

PHYSICAL ACTIVITY, AEROBIC FITNESS, AND INSULIN-LIKE GROWTH FACTOR 1
IN INDIVIDUALS WITH ACUTE STROKE

By

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ABSTRACT

Studies show that individuals with subacute and chronic stroke in inpatient rehabilitation facilities and living in the community engage in very little physical activity. Furthermore, people post-stroke have been shown to have low aerobic fitness (peak VO_2). Lack of physical activity and reduced aerobic fitness in individuals with stroke may affect their ability to ambulate and perform basic activities of daily living (ADLs). Evidence suggests that greater amounts of time spent in bed during inpatient rehabilitation is associated with poorer outcome on the modified Rankin Scale (mRS), a measure of functional independence, at three months post-stroke. However, no studies in the United States have objectively quantified the amount of activity performed in individuals with acute stroke and how sedentary time relates to functional ability at discharge from the hospital. This information is important for clinical practice in understanding how physical activity and exercise influence recovery after stroke.

Moreover, in order to gain a more in-depth understanding of the potential benefits of physical activity, aerobic fitness, and exercise, we need to evaluate the response of potential neuroprotective factors after stroke that are influenced by modifiable lifestyle factors, such as aerobic fitness and physical activity. Insulin-like growth factor-1 (IGF-1), a known neuroprotective agent in animal models, may be among the possible growth factors influenced by lifestyle that are vital to recovery. When IGF-1 is administered by intranasal delivery, core stroke lesion size can be reduced up to 94%, while also improving functional status, compared to vehicle controls. In addition, both circulating levels of IGF-1 and IGFBP-3 can be influenced by physical activity and aerobic fitness in healthy individuals. Therefore, research is needed to better characterize physical activity levels and understand the interaction between physical activity levels, aerobic fitness, and IGF-1 response in individuals with acute stroke. The goal of

the work undertaken in this dissertation is to quantify physical activity levels and determine the relationship between IGF-1 response, estimated aerobic fitness, and stroke outcomes following an acute stroke in humans.

In order to achieve our goal, we first set out to objectively quantify physical activity levels in individuals with acute stroke and examine the relationship between sedentary time during the hospital stay and functional performance at discharge. In Chapter 2, our investigation of 32 individuals showed that individuals with acute stroke spent a large majority of time sedentary during their hospital stay. Sedentary time was positively related to the Physical Performance Test, even when controlling for baseline performance. This suggests that individuals who spent more hospital time sedentary performed worse on functional tasks prior to discharge, regardless of their performance at baseline. These results demonstrate that people recovering from stroke spend most of their hospital stay sedentary. This may have important implications for stroke recovery.

Next, because physical activity is directly related to aerobic fitness and previous literature suggests that aerobic fitness is diminished in individuals with subacute and chronic stroke, in Chapter 3 we set out to examine whether estimated pre-stroke peak VO_2 was related to function at hospital discharge. Our results suggest that non-exercise estimation of pre-stroke peak VO_2 is easily administered within 48 hours of hospital admission in individuals with acute stroke. Analysis of the relationship between estimated pre-stroke peak VO_2 and functional performance at discharge revealed no significant relationships when considering the total sample. However, when stratifying the sample by gender, significant relationships were observed in females between estimated pre-stroke peak VO_2 and the Fugl-Meyer Assessment of lower extremity motor function. Females with higher aerobic fitness prior to stroke exhibited better motor

function of the lower extremities at discharge from the hospital. However, no significant relationships were observed for men. Therefore, estimations of pre-stroke peak VO_2 are feasible to use in individuals during the acute hospital setting. The results of this investigation provide important information for future studies for characterizing fitness prior to stroke and how it may relate to objective measures of physical function during stroke recovery and neuroprotective markers such as IGF-1.

Further, because current literature suggests that IGF-1 is neuroprotective after stroke and in healthy individuals, IGF-1 levels can be influenced by physical activity and aerobic fitness, in Chapter 4 we set out to examine IGF-1 and its relationship to estimated pre-stroke peak VO_2 levels in individuals with acute stroke. The results indicate that in 15 individuals with acute stroke, estimated pre-stroke peak VO_2 is significantly related to circulating IGF-1 levels obtained within 72 hours of hospital admission. Individuals with higher than median IGF-1 levels possessed significantly better aerobic fitness prior to their stroke. These results suggest that improving aerobic fitness prior to stroke may be beneficial and provide neuroprotection by increasing baseline IGF-1 levels.

Finally, many studies have seen that individuals with high IGF-1 levels soon after stroke have a greater chance at survival and more independence 3 months later. However, these studies may have a limited understanding of IGF-1's neuroprotective qualities because they do not consider how the response of IGF-1 during the first weeks of stroke is important and only use general questionnaires to assess outcomes. Therefore, Chapter 5 aimed to characterize the response of IGF-1 during the first week of stroke and how it may be related to outcomes (i.e. discharge placement and independence). Individuals with decreases in IGF-1 levels during the first week of stroke had more desirable outcomes compared to individuals with increases in IGF-

1 levels. Individuals with increases in IGF-1 levels and IGF-1 ratio (defined as IGF-1:IGFBP-3) during the first week had a longer length of stay in the hospital, had less independence and greater stroke severity at one month post-stroke, and went to inpatient facilities instead of directly home when discharged from the hospital. Baseline IGF-1 levels and IGF-1 ratio were not related to any outcomes and were not significantly different between those who went home or those who went to inpatient facilities. While other studies have shown that high baseline levels of IGF-1 are related to positive outcomes, our results may provide preliminary evidence that the change in IGF-1 levels and IGF-1 ratio during the first week of stroke are also important to recovery. Further work should be done to investigate the relationship between change in IGF-1 levels early after stroke and functional recovery.

In conclusion, this body of work describes physical activity and sedentary levels of individuals with acute stroke while they are in the hospital and how activity relates to functional status at discharge. Further, discussion of using non-exercise peak VO_2 in the hospital setting determined that predictive measures of aerobic fitness are practical and easy to use and may potentially have a relationship to both functional recovery and neuroprotection. Our results showed that individuals with acute stroke who have higher pre-stroke fitness levels have higher levels of IGF-1 compared to individuals with acute stroke with lower pre-stroke fitness levels. Finally, this collection of studies revealed that decreases in IGF-1 levels are related to positive short-term outcome during stroke recovery. This is the first investigation of how the response of IGF-1 during the first week post-stroke relates to outcomes and warrants future research. The presented work is novel and significant in that it provides objective measures of activity and neuroprotection very early after stroke and new evidence for the use of easy-to-administer assessments of fitness. These studies set important groundwork for additional research to

provide greater detail accounts of the interaction of fitness, physical activity, IGF-1 levels, and functional recovery.

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«««»»

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by whom I have been *eternally* encouraged and inspired
to change the world in the best way I know how.
Your spirit will forever live on in my heart.**

«««»»

“Agape will carry you through the hardships. Agape is the glue that will hold you together.

With it, you will be something that is greater than the sum of its parts.”

- M.R.M.

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LIST OF ABBREVIATIONS

6MWT	Six-Minute Walk Test
ACSM	American College of Sports Medicine
ADLs	Activities of daily living
Akt	Protein kinase B (Akt)
ALS	Acid-labile subunit
ALT	Alanine aminotransferase
AP-1	Activator protein 1
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BAX	Associate X protein
BBB	Blood brain barrier
BCL2	B-cell lymphoma 2
BI	Barthel Index
BMI	Body Mass Index
BMRS	Brunnstrom motor recovery stages
bpm	Beats per minute
CABG	Coronary artery bypass graft
CI	Confidence intervals
cm ³	Centimeters cubed
CP	Choroid plexus
CR	Cardiorespiratory
CSF	Cerebral spinal fluid

ELISA	Enzyme-Linked Immunoassay
EMR	Electronic medical record
ERK	Extracellular signal-regulated kinase
FIM	Functional Independence Measure
FMA	Fugl-Meyer Assessment
FOXO	Forkhead box O
GDP	Guanosine diphosphate
GRB2	Growth factor receptor-bound protein 2
GTP	Guanosine triphosphate
hrs	Hours
HSC	Human Subjects Committee
IGF-1	Insulin-like growth factor-1
IGF-1R	Insulin-like growth factor-1 receptor
IGF-2	Insulin-like growth factor-2
IGFBP-3	Insulin-like growth factor binding protein-3
ILPs	Insulin-like proteins
kDa	Kilo Daltons
kg	Kilograms
KU	University of Kansas
KUMC	University of Kansas Medical Center
LEFM	Lower extremity Fugl-Meyer
LRP1	Lipoprotein receptor-related protein 1
LRP2	Lipoprotein receptor-related protein 2

IRS-1	Insulin-receptor substrate-1
LOS	Length of Stay
m	Meters
MAPK	Mitogen-activated protein kinase
MCAO	Middle cerebral artery occlusion
MET	Metabolic equivalent
min	Minute
mL	Milliliters
mmHG	Millimeters of mercury
mNSS	modified Neurological Severity Scores
MMP9	Matrix metalloproteinase 9
MRI	Magnetic resonance images
mRNA	Messenger ribonucleic acid
mRS	Modified Rankin Scale
mTOR	Mammalian target of rapamycin
ng	Nanograms
NIHSS	National Institutes of Health Stroke Scale
PGE ₂	Prostaglandin E ₂
PI3K	Phosphatidylinositide 3-kinase
PIP ₂	Phosphatidylinositol 4,5-biphosphate
PIP ₃	Phosphatidylinositol 3,4,5-triphosphate
PPT	Physical Performance Test
RAPA	Rapid Assessment of Physical Activity

RHEB	Ras homolog enriched in brain
rHR	Resting heart rate
RIA	Radioimmunoassay
s	Seconds
S6K	S6 kinase
SD	Standard deviation
SIS	Stroke Impact Scale
SOS	Son of Sevenless
SPD	Steps per day
SSS	Scandinavian Stroke Scale
tPA	Tissue plasminogen activator
TSPD	Time sedentary per day
TUG	Timed-Up and Go
U/L	Units per Liter
VO ₂	Oxygen consumption

CHAPTER 1

Introduction

1.1. Overview

Every 40 seconds someone has a stroke in the United States (Go et al., 2014). Lifetime risk is approximately 1 in 6 individuals, with an estimated 795,000 people experiencing new or recurrent strokes every year. Nearly 610,000 of those cases are first time events and 185,000 recurrent (Go et al., 2014). The incidence of stroke is likely to continue to grow over the next decade with a projected additional 3.4 million individuals (a 20.5% increase from 2012) over the age of 18 years having experienced a stroke by the year 2030 (Go et al., 2014). The prevalence of risk factors for stroke such as elderly age, obesity, diabetes, and heart disease is also ever-growing (Dunstan et al., 2002; Malik, 2004; Ogden et al., 2013). In addition, the average age of stroke onset has decreased, while in recent years, survival of individuals after stroke has begun to grow (Brown et al., 1995). Therefore, there is a growing population of stroke survivors who are also living longer with their disabilities. Stroke is among the leading causes of severe long-term disability in the United States (Go et al., 2014). Many individuals have impaired upper limb mobility and function such as grasping and lifting objects (Knorr et al., 2010; Podsiadlo, Richardson, 1991). Additionally, they often have lower extremity weakness and loss of mobility that affects their ability to ambulate, especially in the community (Podsiadlo, Richardson, 1991). In order to meet the need of the increased number of survivors, researchers and clinicians must work together to better understand recovery following stroke and develop novel strategies for stroke rehabilitation.

To date, research in individuals with stroke has contributed significantly to the care these individuals receive during their recovery. However, a gap still exists between the information gained through research and the implementation of this information during clinical practice. In order to provide the most valuable information to those directly involved in patient care, studies

need to begin looking at this problem from a translational view-point. One such way of achieving this goal is to examine both mechanistic means of neuroprotection, while also considering objective, clinical outcomes and interventions such as pharmaceutical administration or physical exercise. Growth factors and supporting proteins, such as insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) have been shown to be neuroprotective after stroke (Aberg et al., 2011 Bondanelli et al., 2006; De Smedt et al., 2011; Dempsey et al., 2003; Ebinger et al., 2015; Liu et al., 2004; Liu et al., 2001; Liu et al., 2001; Rizk et al., 2007; Schabitz et al., 2001; Wang et al., 2001; Zhu et al., 2008). However, evidence of neuroprotection through these growth factors has primarily been shown in animal models and has been limited in humans by the exclusion of multiple sampling time points, the use of a large variability in the collection window, limited consideration of other factors that affect the availability of IGF-1, and the use of general questionnaires related to stroke recovery.

In current literature, IGF-1 and IGFBP-3 are known to be influenced by modifiable lifestyle factors such as physical activity level, aerobic fitness, and body composition in healthy individuals (Chang et al., 2011 Ploughman et al., 2015; Ploughman et al., 2005 Bang et al., 1990; Elias et al., 2000; Scofield et al., 2011; Voss et al., 2013; Zheng et al., 2014 Carro et al., 2000; Rojas Vega et al., 2010 Bermon et al., 1999; Carlsen et al., 2015; Jeon,Ha, 2015). However, there are no studies to test whether these relationships exist in individuals with stroke. Understanding the interaction between IGF-1, modifiable lifestyle factors, and recovery after stroke could lead to improved rehabilitation interventions and also provide benefits of improving health after stroke.

In order to increase our understanding of how these growth factors might influence stroke recovery in humans, it is important to use a narrow sampling windows to obtain baseline

measures in acute stroke, and we must consider the availability of IGF-1 in circulation. The current study seeks to fill the gaps and answer these important questions by implementing a translational approach that examines growth factors, physical activity, and stroke recovery.

1.2. Physical Activity, Exercise, and Stroke

Physical activity is important for cardiovascular health and for maintaining the ability to perform activities of daily living, especially among aging individuals. Many studies around the world have been employed to examine the amount of physical activity individuals after stroke perform.

1.2.1. Physical Activity and Chronic Stroke

In community dwelling individuals post-stroke with the ability to walk independently, the average steps per day is $5,689 \pm 3,833$ steps (Sakamoto et al., 2008). Individuals in the study by Sakamoto and colleagues had a mean age of 63.4 (7.3) years, were on average 3.4 years from their stroke, and were monitored continuously for 24 hours. Individuals in Sakamoto's study spent 81% of their day either in sitting or lying positions and only 8% of their day walking. When these data are compared to healthy individuals over the age of 64 years, individuals post-stroke spend less time performing physical activity, with healthy individuals spending 21% of their day in light activities (Arnardottir et al., 2013).

Furthermore, one study reports as low as 1,389 steps per day in individuals post-stroke (Michael, Macko, 2007), while most report in the 3,000 range (English et al., 2015; Fulk et al., 2010; Hachisuka et al., 1998; Haeuber et al., 2004; Moore et al., 2010; Roos et al., 2012). Roos and colleagues directly examined the difference in walking amount and structure between

individuals post-stroke with no other neurological diagnoses and healthy older adults, both of which were community dwelling (Roos et al., 2012). All individuals were monitored with the StepWatch for 3 days during waking hours, excluding time performing water activities, such as swimming and showering. Healthy older adults were found to have significantly more steps per day, bouts per day, total time walking, and greater percent time walking. However, steps per bout between groups were not significantly different. This may suggest that the low step count was not limited in individuals who are post-stroke due to a lack of ability to take more steps per bout, but rather the number of step bouts. Furthermore, when comparing walking structure in individuals who are post-stroke between groups of slow and moderate walking speeds, individuals post-stroke who walked slowest, had even significantly fewer steps per day, bouts per day, total time walking, and lower percentage of time walking, but steps per bout remained unchanged. Roos suggested that the difference in walking structure between post-stroke and healthy individuals could be due to a lack of support from family and friends as well as transportation difficulties.

English studied sitting time and its association with physical function in 40 community dwelling individuals post stroke and 23 age-matched, healthy controls (English et al., 2015). Activity was measured continuously for 7 days with both an activPAL3 and an ActiGraph GT3X+. Physical function was assessed in individuals with stroke with the National Institutes of Health Stroke Scale (NIHSS) and Stroke Impact Scale (SIS). People post-stroke spent 10.9 hours per day sitting compared to the 8.2 hours the healthy controls spent sitting and sitting time was negatively related to physical function. Although both healthy older adults and individuals after stroke were shown to spend very little time engaging in physical activity, individuals after stroke are at greater risk for cardiovascular decline, and engaging in increased time sitting may

interfere with their ability to perform activities needed for daily living. However, this high amount of sedentary time may not necessarily be because they are physically incapable of performing activities, but rather they simply choose not to (Rand et al., 2010).

1.2.2. Physical Activity and Inpatient Stroke Rehabilitation

In the in-patient rehabilitation setting, similar patterns exist. Observational studies were performed to determine the amount of time spent in therapies and other activities. In a study by West and colleagues, physical activity of 130 individuals within 14 days post-stroke in an in-patient rehabilitation setting was monitored by observations of 10 minute increments between the hours of 8 am and 5 pm during a single day of the work week (West et al., 2013). They found that on average, individuals spent 45% of their day either out of bed or sitting supported or unsupported in bed and 46% of the day lying inactive in bed or performing passive activities in bed, such as reading or watching television. Additionally, participants were most active when observed with a visitor, usually nursing staff or a family member, supporting the notion that family and social encouragement aid in increasing activity. Askim studied 106 individuals with stroke and found that a greater amount of time spent in bed within the first 14 days of stroke was associated with a poorer outcome 3 months later, tested by the modified Rankin Scale (mRS) (Askim et al., 2014), suggesting that early mobilization may be beneficial to stroke recovery. However, the previously mentioned studies which examined performance of activities and bed sitting were performed anywhere within 14 days of stroke, have primarily been observational, and are short, fragmented periods of time during the day. Valuable information regarding physical activity could be missed from the gaps in observation times and observation methods. This could potentially result in inaccuracies due to the subjective nature of observational studies.

Therefore, objective measures of physical activity very early after stroke are needed, but have not yet been performed nor examined in the United States. The length of stay on an acute stroke unit is shorter in the United States. Therefore, we need to characterize how much physical activity occurs during this short length of stay in the acute stroke unit. The current collection of studies seeks to fill this gap in Chapter 2.

1.2.3. Aerobic Fitness Measures after Stroke

Physical inactivity can result in cardiovascular decline, which may interfere with one's ability to perform activities needed for daily living. Moreover, incorporating physical activity or exercise can improve overall aerobic fitness and walking endurance in people with subacute and chronic stroke. However, having methods available to assess aerobic fitness across all stages of stroke recovery would be advantageous. Many studies have used a metabolic cart to assess aerobic fitness but this method is costly, requires special equipment and trained personnel, and is limited by motor impairments. Recently a submaximal exercise test was developed to predict aerobic fitness in healthy individuals using a recumbent stepper (Billinger et al., 2012; Herda et al., 2014). This exercise device works well for people post-stroke and accommodates those with multiple motor impairments (Billinger et al., 2012; Billinger et al., 2008). Furthermore, this exercise test does not require the expensive metabolic equipment. Predicted peak aerobic fitness from the submaximal exercise test was compared to measured aerobic fitness and was found to be similar in people with subacute stroke (Mattlage et al., 2013). However, evaluating aerobic fitness through maximal or submaximal exercise testing may not always be feasible especially early after stroke. To be able to better understand whether pre-stroke aerobic fitness is related to stroke recovery, simpler tools to assess aerobic fitness, such as prediction equations to estimate peak oxygen consumption, are needed.

In healthy individuals, non-exercise estimations have been used to predict aerobic fitness. One specific non-exercise estimate equation considers factors such as: a self-reported measure of physical activity level, gender, age, body mass index, and resting heart rate (Jurca et al., 2005). Obtaining these variables would be feasible for healthcare professionals during the acute stroke hospital stay. Chapter 3 uses this measure in an acute stroke population. Information gained regarding this topic can be used to set the groundwork for future studies to implement exercise interventions, monitor cardiovascular progress to interventions, to evaluate how aerobic fitness is related to recovery, and how exercise can be used to harness protective benefits.

In summary, individuals after stroke often exhibit increased sedentary behavior compared to their healthy counterparts. In addition, establishing aerobic fitness levels of post-stroke individuals may be beneficial to understanding recovery. However, using exercise to evaluate aerobic fitness soon after stroke is not always feasible due to functional limitations of motor impairments after stroke and limited resources for required equipment and personnel. The use of non-exercise prediction equations for estimating aerobic fitness can provide a feasible and cost-effective alternative. In order to gain a more in-depth understanding of the benefits of aerobic fitness and exercise, we need to evaluate the response of potential neuroprotective factors after stroke that are influenced by modifiable lifestyle factors, such as aerobic fitness and physical activity. Insulin-like growth factor-1 (IGF-1), a known neuroprotective agent in animal models, may be among the possible growth factors that are vital to recovery post-stroke influenced by lifestyle.

1.3. Overview of Insulin-Like Growth Factor-1

Aside from insulin, there are two other insulin-like proteins (ILPs): insulin-like growth factor-1 (IGF-1), and insulin-like growth factor-2 (IGF-2). Both of these proteins are expressed in high quantities during fetal and perinatal development. After this period, both protein levels decrease, with IGF-2 decreasing quite substantially (LeRoith, 1997). Therefore, IGF-2 is less important and plays less of a vital role during childhood, into adulthood, and old age when compared to IGF-1. Although IGF-1 levels steadily decrease throughout the lifespan, this growth factor still remains in abundance within healthy and diseased individuals. This single-chain polypeptide is produced locally in the brain by nearly every cell type, but is primarily produced peripherally from the brain by the liver and secondarily by the pancreas. The scope of IGF-1's function is extensive, as it is involved in almost every organ of the body (Daughday, Rotwein, 1989). It plays a key role in many bodily processes such as bone and cartilage growth, wound healing, metabolism, reproduction, angiogenesis, memory and cognition, neuroprotection, and neurogenesis (Hernandez et al., 1989; Mueller et al., 1991; Schmid et al., 1991; Spencer, 1988). For the purposes of the current study, background information and study design will focus on IGF-1's neuroprotective and neurogenerative potential.

The human IGF-1 gene was discovered to have at least five exons, which code for the protein during transcription, and resides on the long arm of chromosome 12 (Brisenden et al., 1984; De Pagter-Holhuizen et al., 1986; Hoppener et al., 1985; Rotwein, 1986; Tricoli et al., 1984). Mature IGF-1 is comprised of 70 amino acids and is encoded from the second and third exon on the IGF-1 gene. There are six insulin-like growth binding proteins with IGF-1 being most affinitive to IGFBP-3. When in circulation, about 95% of IGF-1 proteins are bound to

IGFBP-3, 4% to other IGFBPs, and 1% are found in free form (Baxter, 1994). A ternary complex of 150 kilo Daltons (kDa) is formed between IGF-1, IGFBP-3, and acid-labile subunit (ALS). Insulin-like binding proteins have two primary functions: 1) to regulate IGF-1 activity; and 2) to act as a storage facility to IGF-1. However, most IGFBPs do have some small function independent of IGF-1, either inhibition or facilitation of apoptosis and cell growth. All IGFBPs act as inhibitors on IGF-1 by binding with them instead of allowing them to act as a ligand to their receptor. Therefore, the actions of IGF-1 are determined by the ratio of total IGF-1 to IGFBP-3, which can also be referred to as the availability of IGF-1. Currently, very few studies have considered IGFBP-3 when investigating the response of IGF-1 blood levels after stroke in humans. Because of IGFBP-3's great influence on the availability of IGF-1, it is extremely important to quantify IGFBP-3 alongside the quantification of IGF-1. Without this information, the ability to draw meaningful conclusions about the response of IGF-1 in individuals with stroke is extremely limited. The current study seeks to fill this gap by quantifying both proteins.

1.3.1. Insulin-Like Growth Factor-1 Signaling, Pathways, and Action

As mentioned previously, IGF-1 is produced both locally in the brain by cells containing IGF-1 messenger ribonucleic acid (mRNA) and also peripherally from the brain by the liver. Because IGF-1 is synthesized from these two locations, it exhibits both endocrine and paracrine signaling. **Figure 1.1** illustrates this phenomenon.

