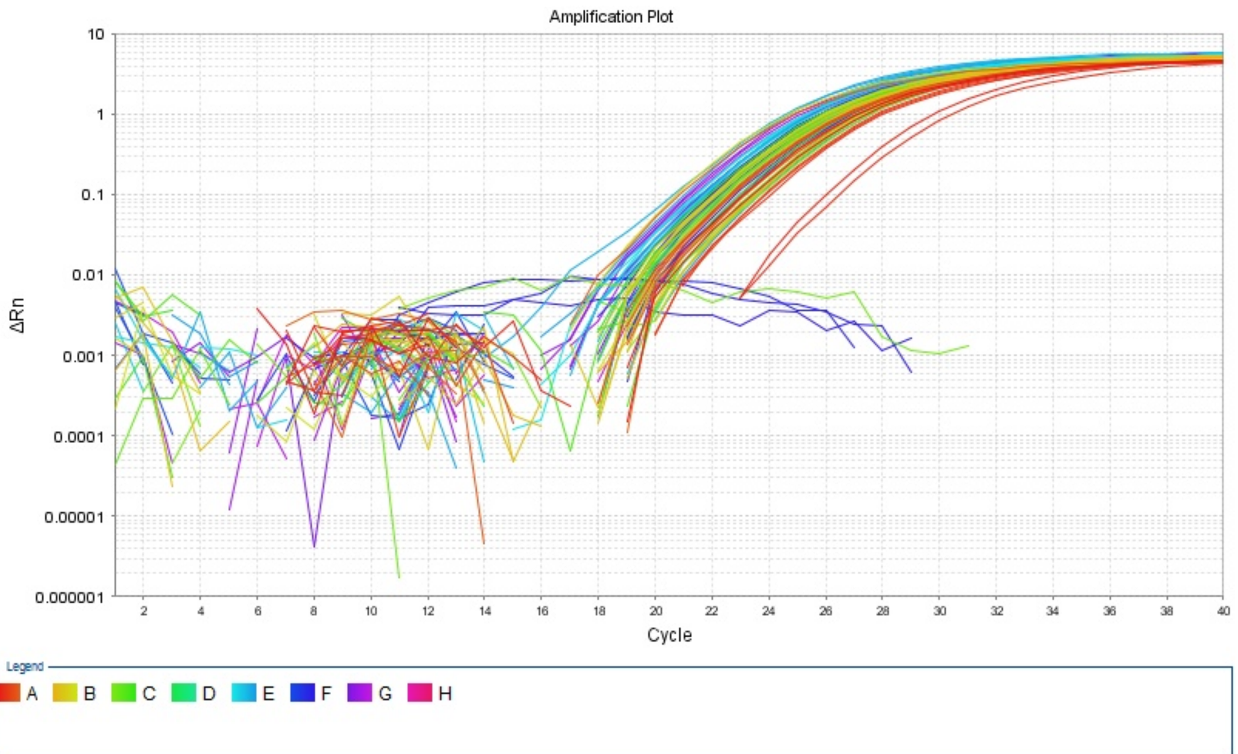


Figure 17: BACE1 Gene Expression Curve. *Representative PCR curve of the human gene BACE1.*

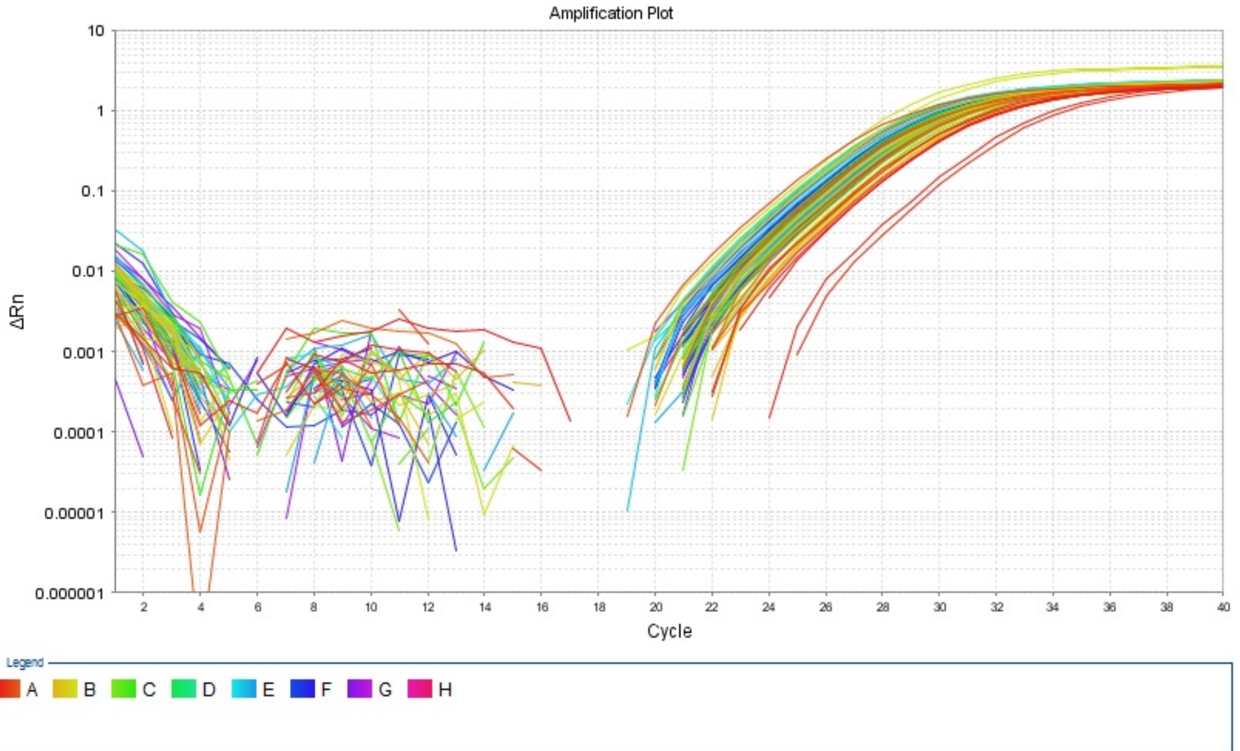


Figure 18: BDNF Gene Expression Curve. *Representative PCR curve of the human gene BDNF.*

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