Figure 1.1. Endocrine/Paracrine Signaling of Neuroprotection

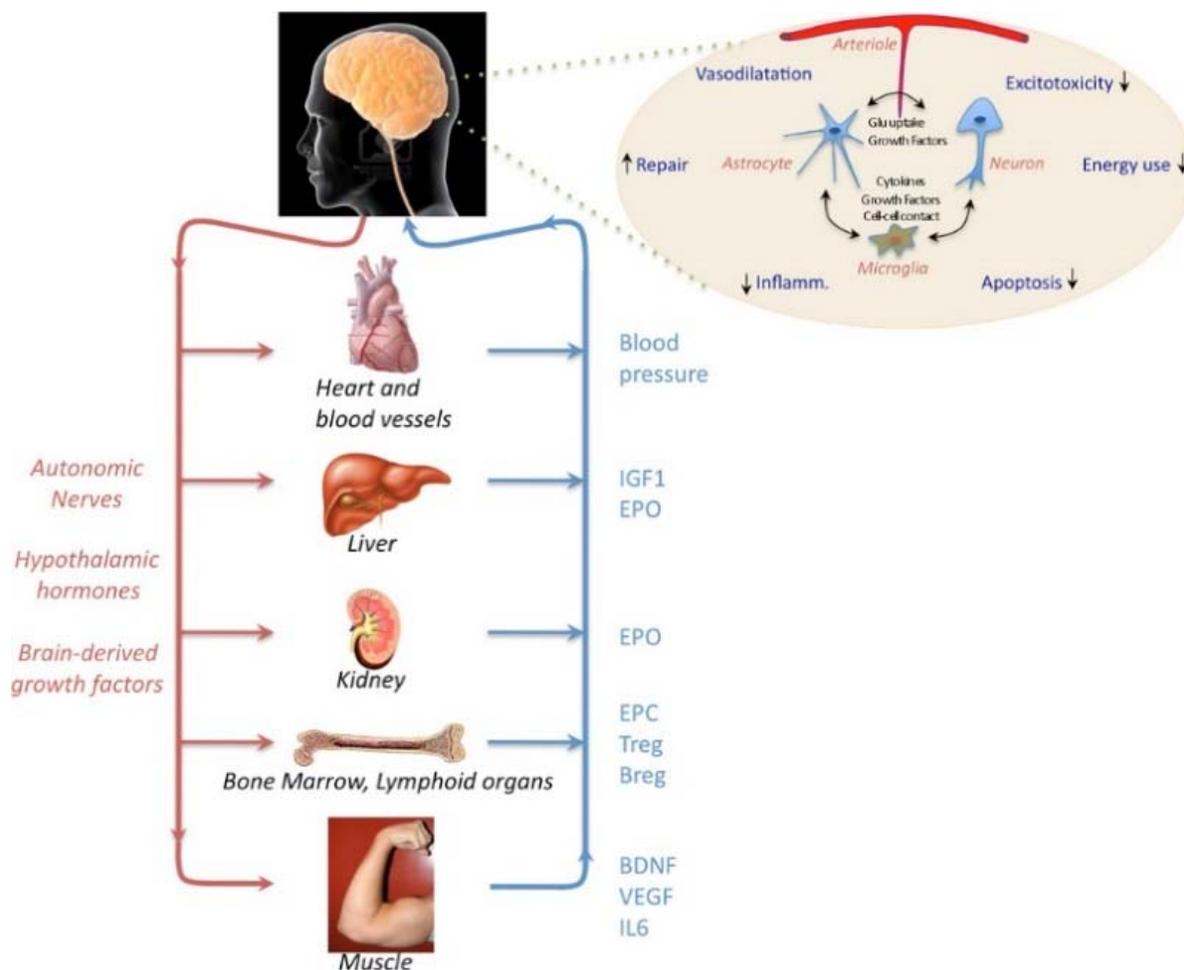


Figure used with permission from Iadecola et al. in Nature Neuroscience, 2011

After injury, there is a protective cell-to-cell communication within the brain between neurons, astrocytes, microglia, and blood vessels through a response to excessive glutamate and growth factor release. This system is involved in homeostasis of the tissues within the brain by sustaining cerebral blood flow, decreasing excitotoxicity, preventing inflammation and apoptosis, and promoting mechanisms of repair. Signaling from the brain supports both central processes and peripheral organs such as the cardiovascular system, while peripheral signaling feeds back to the brain to provide neuroprotection and repair. Although almost every cell type in

the mammalian brain contains IGF-1 mRNA, both endocrine and paracrine signaling play significant roles in cell growth and protection after cerebral injury. It has been shown that in the brain there is a low level of IGF-1, but a high level of IGF-1 receptors (Carro et al., 2000; Trejo et al., 2001), suggesting that additional IGF-1 from the periphery is needed for neuroprotection in the brain. The receptor for IGF-1 (IGF-1R) is a transmembrane, tyrosine-kinase receptor. In the adult mouse, IGF-1Rs are widely distributed and expressed in most abundance in the neocortex, thalamus, and choroid plexus (see **Figure 1.2**). The distribution of IGF-1Rs is similar in the adult human brain (Adem et al., 1989; De Keyser et al., 1994).

Figure 1.2. Distribution of IGF-1R in Mice

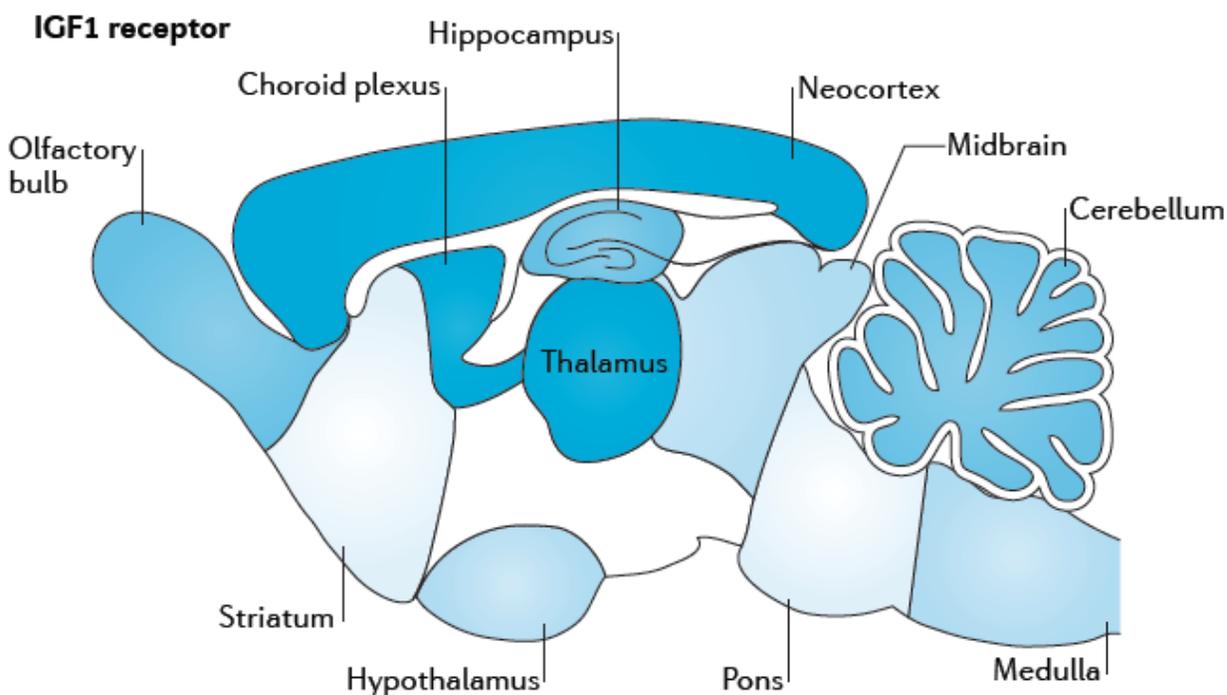


Figure used with permission from Fernandez et al. in Nature Reviews Neuroscience, 2012

Darker areas indicate greater abundance of IGF-1R

In human brains, epithelial cells in the choroid plexus and endothelial cells in brain vessels have the highest distribution of IGF-1 receptors (Bondy, Lee, 1993). Peripherally synthesized IGF-1 can enter the brain from circulation via two routes: 1) through the choroid plexus (CP) to the cerebral spinal fluid (CSF) and finally to brain parenchyma (**Figure 1.3**); or 2) directly from circulation through the blood brain barrier (BBB) (**Figure 1.4**), further supporting the idea that paracrine signaling from circulating IGF-1 plays an important role centrally in the brain.

Figure 1.3. IGF-1 from CP-CSF-Parenchyma

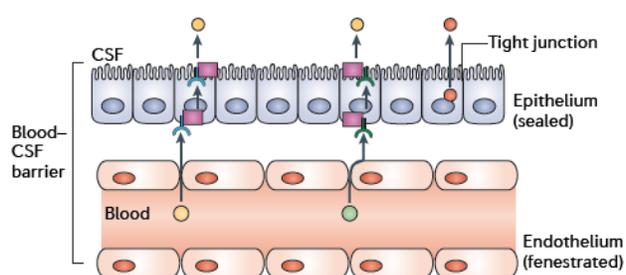
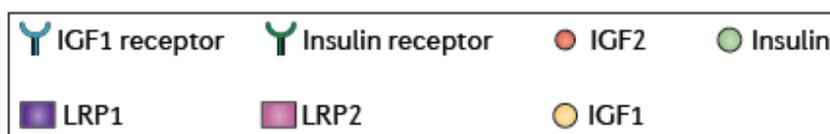
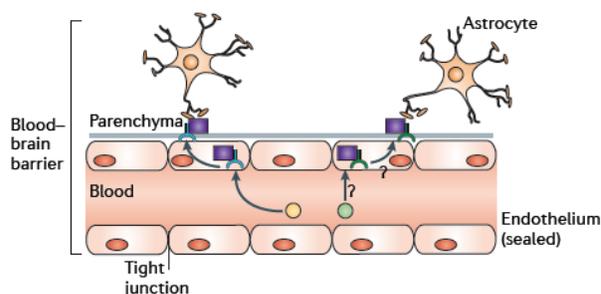


Figure 1.4. IGF-1 from BBB Parenchyma



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The CP is a network of capillaries within the ventricles of the brain with a primary function of producing CSF. Fluid from the blood is filtered through epithelial cells of the CP to eventually become CSF. Amount of IGF-1 entrance into the brain through this process is directly related to the level in circulation. **Figure 1.3** depicts transcytosis of IGF-1 from the CP to its receptor in the CSF and eventually on to the brain parenchyma with the help of low-density

lipoprotein receptor-related protein 2 (LRP2). Insulin-like growth factor-1 that interacts with the brain through the CP typically supplies areas closest to the ventricles such as the hypothalamus and the hippocampus. Areas that are geographically farther away from the CP are less accessible for IGF-1 and must use and rely primarily on either a transportation system or locally synthesized IGF-1. **Figure 1.4** represents the process of IGF-1 entering the brain parenchyma through IGF-1 receptors in endothelial cells in the BBB with the help of LRP1. Injury and the activation of neuronal cells results in glutamate release, which enhances local blood flow. With an enhancement in blood flow, the levels of the ternary complex of IGF-1, IGFBP-3 and ALS is also increased (Chiu et al., 2008; Scolnick et al., 2008). However, in order for IGF-1 to reach its receptor, IGF-1 must be disassociated from its bound proteins. Several mediators such as prostaglandin E₂ (PGE₂) and adenosine triphosphate (ATP) are also released during this processes that stimulates matrix metalloproteinase 9 (MMP9). One important function of MMP9 is to aid in the disassociation of IGF-1 from IGFBP-3, allowing it to attach to its receptor in the endothelium. Once attached to its endothelial receptor, LRP1 helps IGF-1 to be picked up by the glial end-feet of astrocytes and then is transferred to the IGF-1R of a neuron for activation of its function. **Figure 1.5** demonstrates this process.

Figure 1.5. BBB IGF-1 Attachment to Astrocyte and Neuron

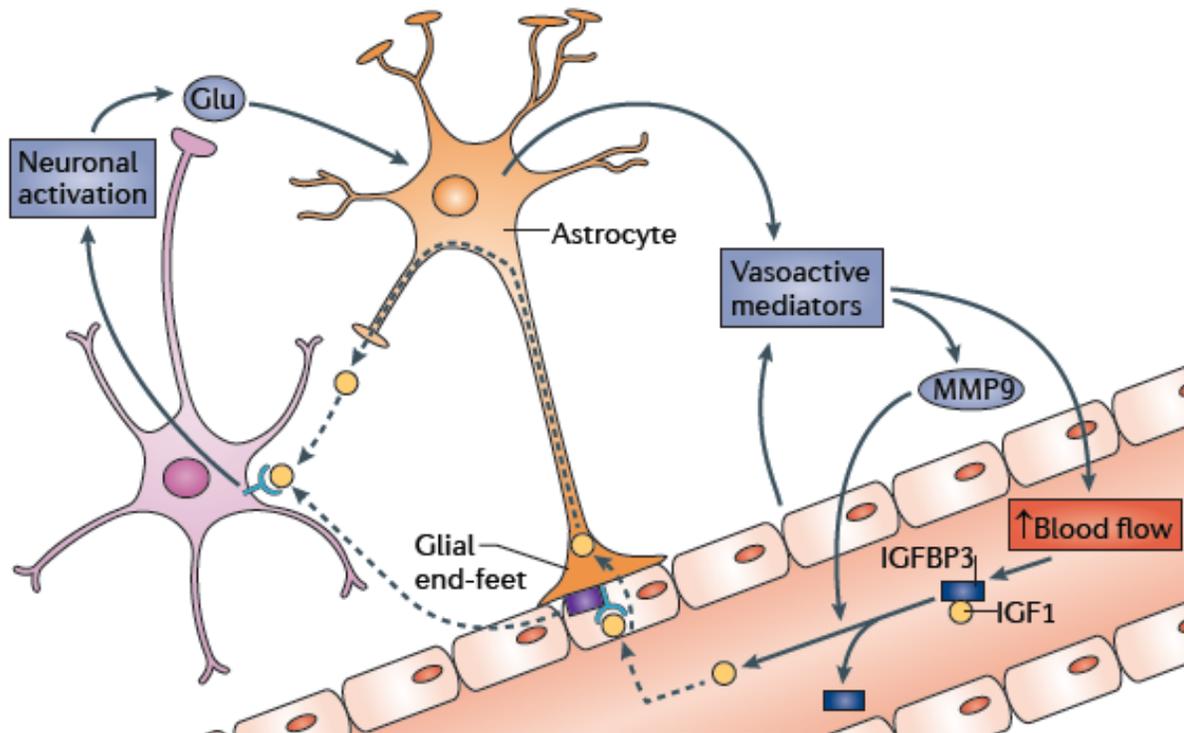


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In the event of a cerebral injury, locally produced IGF-1 and IGF-1 from circulation target activated neurons. An experimental study showed evidence of this by inducing a middle cerebral artery occlusion (MCAO) in rats and administering IGF-1 immediately after. Within 30 minutes of IGF-1 administration, the majority of IGF-1 proteins were associated with neurons of the infarcted (activated) hemisphere (Guan et al., 1996).

As mentioned previously, IGF-1 has a transmembrane, tyrosine-kinase cell surface receptor, IGF-1R. This class of receptors is made up of single subunits that dimerize when a ligand, such as IGF-1, is attached. Each subunit has two terminals, an extracellular N-terminal (amino-terminal) and an intracellular C-terminal (carboxyl-terminal). The extracellular N-terminal is the binding site for the ligands, while the intracellular C-terminal houses the tyrosines

and is also responsible for the kinase, or phosphorylation, activity of the receptor. When IGF-1 attaches to the N-terminal of its receptor, several signaling cascades occur within two major pathways: the Ras/mitogen-activated protein kinases (MAPK) pathway (Ras/MAPK) and the phosphatidylinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway (PI3K/Akt). Each pathway and signaling cascade is responsible for a different action (**Figure 1.6**). The Ras/MAPK pathway activates cell proliferation, while the PI3K/Akt pathway has three functions: 1) inhibition of apoptosis; 2) protein synthesis; and 3) prevention of inhibition of proliferation (or facilitation of cellular proliferation).

The signaling cascade of the Ras/MAPK pathway is initiated by IGF-1 acting as a ligand and binding to its receptor. Two subunits of the tyrosine kinase receptor dimerize and the tyrosines on both C-terminals are phosphorylated, allowing growth factor receptor-bound protein 2 (GRB2) to bind to the phosphorylated receptor. Son of Sevenless (SOS) then binds to both GRB2 and Ras, a membrane bound protein. When Ras is inactivated, it is bound to the nucleotide guanosine diphosphate (GDP). In order for Ras to activate, SOS must catalyze the replacement of GDP with guanosine triphosphate (GTP) attached to Ras. Now the activated Ras, attached to GTP and disassociated with SOS, is able to bind to B-Raf. The kinase B-Raf activates MEK 1 and 2 by phosphorylating them, which in turn phosphorylates extracellular signal-regulated kinase (ERK) 1 and 2. This cascade leads the activation of transcription factors in the activator protein 1 (AP-1) family. Two of the primary transcription factors in this family are fos and jun. Jun and fos move to the nucleus and attach AP-1 expression on the DNA. Finally, this activates cellular proliferation through Ras at the cell membrane.

Signaling of the PI3K/Akt pathway is also initiated in the same manner as the Ras/MAPK pathway by IGF-1 binding to IGF-1R at the cell surface. Again, tyrosines on the C-

terminal are phosphorylated which allows insulin-receptor substrate-1 (IRS-1) to bind to the phosphorylated receptor and PI3K to bind to IRS-1. Phosphorylated PI3K then disassociates with IRS-1 and binds to membrane-bound phosphatidylinositol 4,5-biphosphate (PIP2). Next, PIP2 is phosphorylated and becomes phosphatidylinositol 3,4,5-triphosphate (PIP3), which activates Akt. From here, Akt goes on to follow three different signaling pathways, each with a different function. First, Akt can inhibit apoptosis, or programmed cell death, by binding to B-cell lymphoma 2 (BCL2)-associate X protein (BAX). When not bound to Akt, BAX initiates apoptosis by forming holes in the outer mitochondrial membrane. Second, Akt initiates protein synthesis, or translation, through the Akt/mTOR signaling pathway. After Akt is activated by PIP3, it phosphorylates Ras homolog enriched in brain (RHEB), which activates mammalian target of rapamycin (mTOR). Then, mTOR activates the translation factor S6 kinase (S6K). Finally, S6K binds to ribosomes to activate protein synthesis. The third signaling pathway of Akt facilitates cellular proliferation by decreasing the concentration of forkhead box O (FOXO), which inhibits proliferation. After Akt is activated, it “tags” FOXO to be destroyed by phosphorylating it. Once FOXO is phosphorylated, ubiquitin ligase recognizes it and transfers ubiquitin peptides onto FOXO. Ubiquitinated FOXO is then destroyed by proteasomes.

Figure 1.6. IGF-1 Signaling Pathways

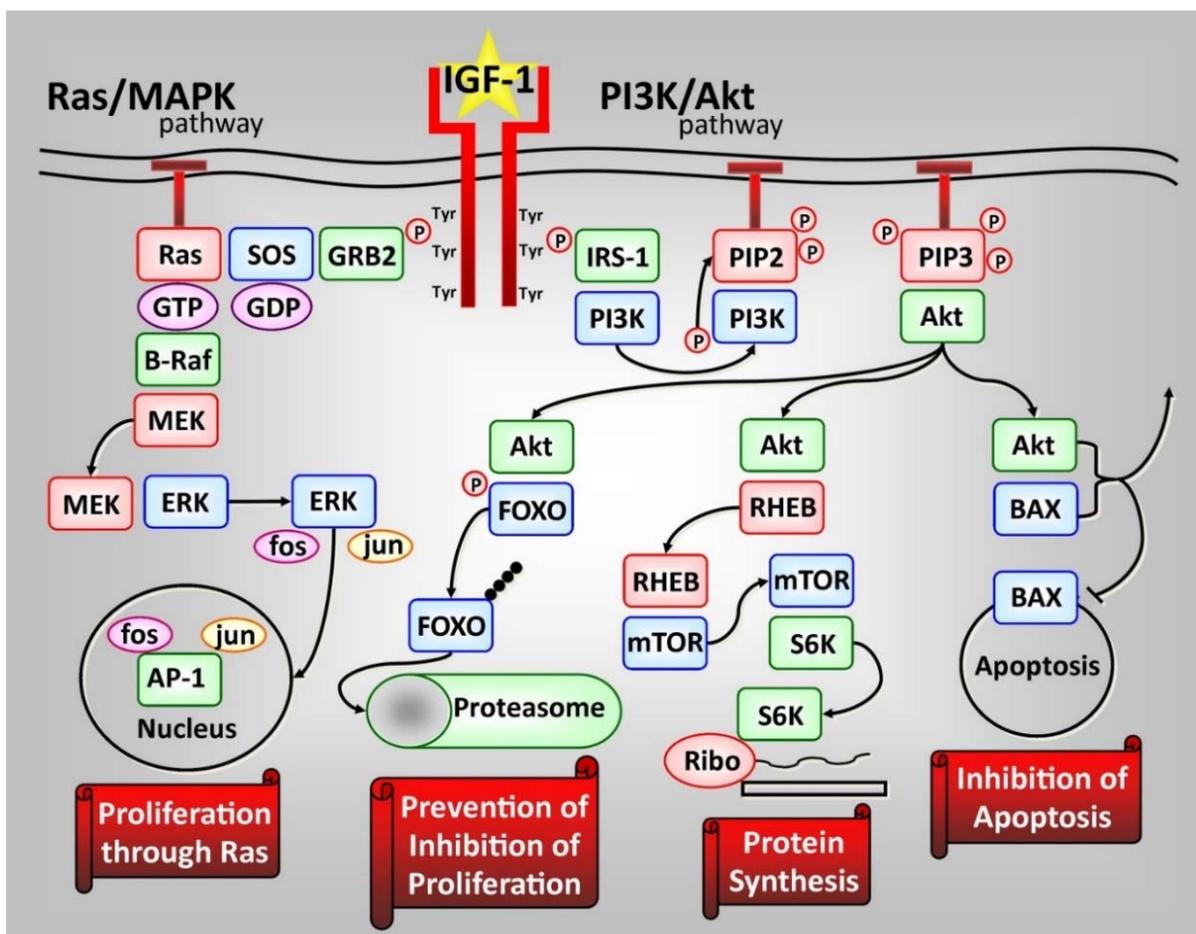


Figure created as part of the current dissertation

Many factors regulate the amount of IGF-1 that is released into blood circulation as well as the level of IGF-1 mRNA. Growth hormone produced from the pituitary is well established as mediator of IGF-1 synthesis. Circulating growth hormone interacts with hepatic receptors and results in stimulation of IGF-1 production and secretion (Johnson et al., 1989; Mathews et al., 1986), which interacts with the whole body as opposed to specific areas or organs. Growth hormone also regulates IGF-1 mRNA expression in the brain. In a mouse model, Mathews and colleagues showed that IGF-1 mRNA in the brain was altered due to growth hormone deficiency

(Mathews et al., 1986). Other hormones have a more target specific effect on the abundance of IGF-1 mRNA. For example, estrogen is a prime regulator in the female reproduction system by stimulating IGF-1 mRNA in the uterus as well as ovarian granulosa cells (Murphy et al., 1987). Another example of this concept is parathyroid hormone, which regulates bone growth by increasing IGF-1 peptide production (McCarthy et al., 1989). Finally, in culture, glucocorticoid dexamethasone, a steroidal hormone that decreases inflammation activity, also decreased IGF-1 mRNA in neuronal and glial cells (Adamo et al., 1988). However, how this translates to clinical populations is still unclear.

1.3.2. IGF-1 Regulation

Nutrition and exercise also have been shown to regulate IGF-1 levels. Elmer and Schalch were the first to observe decreases in IGF-1 mRNA in the rat liver after fasting (Emler, Schlach, 1987). These levels were restored after fasting ended. This concept was supported after Lowe et al observed the same thing (Lowe et al., 1989). In the clinical model, fasting caused a decrease in IGF-1 within 24 hours (Clemmons et al., 1981). On day ten of fasting, IGF-1 levels were 20% of pre-fasting levels. Several studies have also been conducted examining the effect of exercise on IGF-1 levels. A study performed by Cappon et al, aimed at determining the effect of exercise on IGF-1 levels as well as how differences in diet will effect these levels (Cappon et al., 1994). Ten healthy participants between the ages of 22-35 years exercised above their lactate threshold on a cycle ergometer on three separate mornings for 10 minutes each. Each morning they were either given a high-fat, high-glucose, or placebo meal. Blood was sampled 10 minutes prior to exercise, immediately prior to exercise, and every 10 minutes for 90 minutes after the start of exercise. They observed that IGF-1 significantly increased with exercise, peaking at 10 minutes

after the start of exercise. However, they found that the type of meal ingested before exercise had no effect on IGF-1 levels at any time point. Schwarz et al later examined the relationship between aerobic exercise and acute changes in IGF-1 levels using two different intensities (high- and low-intensity) (Schwarz et al., 1996). This study supported Cappon et al.'s results. Ten healthy males who were regular exercisers underwent three separate conditions: resting quietly, 10 minutes of low-intensity exercise, and 10 minutes of high-intensity exercise, and high-intensity exercise. Blood was sampled 5 minutes prior to the onset of exercise, immediately upon onset of exercise, 5 minutes into exercise, immediately upon cessation of exercise, and every 10 minutes after exercise for an hour. Blood was sampled following the same time increments during the resting condition. Blood levels of IGF-1 and IGFBP-3 peaked after 10 minutes of exercise and did so on a dose-dependent basis. High-intensity exercise showed significantly elevated levels of IGF-1 and IGFBP-3 compared to low-intensity exercise and rest. Further, low-intensity exercise elicited significant increases in blood levels compared to rest (Schwarz et al., 1996). The results of Schwarz et al. and Cappon et al. studies are important to stroke and neurological recovery because if evidence continues to be uncovered in support of IGF-1 as a neuronal rescue agent after injury, it is possible that exercise can be used as an intervention technique to utilize and enhance IGF-1's benefits during recovery.

However, not all studies demonstrate an increase in IGF-1 levels following exercise. Suikkari and colleagues had participants complete a three hour cycle ergometer exercise (Suikkari et al., 1989). No increases in IGF-1 were observed after completion, but significant increases in IGFBP-3 were observed.

The observed differences in results from the Cappon and Schwarz studies to the Suikkari study could have been attributed by many factors. Intensity, duration, and mode of exercise

needs to be considered as well as time of sampling in relation to exercise onset. As evident by Schwarz study, there seems to be a dosing effect in the magnitude of IGF-1 elevation due to different intensities of exercise. Mode of exercise (i.e. running, cycling, resistance training) may also have an influence on the magnitude of elevation in IGF-1. Differences in results could also be due to time of sample in relation to exercise onset and completion. If IGF-1 is not sampled frequently enough or at the right time, peak levels could be missed and interpreted incorrectly. Additional studies need to be performed to evaluate the influence of these factors on IGF-1 and IGFBP-3 blood levels, but are not within the scope of the current studies.

Other factors such as age, body mass index (BMI), and gender have been shown to have an effect on circulating IGF-1 levels in the clinical population. Circulating IGF-1 levels steadily decrease across the lifespan (Friedrich et al., 2008; Friedrich et al., 2010; Guven et al., 2013; Kehinde et al., 2005; Mattsson et al., 2008; Morimoto et al., 2005). Higher BMI has been associated with lower circulating IGF-1 (Morimoto et al., 2005). Finally, females typically have higher IGF-1 levels when compared to males (Friedrich et al., 2008; Friedrich et al., 2010; Morimoto et al., 2005). There have been numerous studies performed to determine reference ranges of IGF-1 in healthy individuals (Friedrich et al., 2008; Friedrich et al., 2010; Guven et al., 2013; Kehinde et al., 2005; Mattsson et al., 2008; Morimoto et al., 2005).

Previous studies have shown that IGF-1 follows a circadian pattern in healthy individuals and those with cancer. Mazzoccoli and colleagues quantified IGF-1 levels over 24 hours in men with lung cancer and healthy men (Mazzoccoli et al., 2012). Blood was sampled every six hours. They found that MESOR values, or the arithmetic mean, was significantly higher in healthy men compared to men with cancer. The amplitude of healthy individuals was also significantly larger. Finally, the peak value in healthy individuals occurred later in the morning compared to

men with cancer. Haus also quantified IGF-1 and IGFBP-3 levels over a 24 hour period in women with breast cancer Haus et al., 2001. They found that women with breast cancer also follow a circadian rhythm of IGF-1 and IGFBP-3. However, no studies have determined IGF-1 circadian patterns in neurological injury such as stroke. This proposed study seeks to fill this gap in the literature. This information will give us a more comprehensive look to response of IGF-1 after stroke and how it relates to outcomes after stroke.

1.3.3. Quantification of IGF-1 Blood Levels through Enzyme-Linked Immunosorbent assay (ELISA) Analysis

Radioimmunoassay (RIA) is considered a gold-standard method of quantifying the concentration of an antigen of interest in serum or plasma with the use of antibodies. The antigen of interest is typically labeled, or “tagged”, with a radioactive iodine isotope. An antibody for that antigen is then mixed in, resulting in a complex of the radioactive antigen and the antibody. During the experiment known concentrations are used to determine the concentration in the unknown serum/plasma samples. A gamma-counter is then used to measure the radioactivity of each sample and the concentration of the antigen of each sample can then be derived. However, this method has since been replaced with a newer method, enzyme-linked immunosorbent assay (ELISA). Analysis of antigens using ELISA is typically favored over RIA for several reasons. First, RIA requires special precautions due to the use of radioactive materials. Contrarily, ELISA eliminates the use of radioactive substances and therefore requires fewer precautions. Instead, ELISA analysis uses colorimetric measurements including light absorbance or fluorescence. Analysis by RIA also generally takes several days to complete, whereas ELISAs can be completed in a single day. Finally, both of these methods result in similar sensitivity of measurements.

When using ELISA analysis to determine the availability of IGF-1 to reach its receptor, there are several factors one must consider. First, as mentioned previously, IGF-1 is most abundantly found in a ternary complex with IGFBP-3 and ALS. In order to determine total amount of circulating IGF-1, it must first be disassociated from its ternary complex. To do this, acidic buffer is added to strip IGF-1 from the complex. The acidic samples are then neutralized and an antibody for IGF-1 is added. Then IGF-1 disassociates from IGFBP-3 and IGF-2 takes its place. Cross-reactivity of the IGF-1 antibody with IGF-2 is extremely low. Thus, the ELISA kit is incredibly specific to IGF-1 and measured concentrations are accurate. After determining total concentration of circulating IGF-1, one must also consider the amount of IGFBP-3 to measure the true availability of IGF-1 to reach its receptor. To do this, IGFBP-3 levels can be assayed using ELISA as well.

1.4. Stroke and IGF-1 Levels in the Experimental Animal Model

There are two phases of an ischemic stroke, a primary phase and a secondary phase. The primary phase occurs immediately during the insult. A secondary phase occurs hours later in which there is a delayed cell loss, making up the penumbra (Beilharz et al., 1995; Lorek et al., 1994; Williams et al., 1991; Williams et al., 1992). Stroke research has focused on prevention of the secondary loss of neuronal cells. As mentioned earlier, a cascade of events due to IGF-1 attaching to its receptor result in neuroprotection through stimulation of cellular proliferation and protein synthesis and inhibition of apoptosis. Experimental models have extensively demonstrated the potential of IGF-1 to act as a neuronal rescue agent after stroke. IGF-1 level was increased after injury in proportion to the degree of insult and associated with cells where neuronal death occurred in the injured hemisphere (Beilharz et al., 1998). Further, previous studies have shown that administration of IGF-1 after cerebral insult may be beneficial by

delaying or reducing secondary neuronal loss (Guan et al., 1996; Johnston et al., 1996; Schabitz et al., 2001). Evidence suggests that a single dose of IGF-1 can reduce cortical lesion size from 94% in placebo rats to 24% in experimental rats (Guan et al., 1993). Guan et al demonstrated that there is a dose dependent response of administration of IGF-1 in reduction of neuronal loss in all areas of the brain, such that increased amounts of administered IGF-1 will lead to increases in positive outcomes (Guan et al., 1993). In rats, administration of 75 μ g IGF-1 through intranasal delivery 10 minutes, 24 hours, and 48 hours after the onset of MCAO also showed reduced infarct volume by 60% and hemispheric swelling by 45.6% compared to vehicle controls (Liu et al., 2001). This same study also assessed neurological function through reflex response and demonstrated improvements compared to vehicle controls. Neurological functional assessments included motor-sensory (postural reflexes and flexor responses) and somatosensory (adhesive tape) tests. Rats who were treated with IGF-1 showed significantly lower deficits in all functional assessments compared to vehicle control rats up to 75 hours following MCAO. Results of these studies indicate that using IGF-1 as a pharmaceutical rescue agent or finding ways to up-regulate IGF-1 beginning early after stroke may decrease lesion size of the injured hemisphere and contribute significantly to functional status during recovery.

Experimental models have also shown that exercise and IGF-1 may work together to mediate these neuroprotective effects on the brain. Chang and colleagues demonstrated this by assigning rats to one of four groups [MCAO with and without exercise training as well as MCAO with IGF-1 receptor inhibition with and without exercise training] (Chang et al., 2011). They observed that MCAO rats who participated in exercise had significantly higher levels of IGF-1 in the affected motor cortex when compared to the MCAO rats who did not exercise, indicating that exercise may facilitate IGF-1 entrance into the brain. Further, exercising MCAO

rats showed decreases in lesion size as well as improved motor function. Daily injection of IGF-1 receptor inhibitors mitigated all of these effects, further indicating that IGF-1 may mediate beneficial effects of exercise (Chang et al., 2011). Interestingly, Zheng and colleagues found strong evidence that IGF-1 signaling is a likely mechanism of functional neurological improvement in rats as a result of an exercise intervention after experimental stroke (Zheng et al., 2014). Zheng performed MCAOs on 40 male rats. One group of rats was used as controls, with only a stationary wheel in their cages, while another group was subjected to an exercise intervention that consisted of running for 20 minutes, twice daily, for three days post-ischemia. Neurological function of rats was evaluated using the modified Neurological Severity Scores (mNSS) scale on day 3, 7, and 14 following MCAO. Rats who underwent the exercise intervention scored significantly better on the mNSS on days 7 and even more so on day 14. However, on day 3 following MCAO, both groups showed no difference in neurological function, suggesting that there may be a latency period between an exercise intervention and functional neurological improvements.

Further, this same pattern was observed in infarct lesion volumes, with day 3 showing no significant difference and days 7 and 14 showing increasingly greater significant differences between exercisers and sedentary, with exercisers exhibiting significantly smaller lesion volumes. Even more, Zheng and co-workers showed evidence to suggest that IGF-1 signaling may be a mechanism behind these observed improvements through the sprouting of newborn neurons and decreases in apoptosis of cells in the infarcted hemisphere (Zheng et al., 2014). Immuno-staining and western-blotting showed increases in neural progenitor cells, Nestin and NeuN. Further, exercise activated IGF-1 signaling through the Akt pathway with the expression of IGF-1 and p-Akt drastically increased after exercise. Notably, the same pattern exists once

again with the increases in progenitor cells and expression of IGF-1 and Akt not occurring until days 7 and 14. It is clear that Zheng provides compelling evidence to suggest that exercise after stroke can lead to increases in IGF-1 through the Akt pathway, providing necessary neuroprotection to improve functional recovery in rats. While exercise and IGF-1 look very promising in the animal model, it is still unclear whether these benefits exist in a clinical population.

1.5. IGF-1 in the Clinical Population

Encouraging results of IGF-1 neuroprotection have been extensively shown in animal models of stroke. However, the ability for these concepts to translate over to clinical models is still unclear. Several studies have been performed in the clinical population, however, the application of these studies to interpret IGF-1 response after stroke is limited. Available evidence shows that individuals with low IGF-1 levels have a greater risk of ischemic heart disease, myocardial infarction, coronary artery disease, and a lower incidence of diabetes (Juul, 2002; Laughlin, 2004; Okura, 2001; Vasan et al., 2003). All these conditions are risk factors for stroke. Further, bottom quartile levels of IGF-1 have been directly associated with stroke risk in humans (Bondanelli et al., 2006; Denti et al., 2004; Johnsen, 2005; Tang et al., 2014). Very few studies have been conducted examining IGF-1 levels after clinical stroke and whether this is related to functional recovery and/or lesion size. Denti and colleagues examined IGF-1 and IGFBP-3 levels in 85 acute stroke participants and 88 healthy controls (Denti et al., 2004). At baseline, stroke participants had significantly lower IGF-1 levels compared to healthy controls. Stroke severity by the Barthel Index (BI) was assessed at 3 months and 6 months post-stroke. Low IGF-1 levels early after stroke were associated with a higher stroke severity as well as lower survival rate at 3 months and 6 months post-stroke.

DeSmedt further demonstrated the relationship between IGF-1 levels and stroke outcomes by examining IGF-1 and IGFBP-3 levels very early after stroke and stroke severity by the National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS) (De Smedt et al., 2011). The results of this study showed that even when controlling for risk factors (i.e hypertension, diabetes, atrial fibrillation, coronary artery disease, heart failure, smoking, and previous stroke), lower than median IGF-1 levels were related to increased stroke severity and lower survival rates at 3 and 6 months post-stroke when compared to those with high IGF-1. Baseline stroke severity was not significantly different between low and high IGF-1 individuals, indicating that IGF-1 may not be influenced by the original severity of stroke. Aberg and colleagues studied 407 acute stroke patients and also found individuals with higher than median IGF-1 had improved outcomes 3 and 24 months post-stroke demonstrated by the Scandinavian Stroke Scale (SSS) and mRS (Aberg et al., 2011). Aberg observed that IGF-1 levels were elevated in the acute stage of stroke when compared to healthy controls. The conflicting evidence in baseline IGF-1 levels in individuals with acute stroke and healthy controls between the study by Denti and the study by Aberg, could be attributed to factors such as age of the compared groups, sampling time of IGF-1 in relation to stroke onset, and variation in sampling time of day. Nonetheless, the results of these studies are encouraging evidence to show that IGF-1 may be an important biomarker for neuroprotection after stroke.

1.6. Significance of the Presented Work

This dissertation work is novel and significant as it is a translational project examining physical activity levels, estimated aerobic fitness, and the response of IGF-1, IGFBP-3, and IGF-1 ratio and their relationship to outcomes after stroke in humans. To our knowledge, this is the

first collection of studies in acute stroke to closely examine the ratio of IGF-1 to IGFBP-3, or the availability, across tightly controlled multiple time-points and how it relates to physical activity, estimated aerobic fitness, and clinical outcomes following stroke. This study bridges the gap between current knowledge of the neuroprotective qualities of IGF-1 seen in animals models of stroke, the interaction between physical activity and IGF-1 in healthy humans, and the response of IGF-1 during clinical stroke recovery.

Chapter 2 examines sedentary time on the acute stroke unit using an objective measure, accelerometry. This was the first study conducted in the United States using accelerometry on the acute stroke unit. Current literature has described physical activity during most phases of stroke recovery. Unfortunately, most of this work has been done in the later phases of recovery. The several studies which sought to gain information during the acute and early sub-acute phases have been limited in that they used subjective, observational methodology only. Chapter 2 improves upon this information by providing objective measures of activity using 24-hour monitoring actigraphy for several days after admission to the hospital with acute stroke. The results of this study showed that individuals in the acute hospital setting spend very little time performing activities with an intensity of “light” and above, while they spend most of their time engaging in sedentary behavior. Further, the amount of time spent sedentary was significantly correlated with function at discharge from the hospital, regardless of function upon admission. These important results add to this collection of work by providing a foundation on which to provide beginning evidence that physical activity may provide benefit early after stroke. However, the response of IGF-1 after stroke and how it is related to recovery has still yet to be determined and is sought after through the following chapters. It was the goal of this dissertation

work to provide insight into this important issue and provide groundwork to guide rehabilitation techniques in the future.

From Chapter 2, we learn that physical activity during the acute stroke hospital stay may have a role in improving function. However, it is not always possible or feasible to collect objective measurements of aerobic fitness and physical activity due to limitations in motor impairments and resources, such as cost or time. In Chapter 3, discussion is focused on the use and feasibility of measurement tools to estimate aerobic fitness at the time of stroke admission. The use of this tool will become very useful in later chapters in order to provide knowledge to the field regarding the relationship between estimated aerobic fitness and the change in IGF-1 levels and the availability of IGF-1 during the first week of stroke.

Chapters 4 and 5 seek to outline possible growth factors after stroke that may related to recovery, physical activity, and exercise. It is in these chapters that we have formulated our aims. First, this project seeks to fill the aforementioned gaps in the literature by providing information on the availability of IGF-1 in individuals with acute stroke and how it relates to estimated aerobic fitness (**Aim 1**). Previous research suggests that levels of IGF-1 is influenced by both physical activity levels and aerobic fitness. Additionally, research shows that physical activity after stroke can lead to improved outcomes and decreases in stroke severity. However, no work has been done to understand the intersection of these two early after stroke. If the scientific questions proposed in this study are supported, it will provide evidence to suggest that IGF-1 is an important growth factor related to stroke recovery and may be able to be positively influenced by interventions, such as physical activity. We can use the information gathered from this proposed study to guide future studies and also perhaps assist clinicians in the design of future of rehabilitation protocols. This study has the potential to provide a strong foundation

needed for future studies to focus on understanding ways to up regulate IGF-1 for maximizing neuroprotection.

Second, we will address the complex and conflicting information available to determine the relationship of the response of IGF-1, IGFBP-3, and IGF-1 availability during the first week of stroke to days spent in the hospital, functional independence at one-month post-stroke, and discharge destination (i.e. individuals who discharge home vs. individuals who discharge to inpatient facilities) (**Aim 2 and 3**). Knowledge exists regarding the relationship between IGF-1 and stroke severity, however, these studies did not examine IGFBP-3 levels, nor the availability of IGF-1, an important factor to consider when studying stroke recovery. Further, there is no information regarding hospital length of stay, functional independence at one-month post stroke, and discharge destination and their relationship to IGF-1 in individuals with acute stroke. This information may be beneficial in order to predict outcomes and implement appropriate early interventions to those who are most at risk for sustained impairments due to stroke.

1.7. Summary

Insulin-like growth factor-1 has been shown to be neuroprotective after cerebral injury and plays a key role in inhibition of apoptosis in people and animals. Experimental models of stroke have shown that IGF-1 is necessary for recovery and may act as a neuronal rescue agent. Animals that were injected with IGF-1 after insult showed smaller lesion sizes and better functional recovery than those with vehicle control only. Although, these results seem promising, it is still unknown if this concept is translational to humans. Few studies have been performed examining IGF-1 levels and its relationship to diseased states such as stroke. In the clinical model, studies have shown that above median IGF-1 levels can lead to better scores on

the NIHSS and mRS and even higher survival rates at three months post-stroke (Aberg et al., 2011; De Smedt et al., 2011; Denti et al., 2004). However, these two scales are global questionnaires assessing severity of stroke symptoms, neurological deficit, and independence in activities of daily living, which may not be complete measures of stroke recovery. Therefore, this proposed study aims to add to this current knowledge by providing measures of stroke recovery and improved measures of IGF-1 blood levels. This may be important in guiding clinical rehabilitation in future by providing further evidence of IGF-1's potential for neuroprotection and lay a groundwork for future studies in order to examine methods of facilitating IGF-1 uptake into the brain and other injured regions.

1.8. Specific Aims and Statement of Hypotheses

Insulin-like growth factor 1 (IGF-1) is an important biomarker of neurogenesis and inhibition of apoptosis. Effort has been expended on determining the role of IGF-1 in neuronal recovery and in inhibiting neuronal apoptosis after brain injury. In animal models, IGF-1 appears to be an important factor in providing neuroprotection after cerebral insult by delaying and/or inhibiting apoptosis of brain neuronal cells (Kulik et al., 1997). It has been demonstrated that intranasal administration of IGF-1 to rats reduces infarct volume 60 – 63% and significantly improves assessments of motor, sensory, and reflex function following middle cerebral artery occlusion as compared to control animals (Guan et al., 1996; Johnston et al., 1996; Liu et al., 2001; Liu et al., 2001; Schabitz et al., 2001). Facilitation of IGF-1 is largely modulated by IGF binding protein-3 (IGFBP-3) (Baxter, 1994). Attachment of IGF-1 to IGFBP-3 decreases the availability of the hormone to attach to its receptor. Therefore, it is important to consider the

level of IGFBP-3 and the availability of IGF-1 when studying the potential effectiveness of the hormone in vivo.

However, our understanding of the neuroprotective effect and regulation of IGF-1 after stroke in the clinical population is very limited. Previous studies in humans have shown that individuals after stroke who have above median levels of circulating IGF-1 have higher scores on the National Institutes of Health Stroke Scale and a higher survival rate at 3 months post-stroke than those with lower circulating levels (Aberg et al., 2011; De Smedt et al., 2011). IGF-1 is produced both locally in the brain and peripherally in the liver by cells with IGF-1 gene expression. It has been shown that in the brain there is a low level of IGF-1, but a high level of IGF-1 receptors (Carro et al., 2000; Trejo et al., 2001), suggesting that additional IGF-1 from the periphery is needed for neuroprotection in the brain. A more complete understanding of the regulation and availability of IGF-1 after stroke and the relationship between this hormone and stroke recovery could yield new approaches for overcoming or preventing the stroke-induced neurological damage that is responsible for the long-term disability. The results of the proposed study have the potential to set the groundwork for further studies by providing critical information about important neuroprotective biomarkers after stroke. **The goal of the study was to determine the relationship between IGF-1 response, estimated aerobic fitness, and stroke outcomes following an acute stroke in humans.** To our knowledge, no studies have examined the IGF-1 blood level response, combined with the availability of IGF-1 during acute stroke. Therefore, to measure blood levels of IGF-1 and IGFBP-3, blood samples were collected within 72 hours of admission to the hospital for acute stroke and at one week post-stroke. Physical activity levels prior to the stroke, estimated aerobic fitness at the time of stroke, and outcomes of

stroke recovery were also collected through questionnaires and electronic medical records. We tested our overall hypothesis with the following three specific aims:

Specific Aim 1: Determine the relationship of IGF-1 and IGFBP-3 to estimated aerobic fitness (peak VO₂) in individuals with acute stroke.

We hypothesize that estimated pre-stroke peak VO₂ would be significantly and positively related to IGF-1 and inversely related to IGFBP-3 (H1a). Additionally, we hypothesized that individuals with higher than median circulating IGF-1 levels would have significantly higher estimated pre-stroke peak VO₂ compared to those with lower than median levels (H1b)

Specific Aim 2: Determine the relationship between percent change in IGF-1 and IGF-1 ratio during the first week of stroke and stroke outcomes (i.e. LOS and mRS at one-month post-stroke).

We hypothesize that an increase in IGF-1 and IGF-1 ratio during the first week of stroke would be associated with fewer days spend in the hospital (LOS) and more functional independence at one-month post-stroke (mRS) (H2).

Specific Aim 3: Determine the difference in percent change in IGF-1 and IGF-1 ratio in discharge destination (i.e. individuals who discharged home vs. individuals who discharged to inpatient facilities).

We hypothesized that individuals who discharged home will have significantly greater percent change in IGF-1 and IGF-1 ratio compared to individuals who discharge to inpatient facilities.

CHAPTER 2

Use of Accelerometers to Examine Sedentary Time on an Acute Stroke Unit

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2.1. Abstract

Background and Purpose: Observational studies demonstrate low levels of physical activity during in-patient stroke rehabilitation. There is no objective measure of sedentary time on the acute stroke unit and whether sedentary time is related to functional outcomes. The purpose of this study was to characterize sedentary time after acute stroke and determine whether there is a relationship to functional performance at discharge. **Methods:** Thirty-two individuals (18 males; 56.5 ± 12.7 years) with acute stroke were enrolled within 48 hours of hospital admission. An accelerometer was placed on the stroke-affected ankle to measure 24-hour activity and was worn for 4 days or until discharge from the hospital. Performance of activities of daily living, walking endurance, and functional mobility was assessed using the Physical Performance Test (PPT), Six-Minute Walk Test (6MWT), and Timed-Up and Go (TUG), respectively. **Results:** Mean percent time spent sedentary was $93.9 \pm 4.1\%$ and percent time in light activity was $5.1 \pm 2.4\%$. When controlling for baseline performance, the mean time spent sedentary per day was significantly related to PPT performance at discharge ($r = -0.37$; $p = 0.05$), but not the 6MWT or TUG. **Discussion and Conclusions:** Patients with acute stroke were sedentary most of their hospital stay. Mean time spent sedentary per day was associated with poorer performance at discharge on the PPT, but not the 6MWT and TUG. Patients with acute stroke may benefit from engaging in early mobility or other activities to decrease sedentary time.

2.2. Introduction

Low levels of activity have been documented in those hospitalized for an acute illness other than stroke (Brown et al., 2004; Brown et al.; Lazarus et al., 1991). Inactivity during hospitalization has been associated with functional decline that necessitated nursing home placement even for those who resided in the community prior to hospitalization. Further, inactivity during hospitalization of older adults recovering from a medical illness is an “under-recognized epidemic.” Brown and colleagues monitored activity continuously (24 hours/day) using accelerometers and reported that older adults recovering from an acute illness in the hospital spend approximately 83% of their day lying in bed despite being able to walk independently during their hospital stay (Brown et al., 2009).

In trying to understand activity patterns during stroke recovery, observational studies have examined activity during inpatient stroke rehabilitation and have reported high levels of sedentary time (Askim et al., 2014; Bernhardt et al., 2008). When observing activity in 10 minute intervals from 8:00 am to 5:00 pm, patients during in-patient stroke rehabilitation were seen in bed or sitting 76% of the day and standing or walking 23% of the day (Bernhardt et al., 2008). Further, greater time spent in bed has been associated with a poorer outcome on the modified Rankin Scale (mRS) at 3 months post-stroke (Askim et al., 2014). This evidence suggests that during an inpatient rehabilitation stay, individuals after stroke are spending a large majority of their time engaging in sedentary behavior, which could have a negative impact on functional recovery. Because the acute stroke hospital length of stay in the United States (U.S.) is considerably shorter than those conducted in the early mobilization trials (~14 days) (Bernhardt et al., 2007; Cumming et al., 2011), it is imperative that we better understand activity

patterns during the acute stroke hospital stay in the U.S. so that we can consider strategies aimed at decreasing sedentary time.

Direct observation of people hospitalized with acute stroke is an acceptable method for describing activities performed during the day. However, this has the potential to miss any physical activity, which can limit the interpretation of these findings and may not accurately characterize the degree of inactivity. For example, the observation studies in people with stroke have typically been conducted during the weekday and during usual work hours (i.e.: 8:00 am to 5:00 pm) (Askim et al., 2014; Bernhardt et al., 2007; Bernhardt et al., 2008). This limits documentation of activity in the evening hours, nighttime, or on weekends. However, using an objective measure such as accelerometry would allow for continuous 24-hour monitoring (including sleep hours) without additional personnel burden for observation of activity. The accelerometers can also provide information on intensity of activity such as light or vigorous activity and steps. Additionally, twenty-four-hour monitoring with the use of accelerometers provides accurate characterization of physical activity patterns in patients with acute stroke. To our knowledge, no objective quantification of physical activity using accelerometers has been conducted during the acute stroke hospital stay in the United States.

Therefore, the purpose of this study was to objectively assess sedentary time using tri-axial accelerometers during the acute hospital stay in individuals with stroke. We examined whether the amount of time sedentary was related to functional performance at discharge. A study demonstrated that older adults hospitalized on the medical floor spend 83% of their time lying in bed (Brown et al., 2009). Therefore, we hypothesized that individuals with acute stroke would spend more than 80% of their hospital stay sedentary. Further, we hypothesized that greater mean time spent sedentary over a course of a day would be moderately and significantly

related to poorer functional performance on the Physical Performance Test (PPT), 6 Minute Walk Test (6MWT), and Timed-Up and Go (TUG) at discharge from the hospital.

2.3. Methods

2.3.1. Study Design

This study used a prospective design with a sample of convenience. Approval of the project was obtained from the Human Subjects Committee at KU Medical Center. Institutionally approved written informed consent was obtained prior to study participation.

2.3.2. Participants

Participants were enrolled into the study within 24 – 48 hours of their admission to the certified Comprehensive Stroke Unit at KU Hospital with a diagnosis of acute stroke. Participants were admitted into either the neurological progressive care or neurological intensive care units of the hospital. Patients who are suspected of, or diagnosed with, stroke are commonly admitted to these floors. The neurological progressive care unit is staffed with 1 nurse for every 4 patients and several nursing aids across the floor. The intensive care unit is staffed with 1 nurse for every 2 patients as well as several nursing aids. Rehabilitation services are available 7 days per week. Therapy services on the weekends were identical in duration and intensity to those provided during the week day. A comprehensive list of participant demographics can be found in **Table 2.1**. Individuals were eligible for the study if they were within 48 hours of admission to KU Hospital with a diagnosis of stroke and between the age of 20 and 80 years. Individuals were excluded if they were on physician ordered bed rest.

Table 2.1 Participant Demographics

Characteristics, n = 32	Number or Sample Mean (SD)	Range
Male/Female	18/14	
Age (years)	56.5 (12.7)	(29 – 80)
Body Mass Index	28.3 (5.2)	(16.9 – 39.3)
National Institutes of Health Stroke Scale	3.7 (4.6)	(0 – 21)
Lower Extremity Fugl Meyer Score	25.5/34 (10.1)	(0 – 34)
Days of Activity Monitoring	2.5 (0.9)	(1 – 4)
Race/Ethnicity		
White/Caucasian	23	
African American	5	
Hispanic	2	
Asian	1	
Native American	1	
Functional Performance	<u>Baseline</u>	<u>Discharge</u>
Physical Performance Test score	15.3/36 (10.3)	18.7/36 (11.6)
6 Minute Walk Test (meters)	126.8 (131.8)	191.1 (150.4)
Timed-Up and Go (seconds)	22.0 (21.8)	18.2 (17.1)

2.3.3. Overview

After consent an ActiGraph (ActiGraph LLC. Pensacola, FL) GT3X+ tri-axial accelerometer was placed on the stroke-affected limb at the ankle. When the Fugl-Meyer score was 34/34 on both legs ($n = 6$), we collected data from the right ankle. The Actigraph GT3X+ is more reliable when placed on the ankle vs the hip or spine to measure step count in older adults with and without an assistive device (Korpan et al., 2014).

The water-resistant accelerometers were worn for 24 hours per day until the day of discharge from the hospital, but no longer than 4 days. This timeframe was selected as the maximum length a priori since the average length of stay at KU Hospital for people with acute stroke was 4 days. Our research team would check on the participants throughout the day to make sure the accelerometers were correctly placed and the participants were comfortable with wearing the accelerometers. Nursing staff was given our 24-hour hotline number and if any issues such as non-compliance or skin irritation occurred, they would leave a message and the team would follow up.

ActiGraph GT3X+ accelerometers used 10-second epochs to monitor 24 hour, real-time activity in all three planes of movement. Analysis of sedentary time was performed using ActiLife Version 6.6.3 (ActiGraph® L.L.C. Pensacola, FL) and included time spent in sedentary, light, moderate, and vigorous activity, steps per day, and time sedentary per day (TSPD) in minutes. One day was defined as each 24-hour period starting immediately after consent. For example if a person was consented at 3pm, a day would be defined from 3:00 pm until 2:59 pm the next day and each day thereafter until discharge. If discharge occurred prior to a “day” then sedentary time was calculated based on the time the accelerometers were worn and not a full 24 hour period as this would bias results. Cut points for intensity of activity were based on

information previously reported and validated in adults (Freedson et al., 1998). These were based on the following: light, ≤ 1951 counts; moderate, 1952 – 5724 counts; and vigorous activity, ≥ 5725 counts (Freedson et al., 1998). Sedentary time was defined as any activity that does not amount to more than ≤ 99 counts per ActiLife software. Baseline functional assessments were performed after placement of accelerometers. Follow-up testing was conducted on the day of discharge or four days after baseline testing, whichever came first.

Information regarding participants' lesion size and location, previous medical history, current medications, weight, height, and BMI were obtained from each participant's electronic health record. In addition, we gathered information regarding their physical and occupational therapy minutes. The Fugl-Meyer Assessment (FMA) was used to assess motor performance of the lower extremity (Sanford et al., 1993). Total score on the lower extremity FMA is 34, and a higher score is indicative of lower stroke impairment. Functional assessments included: PPT, 6MWT, and TUG.

The PPT, an assessment of performance of activities of daily living (ADLs), is a valid and reliable test used in many populations including individuals with dementia and Parkinson's disease and those who are elderly (Delbaere et al., 2006; Farrell et al., 2011; Kernan et al., 2005; Lusardi et al., 2003; Reuben, Siu, 1990). Although data has not been published in acute stroke, the PPT has been used in individuals who have experienced transient ischemic attack and stroke (Kernan et al., 2005). The PPT is a test in which the participant is asked to complete 9 tasks of activities of daily living. Each task is timed and then score on a scale of 0 – 4 depending on their time, with 0 indicating unable to complete the task without assistance and 4 indicating completion of the task in the fastest time category. The total score on the PPT is 36, with a higher score indicative of better performance in activities of daily living. The 6MWT was

performed according to previous guidelines outlined by the American Thoracic Society (Crapo et al., 2002). Individuals are asked to walk as far as they can for 6 minutes and the distance walked in meters is recorded. A longer distance walked in 6 minutes is indicative of better walking endurance. Methods of the motor portion of the FMA and the TUG have been described previously (Podsiadlo, Richardson, 1991; Sanford et al., 1993). For the TUG, individuals start the test in a seated position. They are instructed to stand up, walk 3 meters to and around a cone, walk back to the chair, and sit down. A faster time on the TUG is indicative of higher functional mobility. Participants were allowed to rest as needed between functional tasks.

2.3.4. *Data Analysis*

Descriptive statistics were performed to obtain sample mean percent time spent in sedentary, light, moderate and vigorous activities, sample mean steps per day (SPD), sample mean time sedentary per day (TSPD), sample mean score on PPT, sample mean distance on the 6MWT, and sample mean time on the TUG. Mean percent time spent in sedentary, light, moderate, and vigorous activities is reported as an overall percent time over the duration of the hospital stay across participants. Mean time sedentary per day is expressed as $100 * (\text{total minutes spent in sedentary time} * \text{total minutes}^{-1})$. To assess the relationship between TSPD and functional performance of each variable (PPT, 6MWT, and TUG) at discharge we used a Pearson Correlation. To understand the strength of the relationship between TSPD and functional performance, we used criteria defined by Portney and Watkins (LG,MP, 1993): Pearson's coefficient (r) = 0.00 – 0.25, little to no relationship; r = 0.25 – 0.50, fair relationship; r = 0.50 – 0.75, moderate to good relationship; and $r > 0.75$, good to excellent relationship. Since admission (or baseline) functional scores are important predictors of discharge scores in stroke (Pohl et al.,

2013), we took a more conservative approach and controlled for baseline performance in our data analysis of the respective functional assessments. Statistical methods for the linear regressions were based off of methods previously used (Vidoni et al., 2012). Briefly, we used a multistep, hierarchical linear regression with TPSD as the predictor variable and the PPT score at Time 2 as the response variable controlling for baseline PPT performance. Standardized residuals generated from the linear regression were then plotted. P-values were considered significant at $p \leq 0.05$. All statistical analyses were performed using IBM SPSS® Statistics Software Version 20 (Armonk, New York).

2.4. Results

We screened 683 individuals with an acute stroke and enrolled 38 participants in our study. Of the 38 enrolled participants, six were not included in the analysis. The reasons for non-enrollment and exclusion from data analysis are outlined in **Figure 2.1**. Thirty-two people (18 males; mean age of 56.5 ± 12.7 years) were used for data analysis except for the Timed Up and Go ($n = 23$). If participants could not stand up from the chair using the armrests to initiate walking, they were coded as “unable” and not included in the data analysis examining the relationship between sedentary time and functional performance at discharge. These individuals were included in the primary aim, which was describing activity during the acute hospital stay.

Participants spent a mean of 2.5 ± 0.9 days enrolled in our study. Twenty-eight individuals were discharged from the hospital prior to 4 days of enrollment. Therefore, 6 individuals completed 4 full days of accelerometer monitoring. Additionally, 8 individuals were enrolled the same day as their admission to the hospital and 26 were enrolled after their admission date, but within 48 hours.

Figure 2.1. Participant Enrollment Flow Chart

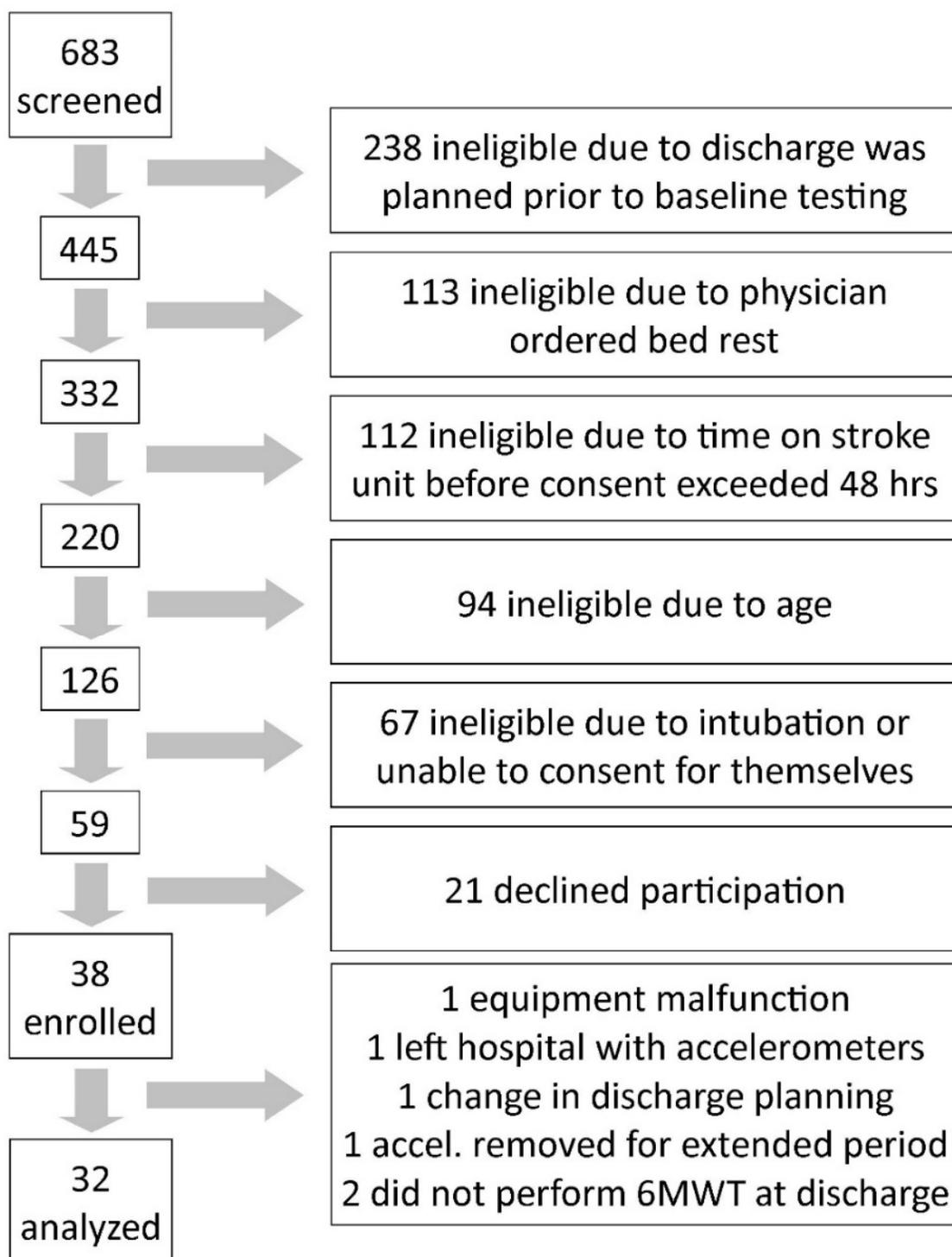


Figure 2.1. Flow chart to describe the reasons for non-enrollment and exclusion from data analysis.

2.4.1. Characterization of Sedentary Time

Our results show that people after acute stroke spend the majority of their time sedentary and almost no time in moderate or vigorous activity (see **Table 2.2**). Participants took a mean of 1907 ± 1594 steps per day. On average, 22.6 ± 15.9 minutes per day were spent in physical therapy and 15.2 ± 12.7 minutes per day were spent in occupational therapy. The total accumulated therapy minutes for physical and occupational therapy are presented in **Table 2.2**.

Sedentary behavior per hour was lowest during the hours of 9:00 – 11:00 am and 2:00 – 4:00 pm. Between the hours of 9:00 and 11:00 am, mean percent time spent sedentary per hour was at its lowest at 91% (**Figure 2.2**) while light activity accounted for 7%. During this time, participants walked a mean of 337 steps per hour.

Table 2.2. Activity Characterization and Functional Performance

n = 32	Number or Sample Mean (SD)	Range
Daily Mean Percent Time in Activities		
Sedentary	93.9 (4.1)	(80 – 99)
Light	5.1 (2.4)	(0 – 10)
Moderate	0.7 (0.7)	(0 – 3.3)
Vigorous	0.2 (0.4)	(0 – 1.4)
Time Sedentary per Day (minutes)	1354.7 (58.6)	(1154 – 1428)
Total Accumulated Therapy Minutes		
Physical Therapy	53.6 (50.6)	(0 – 230)
Occupational Therapy	34.5 (31.6)	(0 – 133)

Figure 2.2. Daily Sedentary Activity Pattern

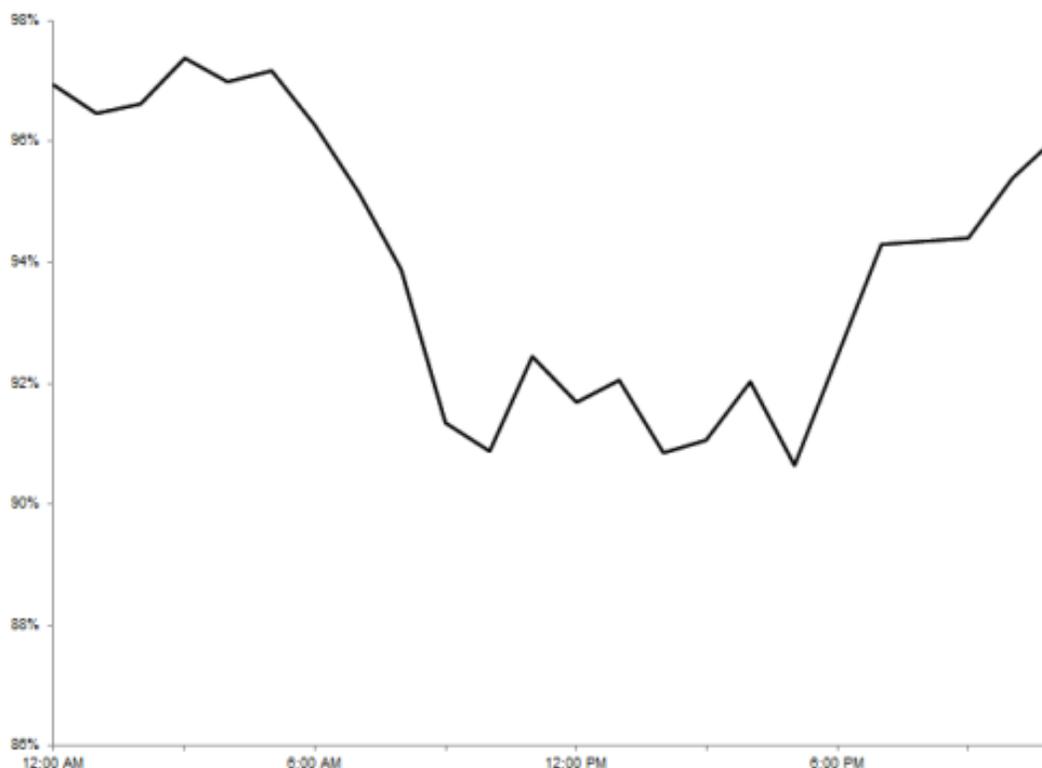


Figure 2.2. Graph of mean percent time spent sedentary per hour over the course of the day

2.4.2. Sedentary Time and Functional Outcome

Baseline performance for all functional measures is presented in **Table 2.2**. A multistep hierarchical linear regression revealed a significant, weak relationship between TSPD and discharge performance on the PPT ($r = -0.37$; $p = 0.05$) when adjusting for baseline performance (**Figure 2.3**). However, when examining the relationship between TSPD and discharge performance on the 6MWT ($r = -0.20$; $p = 0.29$) and TUG ($r = 0.23$; $p = 0.37$), no significant relationship was observed when adjusting for baseline performance. From baseline to discharge, participants improved their PPT score, 6MWT distance, and TUG time a mean of 42%, 37%, and 12%, respectively.

Figure 2.3. Time Sedentary per Day vs. PPT Score at T2

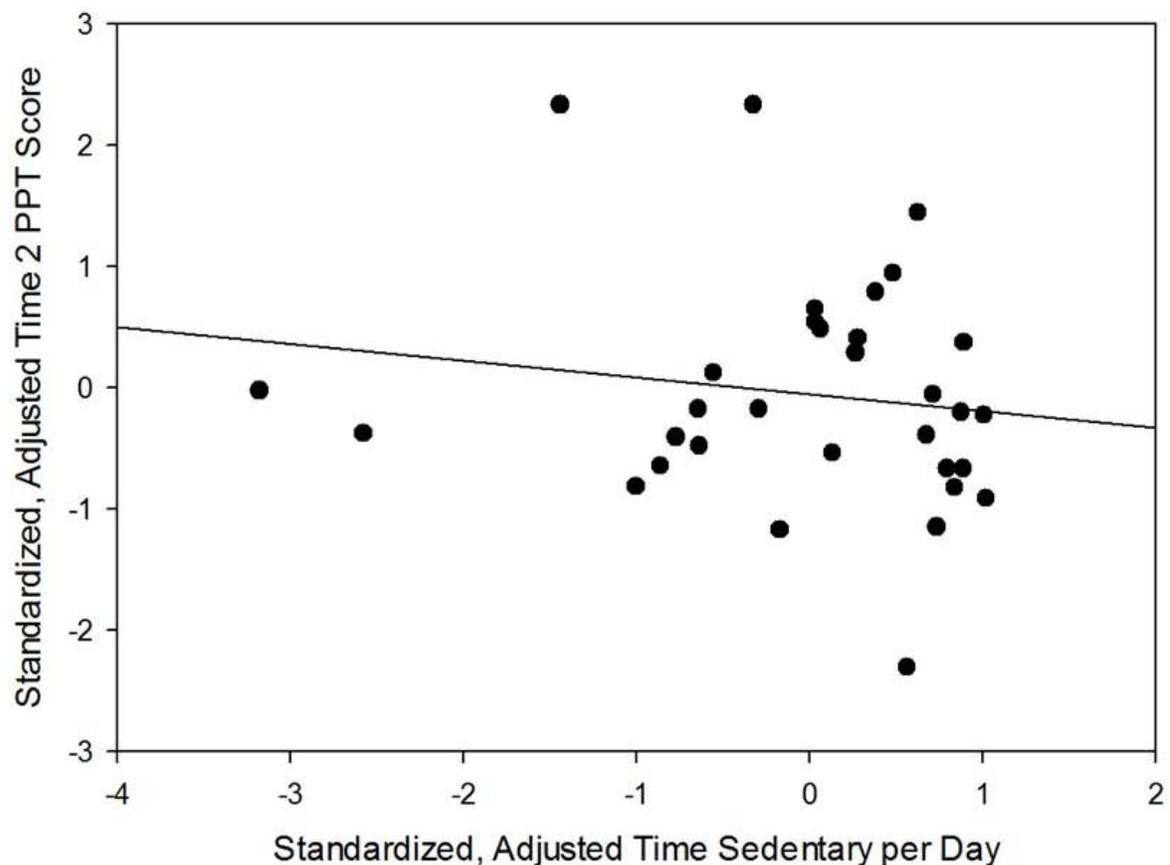


Figure 2.3. Scatter plot of the time sedentary per day plotted against performance on the PPT at Time 2. Longer times in sedentary behavior are associated with poorer performance on the PPT at discharge ($r = -0.37$; $p = 0.05$). Variables are adjusted for performance on PPT at Time 1.

2.5. Discussion

The primary finding of this study was stroke patients spend the majority of the acute hospital stay sedentary. To our knowledge, this is the first study within the U.S. healthcare system to objectively quantify sedentary time using accelerometers during an acute stroke hospital stay.

2.5.1. *Characterization of Sedentary Time*

We hypothesized that stroke patients would spend 80% of their time sedentary as measured by accelerometers. We reported that the mean daily time spent sedentary was approximately 94% of their hospital stay. Our findings are similar to previous observational studies during in-patient rehabilitation in that the majority of the time is spent sedentary (West,Bernhardt, 2013). Our study differs from previous studies in that we used accelerometers to continually assess activity level rather than behavioral mapping and observation (West,Bernhardt, 2013).

Although the percent of time spent sedentary per day is high (94%) or 22.5/24 hours sedentary, this does include nighttime when patients are sleeping. This allowed us to capture all movement and activity during a 24-hour period in a continuous and objective manner without adding bias through observation. Typically, adults 60 years of age and older sleep for an average of 7.4 hours (30% of their day) between the hours of 10:48 pm \pm 1 hour and 6:54 am \pm 1 hour (Klerman,Dijk, 2008). Sedentary time for our participants was highest within the timeframe that was reported by Klerman and colleagues (Klerman,Dijk, 2008). However, some of our participants were not completely sedentary during usual times of sleep (see **Figure 2.2**).

Our study suggests that people hospitalized with an acute stroke spend a higher percentage of time sedentary than what is reported in the literature for an acute medical illness (Brown et al., 2009). Given the negative effects of inactivity, patients with acute stroke appear to be at even greater risk for complications. Bed rest studies have demonstrated negative vascular adaptations (Bleeker et al., 2004) and loss of muscle mass which decreases muscle strength (Kortebein et al., 2007). Considering their low cardiopulmonary fitness levels in acute stroke (Mackay-Lyons,Makrides, 2002), and that the low intensity during rehabilitation is not

sufficient for cardiopulmonary health (MacKay-Lyons, Makrides, 2002), efforts to increase physical activity and minimize decline from sedentary behavior should be encouraged. Therefore, using a multidisciplinary team approach to reduce sedentary time such as early mobilization during the acute hospital stay may slow the further decline of cardiopulmonary health and allow people post-stroke to engage more efficiently in rehabilitation. Due to the stroke-related impairments that often exist, such as hemiparesis and loss of muscle mass (Ryan et al., 2011; Ryan et al., 2002), novel strategies for decreasing the amount of time spent sedentary in the hospital are critical. Further, the recent publication from the American Heart Association/American Stroke Association for Physical Activity and Exercise Recommendations for Stroke Survivors suggests that we need to better understand physical activity patterns during acute stroke and conduct research to gain a better understanding of a dose response to “slow or prevent loss of cardiopulmonary fitness (Billinger et al., 2014).”

While our study is the first to use accelerometers in acute stroke, others have examined physical activity during inpatient rehabilitation and community living. Prajapati and colleagues used accelerometers to assess self-selected walking activity in subacute stroke in one single day on the inpatient rehabilitation unit (Prajapati et al., 2013). The 8 participants ranged from 13 days to 68 days post-stroke and all could ambulate with or without an assistive device independently. The authors report that while their participants were independent with their ambulation (with/without assistive device), they spent little time (about 10%) of their day in activity such as walking. This similar pattern appears to remain consistent when individuals post-stroke return home. Roos and colleagues monitored walking activity using activity monitors in people post-stroke and otherwise healthy older adults dwelling in the community (Roos et al., 2012). Their findings also support that community-dwelling individuals spend less

time in bouts of walking during the day after stroke when compared to healthy adults. Our data suggest that sedentary time begins during acute stroke recovery. Therefore, physical therapists and other healthcare providers should work with people after stroke to minimize inactivity and meet the recommended minutes for physical activity and exercise (Billinger et al., 2014).

2.5.2. *Sedentary Time and Functional Outcome*

This is the first study to examine the relationship between sedentary time using accelerometry and functional performance measures. We hypothesized that we would see a moderate correlation between functional outcome measures and sedentary time. Baseline performance likely influences performance at discharge. Therefore, we chose to be conservative and control for baseline performance on all outcome measures in our analysis. We report that the PPT had a fair and significant relationship with sedentary time. This may be due to the comprehensive activities (balance, walking, feeding, dressing) that are included in the PPT. The 6MWT and TUG (n = 23) had little to no relationship with sedentary time. The 6MWT and TUG are walking activities and perhaps not sensitive to detecting change in a few days following an acute stroke. We must also consider that sedentary time may be higher early after stroke as patients are being evaluated by the therapy team regarding walking performance. Therefore, people after stroke may not be encouraged to move about during this time or wait until therapy or nursing is available to assist.

There are several limitations to the study that must be considered when interpreting results. The sample size for this pilot study was small but provides preliminary data to inform large scale studies of in-hospital sedentary time and the relationship to functional outcome measures such as the 6MWT and TUG. We used the device on the ankle, which appears to

provide the most accurate measure for step counts. However, our primary outcome was physical activity levels and we do not know how accurate the device is for assessing this specific parameter. We did not track the time of day that physical and occupational therapy occurred. Therefore, we do not know how much “activity” time was self-initiated or occurred during therapy time but our data does provide information regarding continuous activity during the acute hospital stay. The PPT is a measure of physical performance and consists of simulated activities of daily living but has not been validated in stroke. Finally, we did not gather information regarding other comorbidities or prior orthopedic injury, joint replacement, or low back pain. It is possible that comorbid conditions could have contributed to reduced mobility during the stroke hospital stay.

Future work should begin to elucidate the benefits of early physical activity and how stroke recovery may benefit from incorporating physical activity early after stroke. A recent review by Zeiler and Krakauer made several excellent points regarding the importance of the “sensitive period” in the early phase of stroke recovery (Zeiler, Krakauer, 2013). The authors discussed the available literature suggesting that enriched environments and engaging in activity (task specific training) enhances recovery from stroke. The data we present suggest that early after stroke the majority of the stroke patients are sedentary and are not spending their time engaging in activity that could be optimal for recovery. Most of the activity occurred early in the morning, which again leaves the remainder of the day inactive and likely alone in their room. Considering ways to encourage more activity whether through early mobility or spending time out of their room, would be advantageous.

2.6. Conclusion

Acute stroke patients are sedentary the vast majority of their time sedentary while in the hospital. Further, sedentary time was inversely related to performance in our outcome measures. Given the well-documented and rapid onset of the negative effects of inactivity, our data suggest that there needs to be a focus of reducing inactivity upon admission. Therapists, with their clinical understanding of movement after stroke, are key providers to initiate and guide increasing activity and mobility.

CHAPTER 3

Use of a Non-Exercise Estimate for Pre-Stroke Peak VO_2 during the Acute Stroke Hospital Stay

Mattlage AE, Redlin SA, Rosterman LR, Harn N, Sisante JFV, Abraham MG, Billinger SA. In
Press, Cardiopulmonary Physical Therapy Journal.

3.1. Abstract

Purpose: In individuals with acute stroke it is difficult to conduct an exercise test to assess peak oxygen consumption (peak VO_2). Therefore, the purpose of this study was to use a clinically feasible tool for assessing pre-stroke peak VO_2 using a non-exercise estimation equation to test whether estimated pre-stroke peak VO_2 was related to functional outcome measures at discharge from the hospital in individuals following an acute stroke. We hypothesized that estimated pre-stroke peak VO_2 would be significantly related to discharge Physical Performance Test (PPT), Six-Minute Walk Test (6MWT), and lower extremity Fugl-Meyer (LEFM). **Methods:** Estimated pre-stroke peak VO_2 was calculated using a previously validated prediction equation using the following variables: BMI, age, gender, resting heart rate, and a self-reported measure of physical activity. Outcome measures were assessed 4 days after enrollment or immediately prior to discharge (whichever occurred first). **Results:** Thirty-four participants (56.0 ± 12.6 years; 20 males) with acute stroke were enrolled within 48 hours of admission. For all individuals, estimated pre-stroke peak VO_2 was $27.3 + 7.4 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and had a weak, non-significant relationship with the PPT ($r = .19$; $p = .28$), 6MWT ($r = .10$; $p = .56$), and LEFM ($r = .32$; $p = .06$). However, when considering sex, females but not males had a significant relationship with LEFM ($r = .73$; $p = .005$) and moderate but non-significant relationship with PPT ($r = .53$; $p = .06$) and 6MWT ($r = .47$; $p = .10$). **Conclusions:** Within 48 hours of stroke admission, we were able to administer a non-exercise equation to estimate pre-stroke peak VO_2 . For the entire sample, functional measures conducted at discharge were not related to estimated pre-stroke peak VO_2 . However, when considering sex, the relationship between pre-stroke VO_2 and the functional measures was strengthened.

3.2. Introduction

Individuals post-stroke are physically inactive, spend small amounts of time walking per day (Roos et al., 2012), and have poor cardiorespiratory (CR) fitness, defined as peak oxygen consumption (peak VO_2) (Billinger et al., 2012; Gordon et al., 2004; Mackay-Lyons, Makrides, 2002; Macko et al., 1997). Assessing peak VO_2 using a metabolic cart with gas analysis is considered the gold standard (ACSM, 2010). Peak VO_2 may be an indicator of one's ability to participate in varying levels of activity such as exercise and activities of daily living (ADLs), especially in older adults (Billinger et al., 2011; Fleg et al., 2013) and people after stroke (Gordon et al., 2004). However, peak exercise testing is difficult, if not impossible, to conduct during the acute stroke hospital particularly with shorter lengths of stay especially in the United States. In our previous work, we reported an average of 2.5 days for an acute stroke hospital stay (Mattlage et al., 2015). This combined with ongoing medical diagnostic testing, patient fatigue, and neuromotor impairments makes exercise testing difficult. Therefore, using non-exercise measurements, such as prediction equations, to estimate peak VO_2 could be a simple, low-cost alternative to healthcare providers and especially physical therapists during the acute stroke hospital stay.

The American Heart Association published a scientific statement, *The Importance of Cardiorespiratory Fitness (VO_2 max) in the United States*, which highlights that CR fitness is recognized as an important marker of both functional ability and cardiovascular health (Kaminsky et al., 2013). Furthermore, CR fitness is currently the only major risk factor that is not routinely and regularly assessed (Kaminsky et al., 2013). People post-stroke already have poor CR fitness in the acute stroke setting (MacKay-Lyons, Makrides, 2002) and our work has demonstrated that a high percent of the acute hospital stay is spent sedentary (Mattlage et al.,

2015). Therefore, using a previously validated, non-exercise estimation for peak VO_2 may be a feasible option for rehabilitation professionals to assess CR fitness during the acute hospital stay when exercise testing may not be available or easily conducted and may be used as a marker of functional ability (Kaminsky et al., 2013).

Jurca and colleagues developed and validated a prediction equation to estimate peak VO_2 in a large population of community dwelling, middle-aged adults (Jurca et al., 2005). Their prediction equation was then cross-validated in a targeted older adult population (60-80 years of age) with 83.3% of the sample having between 1-6 cardiovascular (CV) risk factors and 15.7% having no CV risk factors (Mailey et al., 2010). The measured VO_2 max ($21.58 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and predicted VO_2 max ($21.42 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) were similar in this group of older adults. This non-exercise prediction equation uses the following variables sex, age, body mass index (BMI), resting heart rate (rHR), and a self-reported measure of physical activity. This equation would be simple and easy to use during the acute stroke setting and has clinical utility for estimating peak VO_2 .

Having a measure of CR fitness using an estimated peak VO_2 such as the Jurca equation allows for investigation into whether pre-stroke CR fitness is related to stroke recovery. There is evidence in animal models that suggests exercise pre-conditioning protects against injury following myocardial ischemia (Calvert et al., 2011) and cerebral ischemia (middle cerebral artery occlusion followed by reperfusion) (Davis et al., 2007; Ding et al., 2006; Li et al., 2004; Liebelt et al., 2010). In human studies (Deplanque et al., 2012; Krarup et al., 2008; Stroud et al., 2009) physical activity prior to stroke may be neuroprotective and reduce stroke-related impairment as measured by the Barthel Index (BI) (Deplanque et al., 2012; Stroud et al., 2009) and modified Rankin Score (mRS) (Deplanque et al., 2012; Krarup et al., 2008).

The purpose of this study was to use a clinically feasible tool for assessing pre-stroke peak VO_2 using a non-exercise estimation equation to test whether estimated pre-stroke peak VO_2 was related to functional outcome measures at discharge from the hospital in individuals following an acute stroke. We hypothesized a priori that, after controlling for lesion size, estimated pre-stroke peak VO_2 would be moderately and significantly related to the Physical Performance Test (PPT), our primary outcome measure, and the Six-Minute Walk Test (6MWT) and the lower extremity Fugl-Meyer Assessment (LEFM), at discharge. A post-hoc analysis was conducted to determine whether sex differences existed between estimated pre-stroke peak VO_2 and our outcome measures at discharge.

3.3. Methods

3.3.1. Study Design

This study used a sample of convenience and included individuals admitted to KU Hospital with a diagnosis of acute stroke. The study was approved by the Human Subjects Committee (HSC) at KU Medical Center and institutionally approved written informed consent was obtained from all participants prior to study enrollment.

3.3.2. Participants

Between the months of June 2012 and October 2013, 683 individuals admitted to KU Hospital with a diagnosis of acute stroke were screened for inclusion into the parent study, which has been published (Mattlage et al., 2015). Individuals were enrolled if they were between 20 and 80 years of age with a diagnosis of acute ischemic or hemorrhagic stroke. Individuals were excluded if: 1) consent was not possible without a surrogate consent; 2) the individual was

unable to provide written consent within 48 hours of admission to the stroke unit; 3) discharge from the hospital was to occur before baseline assessments; and 4) the individual was prescribed to physician-ordered bed rest. **Table 3.1** outlines demographic characteristics of study participants. Of the 683 individuals screened, 238 were ineligible due to anticipation of discharge prior to the study team conducting baseline testing, 113 were ineligible due to physician ordered bed rest, 112 were ineligible due to the time on the stroke unit exceeded 48 hours prior to the study team initiating consent, 94 did not meet the inclusion criteria for age, 67 were ineligible due to intubation or the inability to consent, and 21 individuals declined participation.

3.3.3. *Outcome Measures*

All functional performance outcome measures were assessed on either the fourth day following consent (Day 4) or on the day of discharge from the hospital, whichever occurred first (Mattlage et al., 2015). A stroke neurologist performed the NIH Stroke Scale (NIHSS) on admission to the hospital. A team radiologist (N.H.) calculated lesion volumes from structural magnetic resonance images (MRI) performed at admission to the hospital using established standardized neuroimaging techniques for ischemic and hemorrhagic stroke (Majersik et al., 2015; Newman, 2007). An ellipsoidal estimation function was used (Newman, 2007). In cases of multiple lesions, largest lesion volume was used and all volumes are reported in centimeters cubed (cm³) (Newman, 2007).

3.3.3.1. Estimated Peak Oxygen Consumption (Peak VO₂)

We selected the previously established and validated equation by Jurca (Jurca et al., 2005) and (MaileyMailey et al., 2010) based on data from our laboratory examining non-exercise estimated peak VO₂ and measured peak VO₂ in healthy adults (n = 110). The results of our unpublished data were similar to prior reports (Jurca et al., 2005; Mailey et al., 2010) in that estimated peak VO₂ was strongly correlated to measured peak VO₂ (r = 0.88 p < 0.001). Based on these data, we chose to use this non-exercise equation to estimate pre-stroke peak VO₂ in people with acute stroke during the hospital stay.

After consent in acute stroke participants, we recorded the necessary information for the Jurca equation (Jurca et al., 2005). The Jurca equation uses the following variables to estimate pre-stroke peak VO₂: age, sex, BMI, rHR, and a constant variable associated with the participants' self-reported physical activity level prior to being admitted to KU Hospital for acute stroke. The self-reported physical activity score is separated into five categories (Levels 1-5), with a constant variable assigned to each. Each level considers a different duration, frequency, and intensity of activity performed in a typical week. Information regarding participants' activity levels are in **Table 3.2**. The Jurca prediction equation is as follows: Estimated METS = [(Sex, F = 0; M = 1 x 2.77) – (Age in years x 0.10) – (BMI x 0.17) – (rHR x 0.03) + (Physical Activity Score x 1.00) + 18.07] (Jurca et al., 2005). Estimated METS is multiplied by 3.5 mL*kg⁻¹*min⁻¹ (1 MET = 3.5 mL*kg⁻¹*min⁻¹) (ACSM, 2010) to get estimated pre-stroke peak VO₂.

Although the Jurca prediction equation to estimate peak VO₂ has not been validated in the acute stroke population, it has been previously validated in older adults with multiple CV risk factors (Mailey et al., 2010). As mentioned the variables included in the Jurca equation are age,

sex, BMI, physical activity and rHR. We did not expect BMI to change within 1-2 days post-stroke. Physical activity was assessed as “activities performed in a typical week prior to your stroke”. However, resting HR could be influenced after a stroke. Therefore, we wanted to ensure that the rHR at the time of stroke was appropriate to use. To our knowledge, there is no information in the literature regarding whether rHR is similar before and after stroke. Therefore, we examined the medical record of a small subset of the cohort which had rHR captured in the medical record before their stroke. In these individuals (n=19), rHR was not significantly different before and after stroke (before stroke: $76.4 \text{ bpm} \pm 10.8$; after stroke: $76.4 \text{ bpm} \pm 13.8$; $p = 0.983$).

3.3.3.2. Physical Performance Test (PPT)

The PPT has been identified as a useful clinical tool and has demonstrated high inter-rater reliability and concurrent validity for measuring performance of ADLs in older adults and individuals with stroke (Reuben et al., 1995; Rozzini et al., 1997; Sherman, Reuben, 1998). We previously used the 9-item version of the PPT in older adults with and without Alzheimer’s disease (Vidoni et al., 2012) and on the acute stroke unit (Mattlage et al., 2015). The PPT includes tasks individuals complete on a daily basis such as: writing a sentence, simulate eating, lifting a book onto a shelf above shoulder height, putting on and removing a jacket, picking up an item from the floor, walking 50 feet, turning in a circle, rising from a chair 5 times without the use of the upper extremities, and a progressive Romberg test of balance (standing with feet side-by-side, semi-tandem, and full-tandem) (Shah et al., 2004; Vidoni et al., 2012).

3.3.3.3. Six-Minute Walk Test (6MWT)

The 6MWT is a reliable and valid tool for assessing walking endurance in individuals after stroke (Eng et al., 2004; Flansbjer et al., 2005; Fulk, Echternach, 2008; Kosak, Smith, 2004; Patterson et al., 2007; Perera et al., 2006; Wevers et al., 2011). The 6MWT was performed with minimal distractions on a 14-meter section of the walking track outside of the participants' room on the stroke unit. A 14-meter ribbon was secured to the floor and orange cones were placed at both ends. A stopwatch was used to keep time and standardized verbal cues were given every minute according to previously published guidelines (Crapo et al., 2002). All participants wore a gait belt during the test for safety. If the tester needed to provide greater than minimal physical assistance with walking or if the participant needed to sit down to rest, the testing was terminated and the distance walked in that time was recorded. Participants were allowed to use an assistive device (e.g. walker, cane) if needed during the test. If a participant was unable to ambulate with or without an assistive device, a score of 0 meters was recorded.

3.3.3.4. Fugl-Meyer Assessment (FMA)

The FMA is a commonly used tool to measure motor performance and has been shown to be a valid and reliable tool in individuals post-stroke. Sanford et al., 1993 We collected data using only the lower extremity motor portion (LEFM), which was scored on a scale of 0 – 34, with 34 indicating no motor impairment (Mattlage et al., 2015).

3.3.4. *Sample Size Justification*

We calculated sample size for the parent study (Mattlage et al., 2015) and determined we needed 35 participants. We allowed for a small attrition rate of 10% and therefore, planned to enroll a total of 38 individuals.

3.3.5. *Statistical Analysis*

Data analysis was performed using SPSS software Version 20.0 (SPSS Inc, Chicago, IL) for Windows. We report data as mean (standard deviation) where appropriate. All significance levels were set to a .05 alpha-level, with 95% confidence intervals (CI). A partial correlation was performed to determine the relationship of estimated pre-stroke peak VO₂ and functional outcomes (PPT, 6MWT, LEFM) while controlling for lesion size. We chose to be conservative in our analyses and controlled for initial lesion size because of its potential influence on motor performance. Additionally, to examine whether there might be sex differences in estimated pre-stroke peak VO₂ and our selected outcome measures, partial correlations were performed.

3.4. **Results**

In the parent study, we enrolled 38 participants with a total of 34 individuals completed the study and were used for data analysis (**Table 3.1**). Mean time between study enrollment and performance of discharge assessments was 2.5 days.

The majority of our participants reported engaging in light physical activity prior to stroke (**Table 3.2**). Mean estimated pre-stroke peak VO₂ was 27.3 (7.4) mL*kg⁻¹*min⁻¹ and is consistent with prior studies using measured peak VO₂ (Billinger et al., 2012; MacKay-Lyons, Makrides, 2002). This supports prior evidence that people post-stroke had lower CR

fitness pre-stroke for their age, using ACSM categories. **Table 3.3** and **Figure 3.1** demonstrate estimated pre-stroke peak VO_2 and fitness level for gender and age.

Figure 3.1. Estimated Pre-Stroke Peak VO_2 by Gender and Age

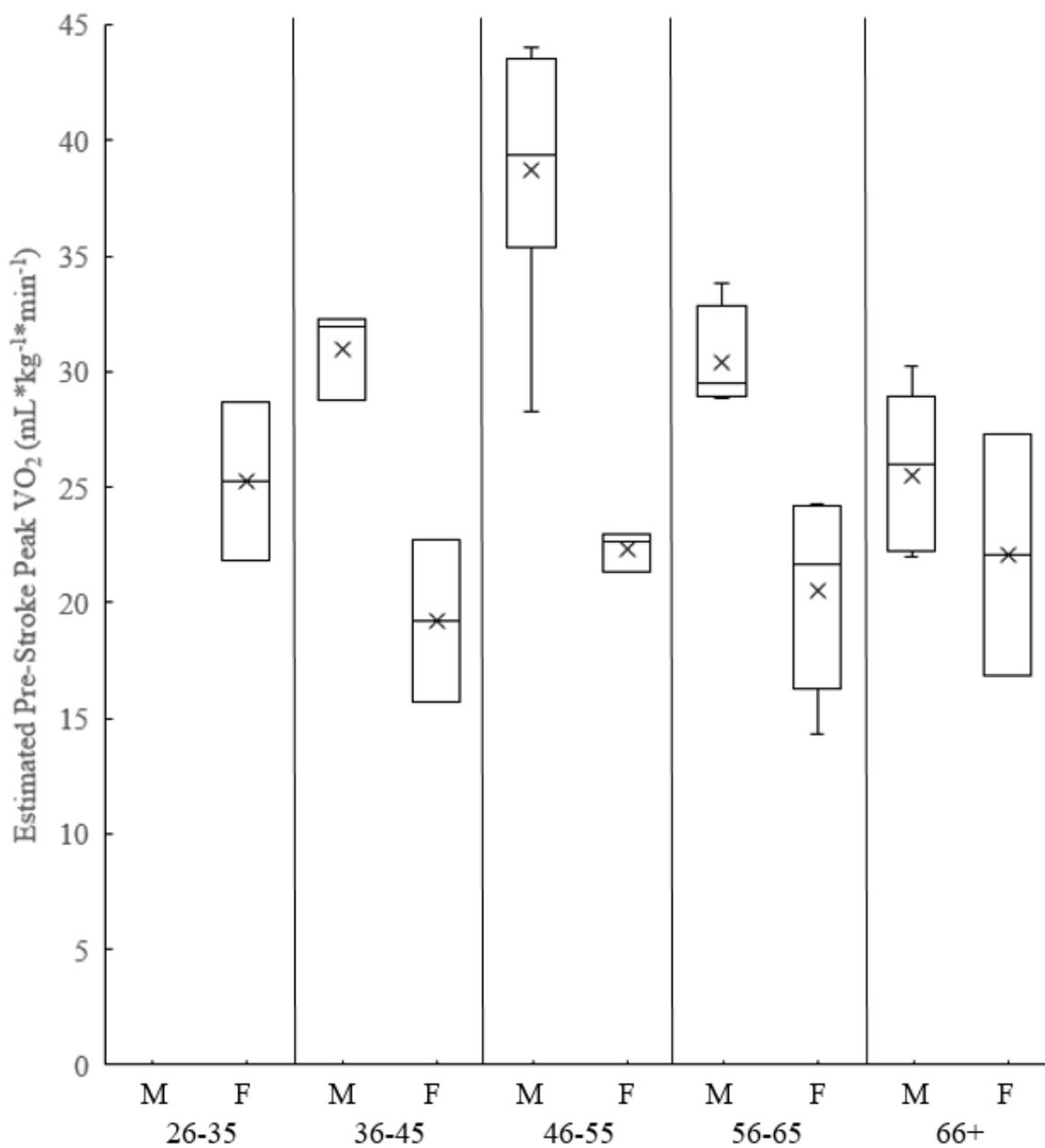


Table 3.1. Participant Demographics

Characteristics, n = 34	Number or Group Mean (SD)
Male	20
Age (years)	56.0 (12.6)
Race/Ethnicity	
Caucasian	24
African American	5
Hispanic	3
Native American	1
Asian	1
Diabetes	
None	18
Type 1	1
Type 2	10
Unknown	5
NIH Stroke Scale on Admission	3.7 (4.4)
Discharge Performance	
Physical Performance Test	18.9 (11.2)
6 Minute Walk Test (meters)	199.7 (149.8)
Lower Extremity Fugl-Meyer Score	27.4 (9.3)
Body Mass Index	28.0 (5.2)

Table 3.2. Self-reported Physical Activity Scores

Physical Activity Score, n = 34	Number	Constant
Level 1	6	0.00
Level 2	19	0.32
Level 3	5	1.06
Level 4	0	1.76
Level 5	5	3.03

Table 3.2. Level 1: Primarily sedentary; Level 2: Five days or more per week of light physical activity for 10 minutes or more at a time; Level 3: Moderate aerobic exercise for 20 to 60 minutes per week; Level 4: Moderate aerobic exercise for 1 to 3 hours per week; Level 5: Moderate aerobic exercise for over 3 hours per week

Table 3.3. Estimated Pre-Stroke Peak VO₂ Levels

Age Group (years)	n	Group Mean (SD) mL*kg ⁻¹ *min ⁻¹	ASCM Fitness Category
ALL	34	27.3 (7.4)	
26-35	2	25.3 (4.9)	Very Poor
36-45	5	26.3 (7.0)	Poor
46-55	9	33.2 (9.4)	Average
56-65	9	24.9 (6.2)	Poor
65+	9	24.7 (4.1)	Below Average
MALES	20	31.3 (6.6)	
26-35	0		
36-45	3	31.0 (2.0)	Average
46-55	6	38.7 (5.7)	Good
56-65	4	30.4 (2.3)	Average
65+	7	25.5 (3.3)	Average
FEMALES	14	21.6 (4.1)	
26-35	2	25.3 (4.9)	Very Poor
36-45	2	19.2 (4.9)	Very Poor
46-55	3	22.3 (0.9)	Poor
56-65	5	20.5 (4.2)	Poor
65+	2	22.1 (7.3)	Average

The secondary objective was to determine whether predicted pre-stroke peak VO_2 was related to the outcome measures at discharge. The findings show that estimated pre-stroke peak VO_2 was weakly, but not significantly, related to discharge outcome scores for the PPT ($r = 0.19$, $p = 0.28$, CI [-.14, .48]), 6MWT distance ($r = 0.10$, $p = 0.56$, CI [-.24, .45]), and LEFM scores ($r = 0.32$, $p = 0.06$, CI [-.03, .61]) when controlling for initial lesion size. When separating data for sex and controlling for initial lesion size, estimated pre-stroke peak VO_2 in males did not have any significant relationships with functional measures (PPT: $r = .13$, $p = .60$, CI [-.32, .55]; 6MWT: $r = .05$, $p = .82$, CI [-.44, .59]; LEFM: $r = .15$, $p = .54$, CI [-.35, .57]). Estimated pre-stroke peak VO_2 in females showed a strong, significant relationship with LEFM ($r = .73$, $p = .005$, CI [.41, .96]) (**Figure 3.2**), but moderate, non-significant relationships with the PPT ($r = .53$, $p = .06$, CI [-.13, .91]) (**Figure 3.3**), and 6MWT ($r = .47$, $p = .10$, CI [-.14, .91]) (**Figure 3.4**) when controlling for initial lesion size.

Figure 3.2. Estimated Pre-Stroke Peak VO₂ vs. Discharge FMA Score

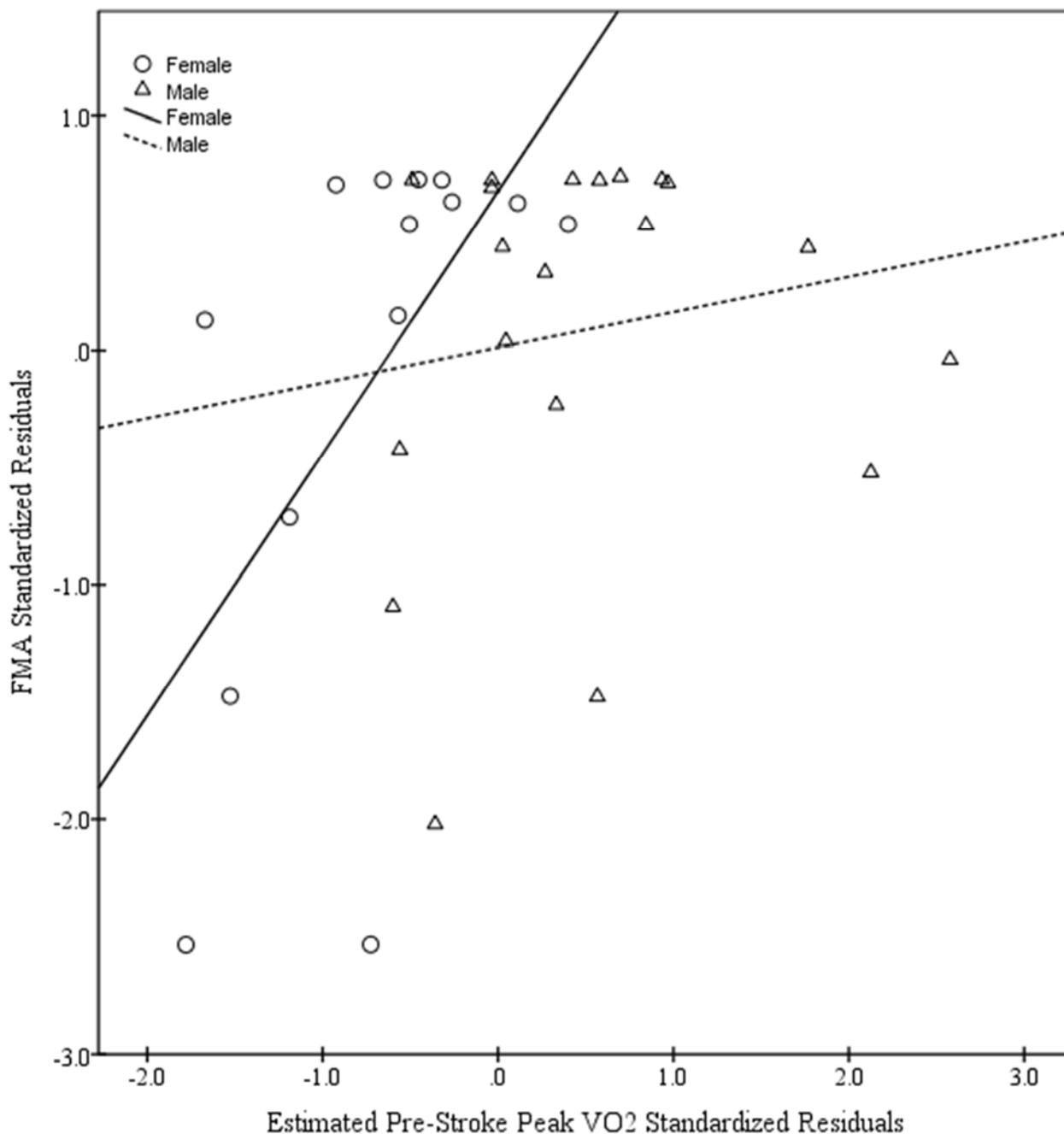


Figure 3.2. Scatter plot of estimated pre-stroke peak VO₂ residuals plotted against FMA score at discharge separated by gender. (Males: $r = .15$, $p = .54$, CI [-.35, .57]; Females: $r = .73$, $p = .005$, CI [.41, .96]). Higher estimated pre-stroke peak VO₂ in females is significantly related to FMA score at discharge. Variables are adjusted for lesion volume calculated from the admitting MRI.

Figure 3.3. Estimated Pre-Stroke Peak VO₂ vs. Discharge PPT Score

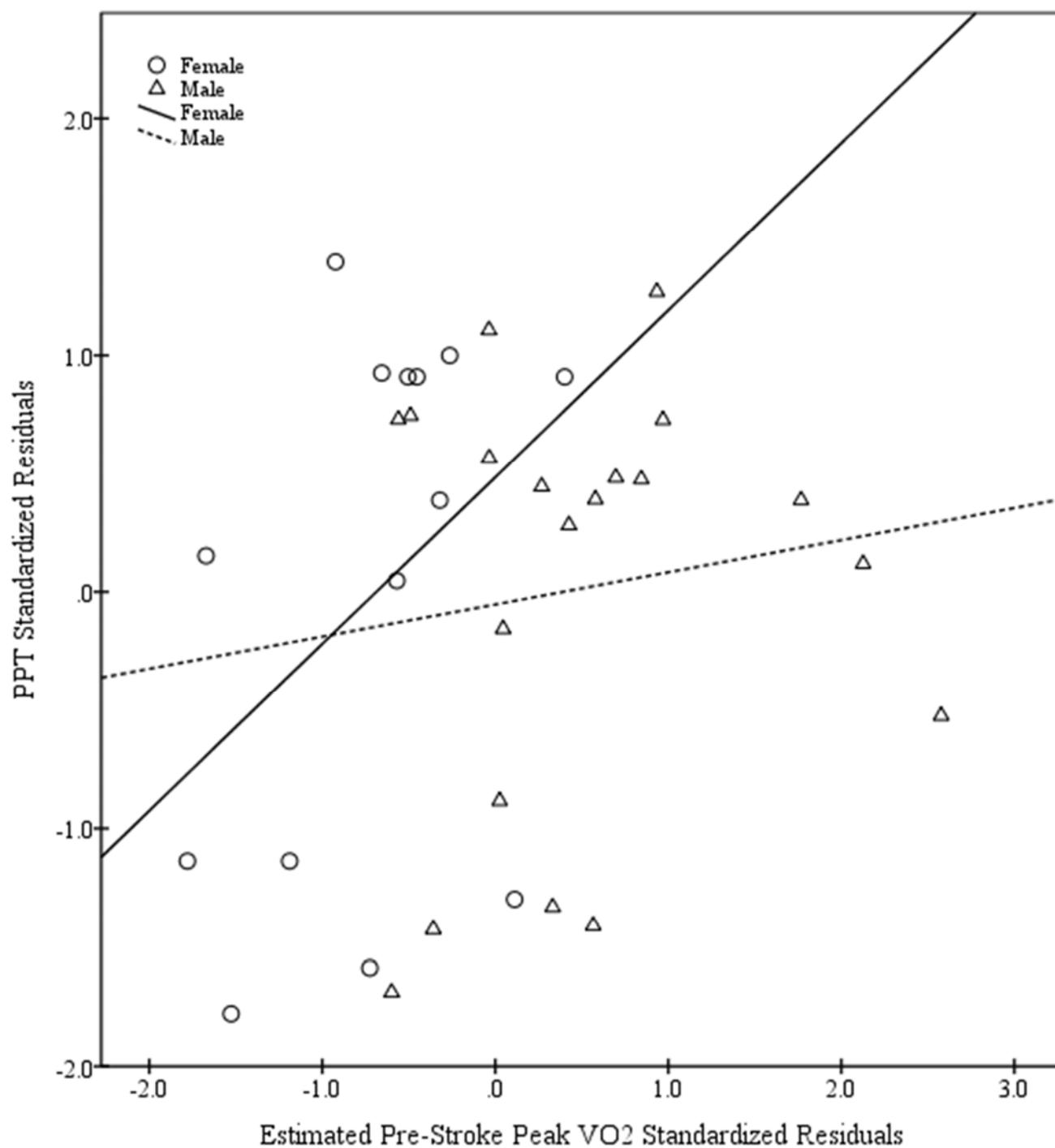


Figure 3.3. Scatter plot of estimated pre-stroke peak VO₂ residuals plotted against PPT score at discharge separated by gender. (Males: $r = .13$, $p = .60$, CI [-.32, .55]; Females: $r = .53$, $p = .06$, CI [-.13, .91]). Variables are adjusted for lesion volume calculated from the admitting MRI.

Figure 3.4. Estimated Pre-Stroke Peak VO₂ vs. Discharge 6MWT Distance

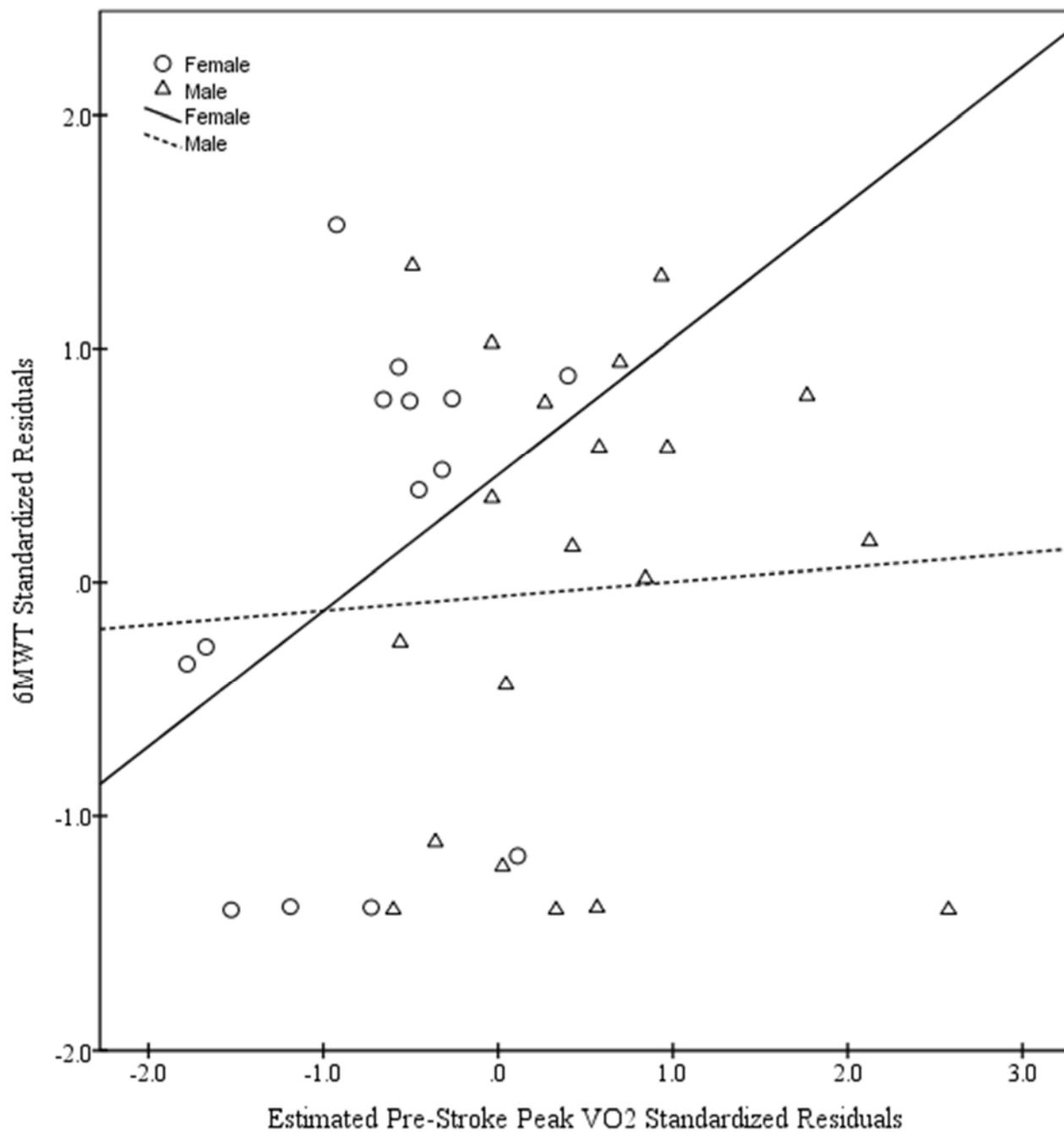


Figure 3.4. Scatter plot of estimated pre-stroke peak VO₂ residuals plotted against 6MWT distance at discharge separated by gender. (Males: $r = .05$, $p = .82$, CI [-.44, .59]; Females: $r = .47$, $p = .10$, CI [-.14, .91]). Variables are adjusted for lesion volume calculated from the admitting MRI.

3.5. Discussion

This study used a non-exercise estimation equation to predict pre-stroke peak VO_2 as an assessment of CR fitness in people with acute stroke during their hospital stay. The mean length of stay for our participants was 2.5 days, which may make conducting an exercise test during the acute hospital stay difficult. Previous data support that approximately 50% of patients with acute stroke return home (Pohl et al., 2013) and do not receive any information regarding CR fitness. These findings support prior work that CR fitness is low in acute stroke, specifically among women (MacKay-Lyons, Makrides, 2002). Using a non-exercise estimation of peak VO_2 would allow rehabilitation professionals, such as physical therapists, the opportunity to evaluate and discuss with their patients the importance of a prescribed home exercise program, where appropriate.

In humans, there is some suggestion that pre-stroke physical activity can have a positive effect on stroke recovery at 8 days (Deplanque et al., 2012), 3 months (Stroud et al., 2009), and 2 years (Krarup et al., 2008) after stroke. One study enrolled participants within 30 days of stroke and asked about their physical activity levels during the year prior to their stroke. The outcome measures were collected by telephone at 2 months post-stroke and were “dichotomized into ‘good’ and ‘bad’ categories according to instrument-specific cut-points” (Stroud et al., 2009). They report that individuals who engaged in moderate or high levels of activity were more likely to have a “good” outcome at 3 months post-stroke. Based on the evidence in the literature and in an attempt to better understand the relationship between CR fitness and stroke recovery, we conducted this study. We hypothesized that estimated pre-stroke peak VO_2 would be moderately and significantly related to discharge PPT, 6MWT, LFM, when controlling for initial lesion size. Our reported results did not support our hypothesis. Our results showed only a weak, non-

significant correlation between estimated pre-stroke peak VO_2 and two of our outcome measures at discharge. This could be due to over- or under-estimation of physical activity prior to stroke. This would influence the predicted peak VO_2 . The acute stroke period is a sensitive time where an individual may not remember their typical physical activity. It is possible that males and females may account for daily physical activity differently. Therefore, in a post-hoc analysis, we examined whether sex differences exist between estimated pre-stroke VO_2 peak and functional performance at discharge.

A novel finding was the sex differences related to estimated pre-stroke peak VO_2 and our functional outcome measures. Our results indicated in females that estimated pre-stroke peak VO_2 was related to functional outcomes but only the LFM was statistically significant. There was no relationship between estimated pre-stroke peak VO_2 and functional outcome measures in males. These results could be due to an over-estimation in self-selected physical activity levels in males or underestimation in females. There could be an influence of stroke size and location between males and females. We did control for the initial lesion size but we acknowledge that lesion location could influence functional performance such as walking in the 6MWT. We also acknowledge participant fatigue could have influenced individual performance on our functional measures especially the 6MWT, which measures walking endurance. We worked with the hospital therapy team to coordinate our testing with therapy sessions to avoid overlap, but previous therapy sessions could have affected performance. In addition, the day of discharge from the acute hospital stay was busy with the participant leaving and could have affected patient performance. It is important to acknowledge that our study was likely underpowered when considering previous work where the sample sizes were much larger (Deplanque et al., 2012; Stroud et al., 2009).

This pilot data will provide important information so that future work can be adequately powered to determine whether estimated pre-stroke peak VO_2 is related to outcome measures such as the PPT or walking endurance (6MWT) and has laid the foundation for future work. The Jurca equation to estimate peak VO_2 has provided an objective measure for CR fitness in people post-stroke. Although we did not compare the predicted peak VO_2 to a measured VO_2 , one previous study by McAuley and colleagues found that these measures were correlated in older adults with chronic disease (McAuley et al., 2011). The authors used the Jurca equation to investigate the association of the non-exercise estimation of peak VO_2 measures of cardiorespiratory fitness (maximal exercise test and 1-mile timed walk) (McAuley et al., 2011). They found that the estimation equation was significantly correlated with the maximal exercise test and the 1-mile timed walk. McAuley suggests that their results serve as evidence that the Jurca equation has utility in other populations, but that future work needs to be done to determine whether relationship exist in other domains. The current work adds to this literature by describing the relationship of the Jurca non-exercise prediction equation and measures of function in a small sample of individuals with acute stroke. McAuley highlights that assessing CR fitness is important in both healthy and diseased populations and may give deeper insight into other domains of health and acknowledge that exercise testing in older adults and individuals with high cardiovascular risk is difficult because of the risks involved, time constraints, and expenses such as obtaining proper equipment, personnel, and medical oversight. Therefore, we believe that there is a need for a short, non-exercise estimation of VO_2 that is easily obtained in the acute, clinical setting.

This study is novel in several ways. First, we enrolled individuals after stroke within 48 hours of admission to the stroke unit, where previous work has only enrolled participants within

the first 30 days (Stroud et al., 2009) or between 5 and 24 days after stroke (Krarup et al., 2008). Second, rather than using a Likert scale for physical activity, we chose to utilize a continuous measure of CR fitness, non-exercise estimated peak VO_2 , which incorporates many variables, including sex, age, self-reported physical activity, and rHR. We acknowledge that rHR may be different pre- and post-stroke, though we demonstrated in a subset of our participants that rHR was not significantly different following stroke when compared to the participant's pre-stroke data. Due to the small sample, we acknowledge that these data may not be generalizable to all people after stroke.

We then examined the relationship between estimated pre-stroke peak VO_2 and selected outcome measures at discharge from the hospital. While most studies have used categorical levels of self-reported physical activity, the intention was to be more specific by using peak VO_2 , a continuous variable. To our knowledge, this is the first investigation to examine the relationship between estimated pre-stroke peak VO_2 and objective measures of functional outcome at discharge from an acute stroke unit.

This pilot study is the first study conducted in acute stroke using an estimated peak VO_2 prediction equation. This data provides important information for assessing CR fitness as recommended in the publication, *The Importance of Cardiorespiratory Fitness (VO_2 max) in the United States* (Kaminsky et al., 2013). The data have provided a foundation for future work to rigorously examine the relationship between peak VO_2 (estimated or measured) and outcome at discharge following stroke.

3.5.1. Limitations

We must address the large number of participants not enrolled and the potential for systematic bias. We did have participants ($n = 113$) on bed rest while 238 were ineligible due to anticipation of discharge prior to the study team conducting baseline testing. We recognize that this may appear to be a systematic bias towards non-enrollment for those with severe and mild stroke, which would bias the study towards accepting the null hypothesis. The reason for non-enrollment lies with the parent study (Mattlage et al., 2013), for which we aimed to consent, perform baseline testing, and equip accelerometers all within 48 hours of admission to the hospital. Participants also needed a confirmed diagnosis of stroke by neuroimaging. The challenge we faced was the short lengths of stay (mean 2.5 days) for all patients regardless of stroke severity. If the study team was unable to consent and conduct the baseline testing within 48 hours, many potential participants were not approached for consent. This could have been due to the individual unavailable such as out for other medical tests, our study team did not get the neuroimaging report within 48 hours or we had current participant testing and could not screen and consent with the potential participant in a timely manner. We believe we enrolled participants with a wide range of function from mild to severe stroke (**Table 3.1** and **Figure 3.2**, **Figure 3.3**, and **Figure 3.4**). It is important to note that while the functional performance of participants at discharge is varied, the propensity for low peak VO_2 values especially in females is observed across the x-axis for estimated pre-stroke VO_2 (**Figure 3.2**, **Figure 3.3**, and **Figure 3.4**). This may be a result of an underestimation of physical activity prior to stroke or people who have a stroke tend to have low levels of aerobic fitness (Billinger et al., 2014). Finally, we must acknowledge that this study was powered for the parent study and not powered to detect the relationship between the functional outcome measures and estimated pre-stroke peak VO_2 . This

study does provide data for future studies to adequately power and address scientific questions in this area of research.

3.6. Conclusion

There is limited information regarding CR fitness early after stroke, especially during the acute hospital stay. We believe given the challenges to conducting a maximal or submaximal exercise test within the first few days post-stroke, a prediction equation for assessing CR fitness (peak VO_2) was a reasonable tool to use during the acute stroke hospital stay. Our results show weak, non-significant relationships between estimated pre-stroke peak VO_2 early after stroke and our selected functional outcome measures. When considering sex, females demonstrated a stronger relationship with the functional measures at discharge but only the LEFM was statistically significant. This study lays the groundwork for future exploration in this area of research.

CHAPTER 4

Estimated Pre-Stroke Peak VO_2 is Related to Circulating IGF-1 Levels during Acute Stroke

Mattlage AE, Rippee MA, Abraham MG, Sandt J, Billinger SA. In Revision,
Neurorehabilitation and Neural Repair.

4.1. Abstract

Background: Insulin-like growth factor-1 (IGF-1) is neuroprotective after stroke and is regulated by insulin-like binding protein-3 (IGFBP-3). In healthy individuals, exercise and aerobic fitness (peak oxygen uptake; peak VO₂) increases IGF-1 in circulation. Understanding the relationship between pre-stroke aerobic fitness and IGF-1 and IGFBP-3 levels after stroke may provide insight into the benefits of exercise and aerobic fitness on stroke recovery.

Objective: The purpose of this study was to determine the relationship of IGF-1 and IGFBP-3 to estimated pre-stroke peak VO₂ in individuals with acute stroke. We hypothesized that: 1) pre-stroke peak VO₂ would be related to IGF-1 and IGFBP-3; and 2) individuals with higher-than median IGF-1 levels will have higher pre-stroke peak VO₂ compared to those with lower-than median levels. **Methods:** Fifteen individuals with acute stroke had blood sampled within 72 hours of hospital admission. Pre-stroke peak VO₂ was estimated using a non-exercise prediction equation. IGF-1 and IGFBP-3 levels were quantified using enzyme-linked immunosorbent assay. **Results:** Estimated pre-stroke peak VO₂ was significantly related to circulating IGF-1 levels ($r = 0.60$; $p = .02$), but not IGFBP-3. Individuals with higher-than median IGF-1 levels (117.9 ng/mL) had significantly better aerobic fitness ($32.3 \pm 7.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) than those with lower-than median IGF-1 ($22.3 \pm 8.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p = .03$). **Conclusions:** Improving aerobic fitness prior to stroke may be beneficial by increasing baseline IGF-1 levels. These results set the groundwork for future clinical trials to determine whether high IGF-1 and aerobic fitness are beneficial to stroke recovery by providing neuroprotection and improving function.

4.2. Introduction

Insulin-like growth factor-1 (IGF-1) is known to be neuroprotective after middle cerebral artery occlusion (MCAO) in animals (Chang et al., 2011; Guan et al., 1993; Guan et al., 1996; Zheng et al., 2014) and may even act as a neuronal rescue agent when given through intranasal delivery (Guan et al., 1993; Liu et al., 2001). When administered through intranasal delivery, core lesion size can be reduced up to 94% (Guan et al., 1993) while also improving functional status compared to vehicle controls (Zheng et al., 2014). In humans, those with high circulating levels of IGF-1 early after stroke have greater survival rates and fewer impairments, tested by the National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS), at 3 and 24 months post-stroke compared to those with low IGF-1 levels (Aberg et al., 2011; De Smedt et al., 2011; Denti et al., 2004). This suggests that IGF-1 may also have a neuroprotective effect in humans. Further, the molar ratio of IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3), IGF-1's primary regulatory protein, has also been observed to be related to stroke recovery (De Smedt et al., 2011). Individuals with a high molar ratio of IGF-1 to IGFBP-3 exhibited better stroke outcomes at 3 months post-stroke, indicating that having low IGFBP-3 levels may also be beneficial to stroke recovery. Therefore, it is important to understand what may influence levels of IGF-1 and IGFBP-3 in individuals with stroke.

In healthy individuals, both circulating levels of IGF-1 and IGFBP-3 can be influenced by physical activity and aerobic fitness (peak oxygen consumption, peak VO_2) (Cappon et al., 1994; Eliakim et al., 1996; Morimoto et al., 2005; Nindl et al., 2011; Schwarz et al., 1996). Prior work has demonstrated that people who are more physically fit have better white matter integrity (Tseng et al., 2013), regional brain volume (Tseng et al., 2013), and brain blood flow (Zhu et al., 2015). Therefore, aerobic fitness prior to a stroke may not only benefit overall brain health, but

also provide some level of neuroprotection via circulating IGF-1, which has been shown to improve stroke outcomes (Aberg et al., 2011; De Smedt et al., 2011; Denti et al., 2004).

However, even though these studies have demonstrated that higher than median levels of circulating IGF-1 improve outcomes after stroke, information is lacking regarding the relationship of pre-stroke aerobic fitness, IGF-1, and IGFBP-3 in individuals with acute stroke.

Assessing peak VO_2 during the acute stroke hospital stay via a maximal exercise test and direct measurement of expired gases could be difficult if the person has unstable blood pressure, activity restrictions, or time constraints due to other standard of care testing. However, one potential alternative method for assessing pre-stroke aerobic fitness is through the use of a non-exercise prediction equation to estimate peak VO_2 . Current literature has shown that non-exercise equations to predict peak VO_2 can be easily administered and provide useful alternatives to exercise testing in healthy adults and older adults (Jurca et al., 2005; Topolski et al., 2006). Further, we recently demonstrated the use of a non-exercise prediction equation (Jurca et al., 2005) to estimate pre-stroke peak VO_2 during the acute stroke hospital stay (Matlaga et al., 2016). Quick, non-exercise, prediction equations would feasibly allow for the study of pre-stroke aerobic fitness (peak VO_2) and its relationship to IGF-1 and IGFBP-3.

Therefore, the purpose of this study was to determine the relationship of IGF-1 and IGFBP-3 to estimated pre-stroke aerobic fitness (peak VO_2) in individuals with acute stroke. We hypothesized that: 1) estimated pre-stroke peak VO_2 would be significantly and positively correlated to IGF-1 and inversely correlated to IGFBP-3; and 2) individuals with higher than median circulating IGF-1 levels would have significantly higher estimated pre-stroke peak VO_2 compared to those with lower than median levels.

4.3. Methods

4.3.1. Study Design

Institution approval from the Human Subjects Committee at the University of Kansas (KU) Medical Center was obtained before beginning the study (HSC#00000972). Written informed consent was obtained by every individual or their surrogate decision maker prior to study participation.

4.3.2. Participants

Individuals were eligible to participate in the study if they had a confirmed diagnosis of acute ischemic or hemorrhagic stroke, were admitted to the certified Comprehensive Stroke units at the KU Hospital, and were between the ages of 25 and 85 years. Individuals were excluded from the study if they had: 1) acute renal failure; 2) a history of ischemic or hemorrhagic stroke, ischemic cardiovascular event, or coronary artery bypass graft (CABG) surgery less than 3 months prior to their current hospital admission; and 3) congestive heart failure; or 4) severe peripheral artery disease.

4.3.3. Estimated Pre-Stroke Peak VO₂

Pre-stroke peak VO₂ was estimated using the Jurca prediction equation (Juil, 2002). The Jurca prediction equation uses the following variables: sex, age, BMI, resting heart rate (rHR), and a self-reported measure of physical activity. Using a questionnaire, participants or their surrogate decision maker were asked to estimate their average physical activity level prior to their stroke. The questionnaire consists of five categories that describe varying levels of physical activity over the course of a typical week. Participants (or their surrogate decision maker) were

asked to select one of the five categories that most accurately describes their normal activities during a typical week prior to being admitted to the hospital. Each of the 5 categories is associated with a constant variable that is used in the prediction equation for calculation of estimated aerobic fitness (Level 1: 0.00 – Primarily sedentary; Level 2: 0.32 – Five days or more per week of light physical activity for 10 minutes or more at a time; Level 3: 1.06 – Moderate aerobic exercise for 20 to 60 minutes per week; Level 4: 1.76 – Moderate aerobic exercise for 1 to 3 hours per week; Level 5: 3.03 – Moderate aerobic exercise for over 3 hours per week). Jurca et al., 2005 Other variables for the prediction equation (sex, age, BMI, rHR) were recorded immediately after consent into the study and within 72 hours of admission to the hospital. Resting heart rate was obtained after the participant was sitting or lying quietly for at least 30 minutes. Our previously published work showed that in a sample of individuals with acute stroke (n = 19), rHR was not significantly different between the rHR recorded during an outpatient, clinic visit from the electronic medical record prior to their stroke and the rHR recorded post-stroke (Mattlage et al., 2016). Further, we used electronic medical record to obtain height and weight, measured upon admission to KU Hospital, to calculate BMI. The equation to estimate peak VO_2 is as follows: $\text{METs} = [\text{Sex, F} = 0; \text{M} = 1 * 2.77) - (\text{Age in years} * 0.10) - (\text{BMI} * 0.17) - (\text{rHR in bmp} * 0.03) + (\text{Physical Activity Constant} * 1.00) + 18.07]$. METs were then multiplied by 3.5 in order to obtain estimated pre-stroke peak VO_2 (1 MET = $3.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

4.3.4. *Quantification of Biomarkers*

Blood for quantification of IGF-1 and IGFBP-3 was obtained after an overnight fast, between the hours of 7:30 am and 9:00 am, and within 72 hours of stroke admission. Ten

milliliters (mL) of blood from each participant was collected in a sodium heparinized tube from BD Biosciences (Becton, Dickinson and Company: Franklin Lakes, NJ). Collection tubes were immediately put on ice and transferred to the laboratory for processing. Within one hour of collection, blood samples were centrifuged to obtain plasma, aliquotted into 3, 1.5-mL polypropylene tubes, and frozen at -80 degrees Celsius until assaying. New aliquots were used for each assay to avoid multiple freeze/thaw cycles.

After all samples were collected, total IGF-1 (Alpco: Salem, NJ; cat#22-IGFHU-E01) and IGFBP-3 (Alpco; Salem, NJ; cat#22-BP3HU-E01) were quantified using enzyme-linked immunoassays (ELISAs). To avoid protein shock, samples were slowly thawed to room temperature with ice prior to assaying. Procedures for assaying were performed exactly to the recommendations of the manufacturer in the manual provided with the ELISA kits.

4.3.5. *Data Analysis*

Pearson correlations were performed to determine the relationship of proteins (IGF-1 and IGFBP-3) to estimated pre-stroke peak VO_2 . Median levels of IGF-1 and IGFBP-3 were determined using descriptive statistics. T-tests were performed to determine the difference in estimated pre-stroke peak VO_2 between above and below median values for both IGF-1 and IGFBP-3. All statistical tests were considered significant at the alpha-level of .05 and were performed using IBM ® SPSS ® Statistics Version 22 (SPSS, Inc., Chicago, IL).

4.4. **Results**

Fifteen individuals with acute stroke (8 males, 61.1 ± 10.7 years) were enrolled into the study and completed data collection within 72 hours of hospital admission. Thirteen individuals

completed data collection within 48 hours of admission. **Table 4.1** describes the demographics of all participants, mean levels of IGF-1 and IGFBP-3, and the results of the non-exercise pre-stroke peak VO₂ estimation equation.

Table 4.1. Participant Demographics

Characteristics, n = 15	Number or Sample Mean (SD)	Range
Male	8	
Age (years)	61.1 (10.7)	(38-75)
Body Mass Index	30.3 (5.7)	(22.8-45.7)
National Institutes of Health Stroke Scale	8.5 (8.0)	(1-22)
Type of Stroke		
Ischemic	13	
Hemorrhagic	2	
Blood Levels (ng/mL)		
IGF-1	123.1 (57.4)	(40.6-217.6)
IGFBP-3	2220 (676)	(636-2937)
Estimated Pre-Stroke Peak VO ₂ (mL*kg ⁻¹ *min ⁻¹)	27.0 (9.3)	(8.8-45.9)
Resting Measures		
Heart Rate (bpm)	70 (12)	(53-99)
Systolic Blood Pressure (mmHg)	145 (32)	(115-222)
Diastolic Blood Pressure (mmHg)	73 (19)	(46-110)

Estimated pre-stroke peak VO_2 was significantly and positively related to IGF-1 levels sampled within 72 hours of stroke ($r = .60$; $p = .02$) (**Figure 4.1**), but not significantly related to IGFBP-3 ($r = -.09$; $p = .75$). Median level of IGF-1 and IGFBP-3 was 117.9 and 2464 ng/mL, respectively. Individuals with higher than median levels of IGF-1 during acute stroke had significantly better estimated peak VO_2 ($n = 7$; $32.3 \pm 7.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) compared to individuals with lower than median IGF-1 levels ($n = 8$; $22.3 \pm 8.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p = .03$) (**Figure 4.2**). There were no significant differences in estimated peak VO_2 between higher- and lower-than median levels of IGFBP-3.

Figure 4.1. Relationship of Circulating IGF-1 and Estimated Pre-Stroke Peak VO_2

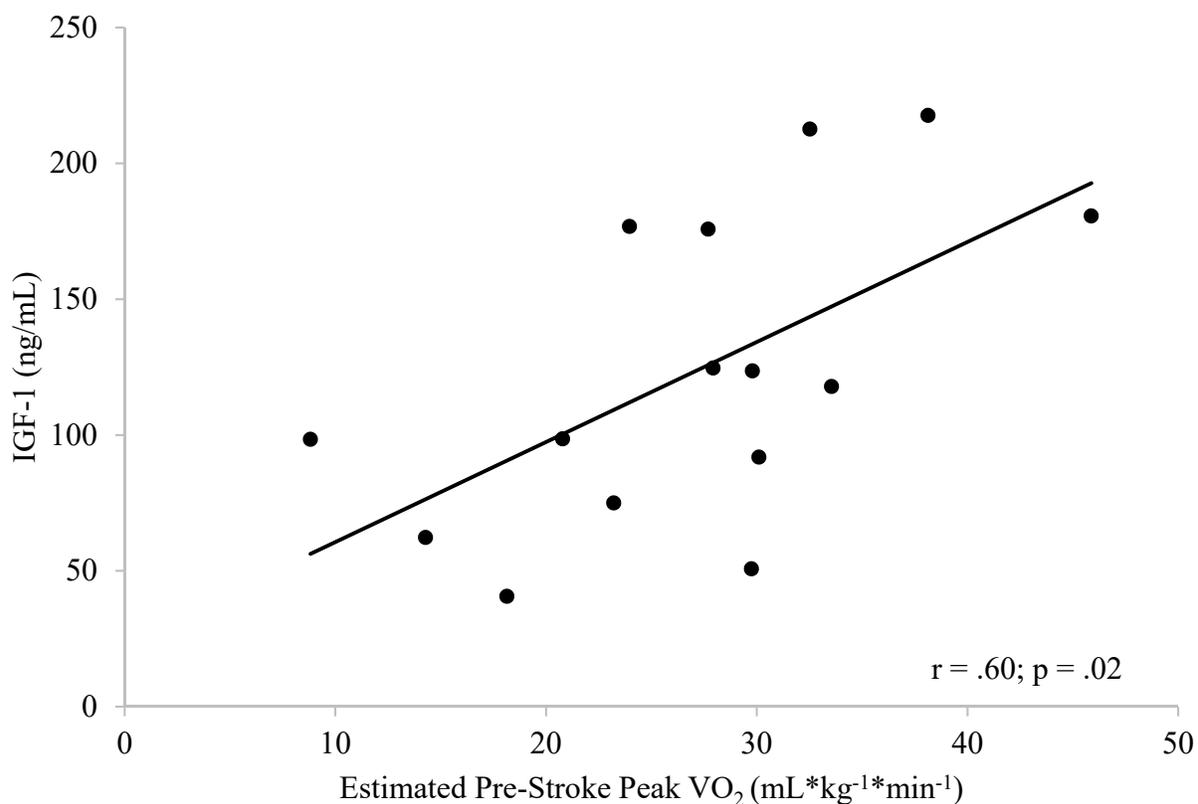


Figure 4.1. Individuals with higher estimated pre-stroke peak VO_2 have elevated circulating IGF-1 levels during acute stroke.

Figure 4.2. Difference in Estimated Peak VO_2 Between Above and Below Median IGF-1 Levels

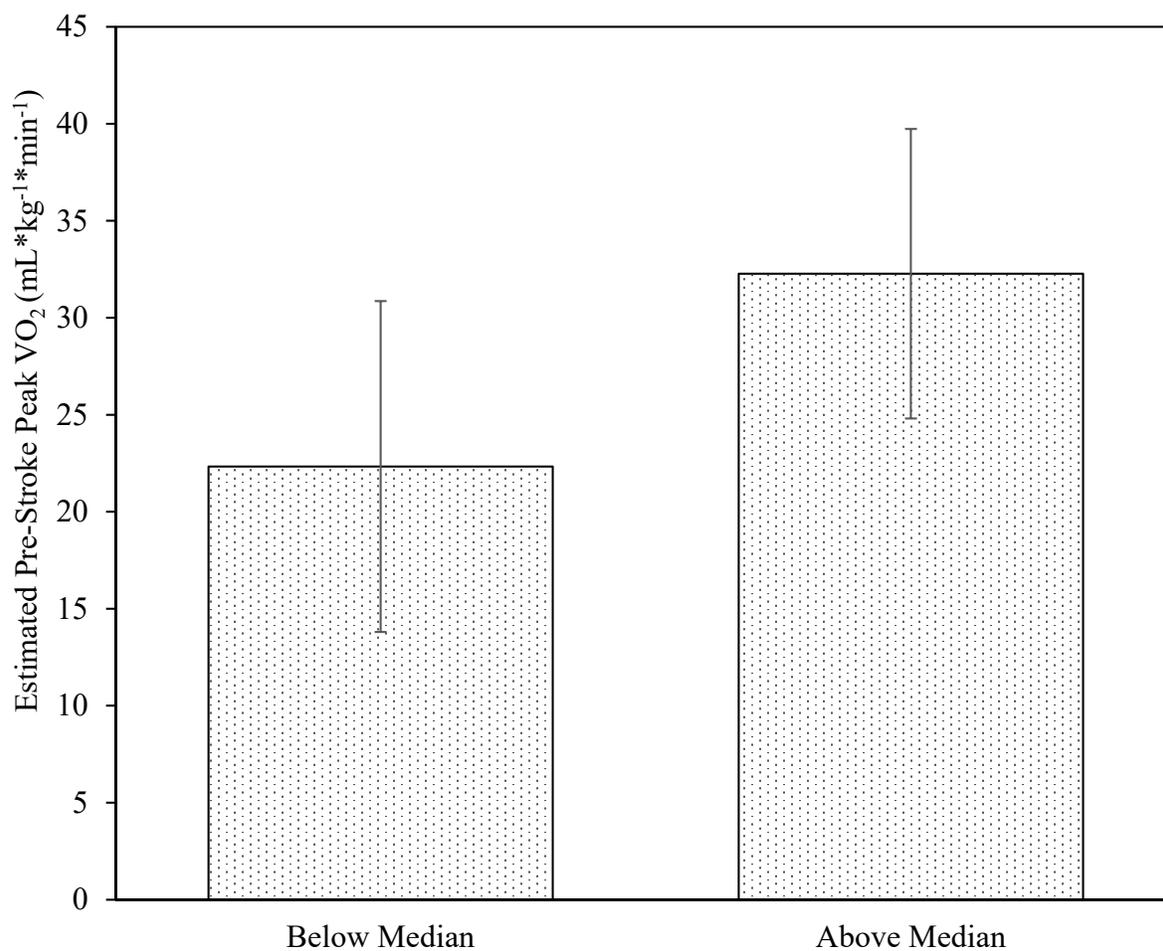


Figure 4.2. Individuals with above median levels of IGF-1 ($>117.9 \text{ ng/mL}$) during acute stroke have significantly higher estimated pre-stroke peak VO_2 ($32.3 \pm 7.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) compared to individuals with below median IGF-1 levels ($22.3 \pm 8.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p = .03$).

4.5. Discussion

This study examined the relationship between estimated pre-stroke peak VO_2 , a known neuroprotective protein (IGF-1), and its primary regulatory protein (IGFBP-3) in individuals admitted to the hospital with a diagnosis of acute stroke. To our knowledge, this is the first investigation to demonstrate that pre-stroke aerobic fitness (peak VO_2) is related to circulating IGF-1 levels in people with acute stroke. Our hypotheses were partially supported in that estimated pre-stroke peak VO_2 was related to circulating levels of IGF-1 early after stroke, but not to levels of IGFBP-3. Further, we sought to determine whether individuals with higher than median levels of IGF-1 would have greater aerobic fitness (peak VO_2) when compared to individuals with lower than median levels, and found our hypothesis to be supported by our data. The results of this work have important implications for future studies to investigate whether circulating IGF-1 levels and aerobic fitness are related to functional recovery following a stroke. These studies would be important for further development of future rehabilitation techniques to improve functional recovery in individuals with stroke.

4.5.1. Neuroprotection

IGF-1 is known to be neuroprotective after stroke (Chang et al., 2011; Guan et al., 1993; Guan et al., 1996; Zheng et al., 2014). In humans, individuals with higher than median levels of IGF-1 during the early phase of stroke have higher survival rates and less severe impairments at 3, 6, and 24 months post-stroke compared to those with lower than median IGF-1 levels (Aberg et al., 2011; De Smedt et al., 2011; Denti et al., 2004). Denti and colleagues examined IGF-1 and IGFBP-3 levels in 85 acute stroke participants (Denti et al., 2004). Stroke severity was assessed by the Barthel Index (BI) at 3 and 6 months post-stroke. Individuals with acute stroke

who had IGF-1 levels less than 60 ng/mL had higher stroke severity and lower survival rates at 3 and 6 months post-stroke (Denti et al., 2004). DeSmedt further demonstrated the neuroprotective quality of baseline IGF-1 by examining IGF-1 and IGFBP-3 levels and stroke severity with the NIHSS and mRS (De Smedt et al., 2011). The results showed that even when controlling for cardiac risk factors, such as hypertension, diabetes, atrial fibrillation, coronary artery disease, heart failure, smoking, and previous stroke, lower than median IGF-1 levels were related to increased stroke severity and lower survival rates at 3 and 6 months post-stroke when compared to those with higher than median levels of IGF-1 (De Smedt et al., 2011). There were no associations between IGFBP-3 levels and stroke severity. However, individuals with higher molar ratios of IGF-1 to IGFBP-3 had better stroke outcomes at 3 months post-stroke (De Smedt et al., 2011), indicating that IGFBP-3 may not directly be related to stroke recovery, but plays a role in regulating the availability of IGF-1, and is therefore important to consider. The studies by DeSmedt and Denti have demonstrated that higher than median circulating IGF-1 improves outcomes after stroke and, along with IGFBP-3, is important to stroke recovery. However, there is missing knowledge regarding the potential influence of factors such as pre-stroke peak VO₂ and its relationship to IGF-1 and IGFBP-3 in individuals with acute stroke.

4.5.2. *Aerobic Fitness*

Aerobic fitness, tested by peak aerobic capacity (peak VO₂), has been directly related to circulating IGF-1 levels in 846 healthy men (Nindl et al., 2011). Blood was collected in the morning after an overnight fast for the quantification of IGF-1. Individuals then underwent a graded maximal exercise test on a cycle ergometer with direct gas analysis to obtain peak VO₂. A repeated squats test was also performed to assess leg strength. Individuals with higher peak

VO₂ exhibited higher levels of circulating baseline IGF-1. Further, better aerobic fitness was associated with increased performance on the repeated squats test, indicating a greater functional performance in the lower extremities (Nindl et al., 2011). Nindl's study in healthy men could potentially have very large implications for individuals post-stroke. However, the relationship of exercise performance, specifically, aerobic fitness, to IGF-1 levels had not yet been tested in individuals with stroke prior to the current study, but gives important insight for which future studies could be based.

Testing aerobic fitness in individuals with stroke can be extremely difficult due to neuro-motor limitations in the lower extremities. In the acute setting, it is particularly challenging because of various factors such as time constraints for standard of care diagnostic assessments, a lack of trained professionals and specialized equipment, and extreme fatigue of the participant. However, our previous work displays feasibility of the use of a non-exercise prediction equation to estimate pre-stroke peak VO₂ in individuals admitted to the hospital with a diagnosis of acute stroke (Mattlage et al., 2016). Our work showed that estimated pre-stroke peak VO₂ was easily administered. The results of the current study corroborates these findings, but also shows that this easily-administered tool to estimate pre-stroke aerobic fitness may also influence IGF-1 levels in individuals with acute stroke.

The results of the current study are novel and important as we found a strong and significant relationship between pre-stroke aerobic fitness (peak VO₂) and IGF-1. Although this requires further exploration, it is plausible that pre-stroke aerobic fitness may have a potential benefit following stroke. In healthy men, those with higher peak VO₂ and greater lower extremity functional ability, had higher levels of circulating baseline IGF-1 (Nindl et al., 2011). In our study, we also report that individuals with higher estimated pre-stroke peak VO₂ have

above median levels of IGF-1 when compared to individuals with lower than median levels of IGF-1. However, the current study does not answer questions regarding IGF-1 levels and functional performance of the lower extremity, but provides strong rationale for further investigation. Further, we did not observe a significant relationship between estimated pre-stroke peak VO_2 and circulating IGFBP-3 levels. The lack of the hypothesized relationship could be due to many factors. In the study by DeSmedt, IGFBP-3 was not directly related to stroke outcomes, but the ratio of IGF-1 to IGFBP-3 was (De Smedt et al., 2011). It is possible that IGFBP-3 can be influenced by exercise short term, but baseline levels may not be directly related to aerobic fitness. Further, changes in IGFBP-3 circulating levels may depend on the type or duration of exercise (Chadan et al., 1999).

4.5.3. Limitations

We must acknowledge that the participants, or their surrogate decision maker, may report higher or lower activity levels, and thereby, influence the pre-stroke peak VO_2 estimation. From our prior work using the Jurca non-exercise estimation equation in acute stroke (Mattlage et al., 2016) and others using the equation in community-dwelling adults and older adults with several cardiovascular risk factors (Jurca et al., 2005; Mailey et al., 2010) we believe this is a feasible and safe alternative in comparison to conducting a maximal exercise test on individuals who are within 72 hours of stroke

Further, the current study was limited by a small sample size and the results should be interpreted with caution. Despite this, we believe the results of our study give rise to further questions regarding pre-stroke aerobic fitness, IGF-1, and functional recovery. Future work should address whether pre-stroke aerobic fitness and IGF-1 in acute stroke influence functional

recovery and stroke outcomes. Finally, blood was sampled, on average, within 48 hours of stroke admission. The sampling window used was more narrow than one study which sampled blood anywhere between 1 and 10 days post-stroke (Aberg et al., 2011). Although we sampled blood within at most 72 hours, our time window for sampling blood was wider than previous studies examining IGF-1 and stroke outcomes (De Smedt et al., 2011; Denti et al., 2004). However, we believe that this would have minimal effect on our study as there is no suggested optimal time to obtain blood for the quantification of IGF-1 and IGFBP-3.

4.6. Conclusion

This study investigated the relationship between IGF-1 and IGFBP-3 levels and pre-stroke estimated peak VO_2 in individuals with acute stroke. We hypothesized that 1) pre-stroke estimated peak VO_2 would be positively and significantly related to IGF-1 and negatively related to IGFBP-3; and 2) individuals with higher than median circulating IGF-1 levels would have significantly better estimated pre-stroke peak VO_2 compared to individuals with lower than median levels of IGF-1. Our results partially supported this hypothesis in that we observed a significant, positive relationship between pre-stroke peak VO_2 and circulating IGF-1 levels, but a non-significant relationship with IGFBP-3. Further, our second hypothesis was supported and demonstrated that individuals with higher than median levels of IGF-1 had higher pre-stroke estimated peak VO_2 , while individuals with lower than median levels of IGF-1 had a lower pre-stroke estimated peak VO_2 . The results of this study are novel and significant to the field in that they provide support for future studies for examining how aerobic fitness, IGF-1, and IGFBP-3 levels are related to recovery and physical function after stroke.

CHAPTER 5

Decrease in IGF-1 Levels and IGF-1 Ratio during the First Week of Stroke is Related to Positive
Outcomes

Mattlage AE, Rippee MA, Sandt J, Billinger SA. In Press, Journal of Stroke and
Cerebrovascular Diseases.

5.1. Abstract

Background: High insulin-like growth factor-1 (IGF-1) levels, measured once during acute stroke, is associated with greater survival rates and lower stroke severity. However, information is lacking regarding how IGF-1 availability, determined by IGF-1's ratio to insulin-like growth factor binding protein-3 (IGFBP-3), relates to recovery and how the response of IGF-1 during the first week of stroke relates to stroke outcomes. The purpose of this study was to determine: 1) the relationship between percent-change in IGF-1 and IGF-1 ratio during the first week of stroke and stroke outcomes; and 2) the difference in percent-change in IGF-1 and IGF-1 ratio in individuals who discharged home and individuals who discharged to inpatient facilities.

Methods: IGF-1 and IGFBP-3 were quantified from blood sampled twice (<72 hours of admission; 1-week post-stroke) in fifteen individuals with acute stroke. Length of stay (LOS), modified Rankin Scale (mRS) at one-month, and discharge destination were obtained from electronic medical records. **Results:** Percent-change in IGF-1 ratio was related to LOS ($r=.54$; $p=.04$). mRS ($n=10$) was related to percent-change in IGF-1 ($r=.90$; $p<.001$) and IGF-1 ratio ($r=.75$ $p=.01$). Those who went home ($n=7$) had decreases in IGF-1 levels ($-24\pm 25\%$) and IGF-1 ratio ($-36\pm 50\%$), while those who went to inpatient facilities ($n=8$) had increases in IGF-1 levels ($37\pm 46\%$) and IGF-1 ratio ($30\pm 40\%$). These differences were significant (IGF-1: $p=.008$; IGF-1 ratio $p=.01$). **Discussion:** Our findings suggest that a decrease in IGF-1 and IGF-1 ratio during the first week of stroke is associated with favorable outcomes: shorter LOS, lower mRS at one-month, and discharging home.

5.2. Introduction

In humans, low levels of insulin-like growth factor-1 (IGF-1) are associated with stroke risk factors such as ischemic heart disease, myocardial infarction, diabetes, and coronary artery disease, while high insulin-like growth factor binding protein-3 (IGFBP-3) levels are associated with ischemic heart disease (Juul, 2002; Rajpathak et al., 2012; Vasan et al., 2003). Moreover, low IGF-1 has been directly correlated to greater stroke risk in humans (Denti et al., 2004; Dong et al., 2014; Johnsen, 2005). Higher levels of IGF-1 with simultaneously low levels of IGFBP-3 allow for greater amounts of free, unbound IGF-1 in circulation, which, in turn, elicits IGF-1 to trigger cascades in neuroprotective pathways. In individuals with stroke, many studies have agreed that high baseline levels of IGF-1 in circulation, measured once within the first few weeks of stroke, are linked to lower stroke severity and greater rates of survival at one-month post stroke (Aberg et al., 2011; Bondanelli et al., 2006; De Smedt et al., 2011; Denti et al., 2004; Ebinger et al., 2015; Tang et al., 2014).

While some studies have examined IGFBP-3 after stroke (De Smedt et al., 2011; Ebinger et al., 2015), there still remains a disparity in knowledge regarding the relationship of IGF-1 ratio (IGF-1:IGFBP-3) to stroke recovery. Because IGF-1 ratio reflects the amount of free IGF-1 available to reach its receptor, it is important to consider. Furthermore, while high baseline IGF-1 levels have been shown to be related to positive outcomes, the response of IGF-1 during the first week is not yet known and may provide further insight into IGF-1's potential neuroprotective benefit to stroke outcomes.

Outcomes such as length of stay (LOS) in the hospital, modified Rankin Scale (mRS), and discharge destination have been used in individuals with stroke to evaluate the relationship between easily-assessable and short-term outcomes, functional status, and other measures of

stroke recovery. One study showed that motor recovery was improved after the first year of stroke in people with a shorter LOS during their first hospital admission for stroke (Kuptniratsaikul et al., 2016). LOS was also negatively related to improvements on the Functional Independence Measure (FIM) (Ifejika et al., 2015). Moreover, discharge destination has been associated acute FIM measures, mortality rates, and global disability at 3 months post-stroke (Bekelis et al., 2016; Roberts et al., 2015; Zhang et al., 2015). However, the relationship between these stroke outcomes and IGF-1 and IGF-1 are yet to be determined.

Therefore, the purpose of this study was to: 1) determine the relationship between percent change in IGF-1 and IGF-1 ratio during the first week of stroke and stroke outcomes (LOS and mRS at one-month post-stroke); and 2) determine the difference in percent change in IGF-1 and IGF-1 ratio in discharge destination (i.e. individuals who discharged home vs. individuals who discharged to inpatient facilities). We hypothesized that: 1) an increase in IGF-1 and IGF-1 ratio during the first week of stroke would be associated with fewer days spent in the hospital (LOS) and more functional independence at one month post-stroke (mRS); and 2) individuals who discharge home will have a significantly greater percent change in IGF-1 and IGF-1 ratio compared to individuals who discharge to inpatient facilities.

5.3. Methods

5.3.1. Study Design

This prospective study used a sample of convenience from University of Kansas (KU) Hospital's certified Comprehensive Stroke neuro-progressive and neuro-intensive care units. Institution approval from the Human Subjects Committee at KU Medical Center was obtained before commencement of the study. Written informed consent was obtained by the participant or their surrogate decision maker prior to study participation.

5.3.2. Participants

Individuals with stroke were eligible for participation if they were admitted to KU Hospital with a diagnosis of acute ischemic or hemorrhagic stroke and were between 25 and 85 years of age. Individuals were excluded from the study if they: 1) had acute renal failure; 2) had a history of ischemic or hemorrhagic stroke, ischemic cardiovascular event, or coronary artery bypass graft (CABG) surgery less than 3 months prior to their current hospital admission for stroke; 3) had a diagnosis of congestive heart failure; or 4) had severe peripheral artery disease. Demographics of enrolled participants can be found in **Table 5.1**.

Table 5.1. Participant Demographics

Characteristics, n = 15	Number or Sample Mean (SD)	Range
Male	8	
Age (years)	61.1 (10.7)	(38-75)
Body Mass Index	30.3 (5.7)	(22.8-45.7)
National Institutes of Health Stroke Scale	8.5 (8.0)	(1-22)
Modified Rankin Scale	2 (1.4)	(1-5)
HbA1C	5.9 (1.3)	(5.1-9.8)
Race/Ethnicity		
White/Caucasian	13	
African American	2	
Marital Status		
Married/Partner	7	
Divorced	5	
Single	3	
Side of Impairment		
Left	7	
Right	6	
Neither	2	
Type of Stroke		
Ischemic	13	
Hemorrhagic	2	
Received tPA	4	

Table 5.1. Participant Demographics (Continued)

Characteristics, n = 15	Number or Sample Mean (SD)	Range
<hr/>		
Comorbidities		
Smoker	4	
Atrial Fibrillation	2	
Seizures	1	
Medicines		
Anti-Hypertensive	8	
Blood Thinners	4	
Cholesterol	7	

5.3.3. *General Methods*

Individuals were enrolled into the study and had baseline blood samples collected within 72 hours of hospital admission for acute stroke. Blood at baseline was sampled between 7:30 and 9:00 am after an overnight fast. One week following stroke, blood was sampled again during similar hours, after an overnight fast. Demographic data (age, height, weight, and body mass index), results of laboratory chemistry testing on admission (hemoglobin A1c), enzyme analysis of liver function on admission (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), and other stroke related information (admitting National Institutes of Health Stroke Score (NIHSS) and lesion volume) were obtained from each participants' electronic medical record (EMR).

5.3.4. *Stroke Outcomes*

Calendar days spent in the hospital (LOS), discharge destination (i.e. home vs. inpatient facility), and functional independence at 1-month post-stroke, assessed by the mRS, was also obtained from each participant's EMR.

5.3.5. *Quantification of Biomarkers*

One, ten-milliliter (mL) sodium heparinized tube was used for obtaining blood from each participant. Collection tubes were immediately put on ice and transferred to the laboratory for processing. Within an hour of collection, blood samples were centrifuged to separate and obtain plasma. Plasma was then aliquotted into 3, 1.5-mL polypropylene PP tubes and stored frozen at -80 degrees Celsius until assaying. Freeze/thaw cycles were limited by using a new, once-thawed aliquot for each assay.

After all samples were obtained from each participant, enzyme-linked immunosorbent assays (ELISAs) were used to quantify total circulating IGF-1 (Alpco: Salem, NJ; cat#22-IGFHU-E01) and IGFBP-3 (Alpco; Salem, NJ; cat#22-BP3HU-E01). All samples from one participant were quantified on the same ELISA plate. Samples were slowly thawed to room temperature using ice before assaying in order to avoid protein-shock. Procedures were performed exactly to the manufacturer's recommendations. Six standards were made in order to create a standard curve. Two internal controls of known concentrations were provided by each kit. Observed values of both the standard curve and the known concentrations were determined to be within acceptable ranges before interpreting the data.

5.3.6. *Statistical Analysis*

Baseline demographics were calculated using descriptive statistics. IGF-1 ratio (the ratio of IGF-1 to IGFBP-3) was calculated by dividing the amount of total circulating IGF-1 by the amount of total circulating IGFBP-3. Percent change in IGF-1 and IGF-1 ratio during the first week of stroke was calculated as: $\text{Percent Change} = [(1 \text{ week} - \text{baseline}) / (\text{baseline}) * 100]$. For aim 1 of the study, Pearson correlations were performed to determine the relationship between percent change in IGF-1 and IGF-1 ratio and stroke outcomes (LOS and mRS). For the second aim of the study, independent t-tests were performed to determine the difference in percent change in IGF-1 and IGF-1 ratio between those who discharged home and those who discharged to an inpatient facility. All analyses were performed using IBM® SPSS® Statistics Version 22 (SPSS, Inc, Chicago, IL) and alpha levels were set at $p < .05$.

5.4. Results

Fifteen individuals (8 males, 61.1 ± 10.7 years) were enrolled into the study and started data collection within 72 hours of hospital admission. **Table 5.2** outlines IGF-1 and IGF-1 ratio levels at each time point and percent changes in IGF-1 and IGF-1 ratio from baseline to 1-week post-stroke. Additionally, since the majority of circulating IGF-1 is synthesized in the liver, enzymes to assess liver function (AST and ALT) were analyzed. Liver enzymes, AST and ALT, were within normal ranges for all subjects (**Table 5.3**).

Table 5.2. Blood Levels

n = 15	Visit 1 (<72 Hours)	Visit 2 (1 Week)	% Change
IGF-1 (ng/mL)	123 (57)	129 (89)	8.5 (47.7)
IGFBP-3 (ng/mL)	2220 (675)	2536 (589)	41.2 (109.9)
IGF-1 Ratio	.07 (.06)	.05 (.03)	-.67 (55.2)

Table 5.3. Characteristics of Individuals Who Discharged Home vs. Individuals Who Discharged to Inpatient Facilities

n = 15 value	Home	Inpatient	p-
Age (years)	62 (10)	60 (11)	.75
Body Mass Index	28 (4)	32 (6)	.17
Lesion Volume (cm ³)	2 (3)	25 (29)	.06
Length of Stay (days)	6(5)	10 (6)	.27
Baseline IGF-1 (ng/mL)	145 (48)	103 (60)	.17
1 Week IGF-1 (ng/mL)	112 (59)	144 (110)	.51
Baseline IGFBP-3 (ng/mL)	2036 (906)	2380 (380)	.38
1 Week IGFBP-3 (ng/mL)	2610 (829)	2471 (308)	.67
Baseline IGF-1 Ratio	.04 (.02)	.1 (.08)	.11
1 Week IGF-1 Ratio	.01 (.03)	.05 (.01)	.38
Aspartate Aminotransferase (U/L)	14 (4)	19 (8)	.68
Alanine Aminotransferase (U/L)	19 (8)	24 (12)	.46

5.4.1. Length of Stay

Our results showed that percent change in IGF-1 ratio was significantly related to LOS ($r = .54$; $p = .04$) (**Figure 5.1**), suggesting that as the percent change in IGF-1 ratio during the first week of stroke increases, individuals are staying in the hospital longer. However, percent change in IGF-1 alone was not significantly related to LOS.

Figure 5.1. Relationship of Percent Change in IGF-1 Ratio and Length of Stay

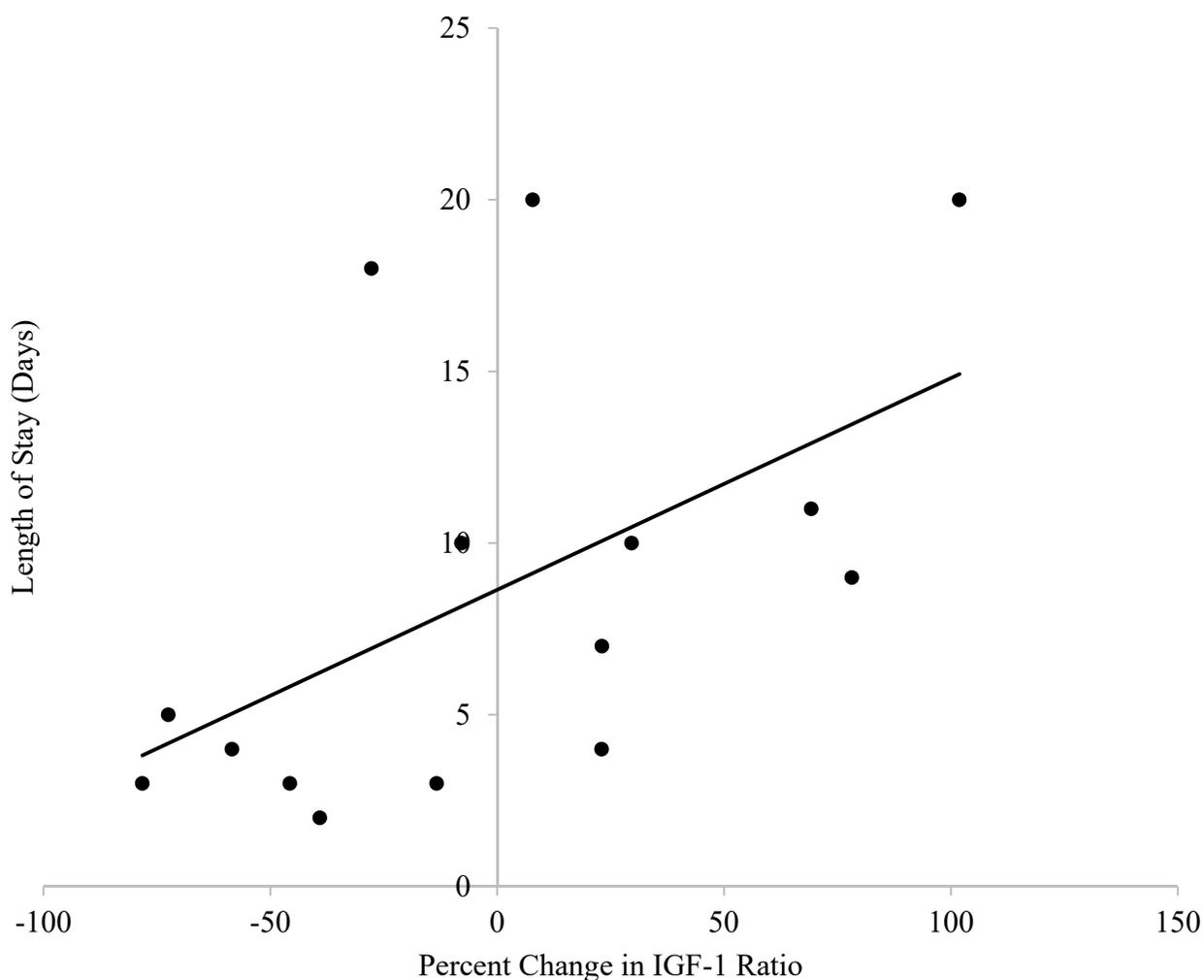


Figure 5.1 Individuals with a higher percent change in IGF-1 Ratio during the first week of stroke stay more days in the hospital.

5.4.2. Modified Rankin Scale

For individuals with a mRS at 1 month post-stroke ($n = 10$), our results showed that there was a significant relationship between mRS and percent change in both IGF-1 ($r = .901$; $p < .001$) (**Figure 5.2**) and IGF-1 ratio ($r = .750$; $p = .012$) (**Figure 5.3**) during the first week of stroke. As individuals had larger increases in IGF-1 and IGF-1 ratio during the first week, they also had greater scores on the mRS (lower functional independence) at one month post-stroke. These results corroborate the observation of the relationship of IGF-1 ratio to LOS and provide additional evidence to suggest that a decrease in circulating IGF-1 and IGF-1 ratio is associated with positive, or more favorable, outcomes.

Figure 5.2. Correlation Between Percent Change in IGF-1 and mRS at One Month

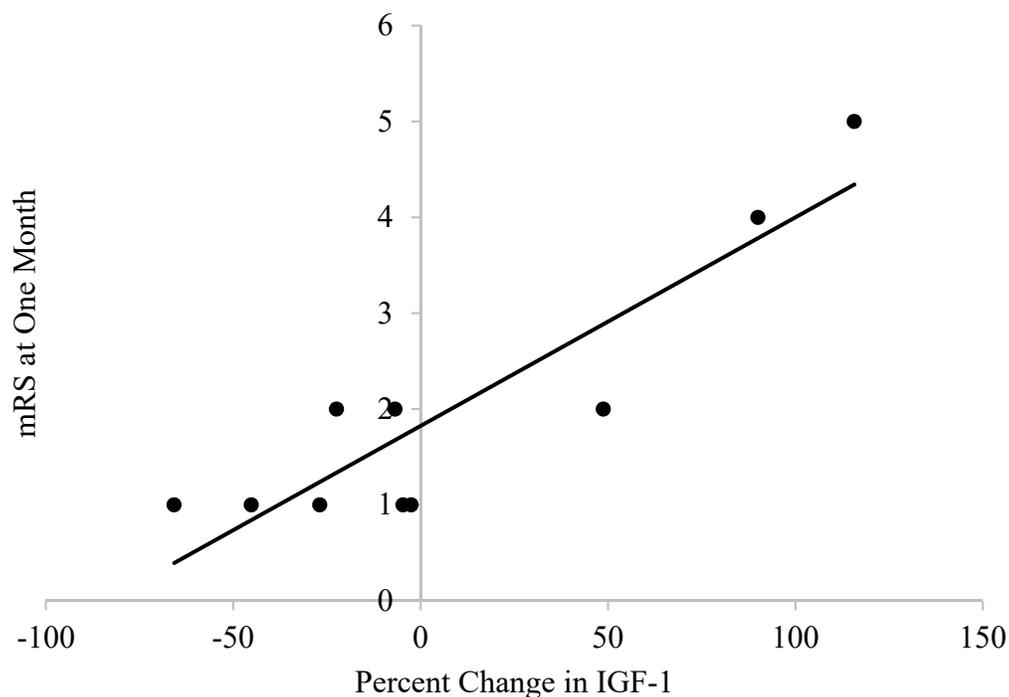


Figure 5.2 Individuals with a higher percent change in IGF-1 during the first week of have a poorer outcome on the mRS at one month post-stroke.

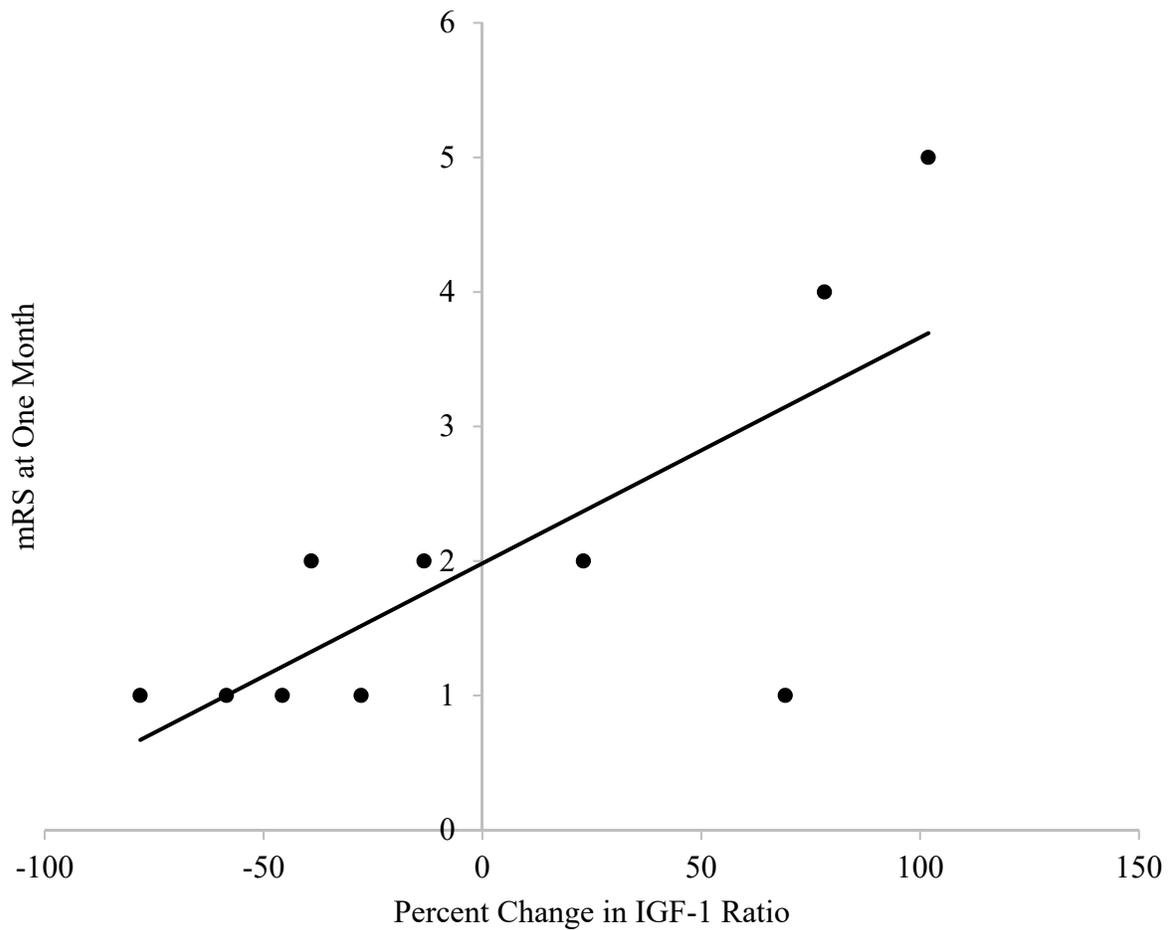
Figure 5.3. Correlation Between Percent Change in IGF-1 Ratio and mRS at One Month

Figure 5.3 Individuals with a higher percent change in IGF-1 Ratio during the first week of have a poorer outcome on the mRS at one month post-stroke.

5.4.3. Discharge Destination

Finally, those who went home ($n = 7$) had negative percent changes (decreases) in IGF-1 ($-24 \pm 25\%$) (**Figure 5.4**) and IGF-1 ratio ($-36 \pm 50\%$) (**Figure 5.5**) during the first week of stroke. Contrarily, those who went to inpatient facilities ($n = 8$) had positive percent changes (increases) in IGF-1 ($37 \pm 46\%$) and IGF-1 ratio ($30 \pm 40\%$) during the first week of stroke. The difference in percent change of IGF-1 ($p = .008$) and IGF-1 ratio ($p = .014$) between discharge destinations (home vs. inpatient) was significant.

Baseline demographics were not significantly different between individuals who went home and individuals who discharged to inpatient facilities (**Table 5.3**). Further, because both groups did not have a significant difference in either liver enzymes, we cannot contribute the differences in IGF-1 and IGF-1 ratio to impaired liver function.

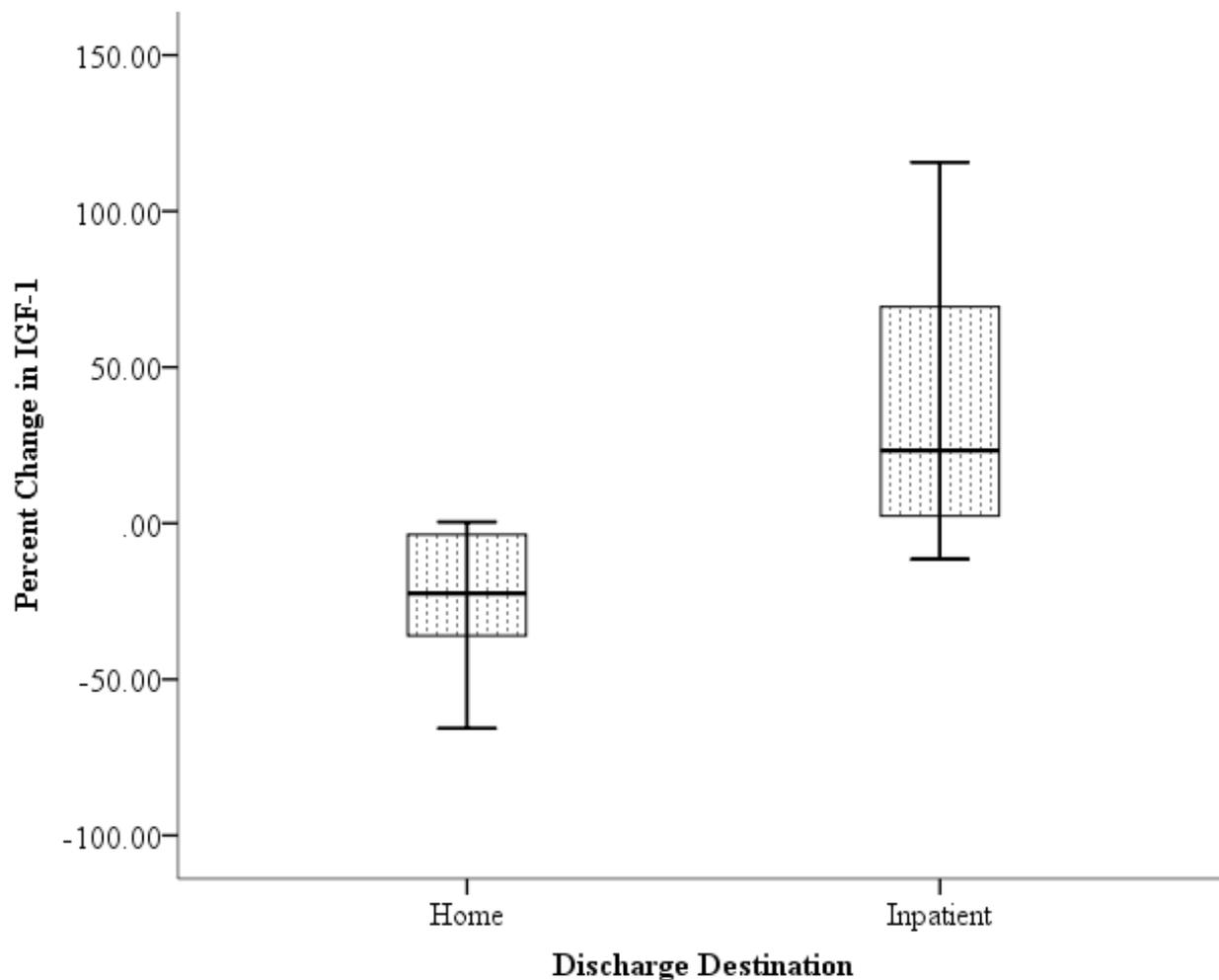
Figure 5.4. Change in IGF-1 and Discharge Destination

Figure 5.4 Individuals who discharged home from the hospital had decreases in IGF-1 levels during the first week of stroke, while individuals who discharged to inpatient facilities had increases in IGF-1 levels ($p = .008$).

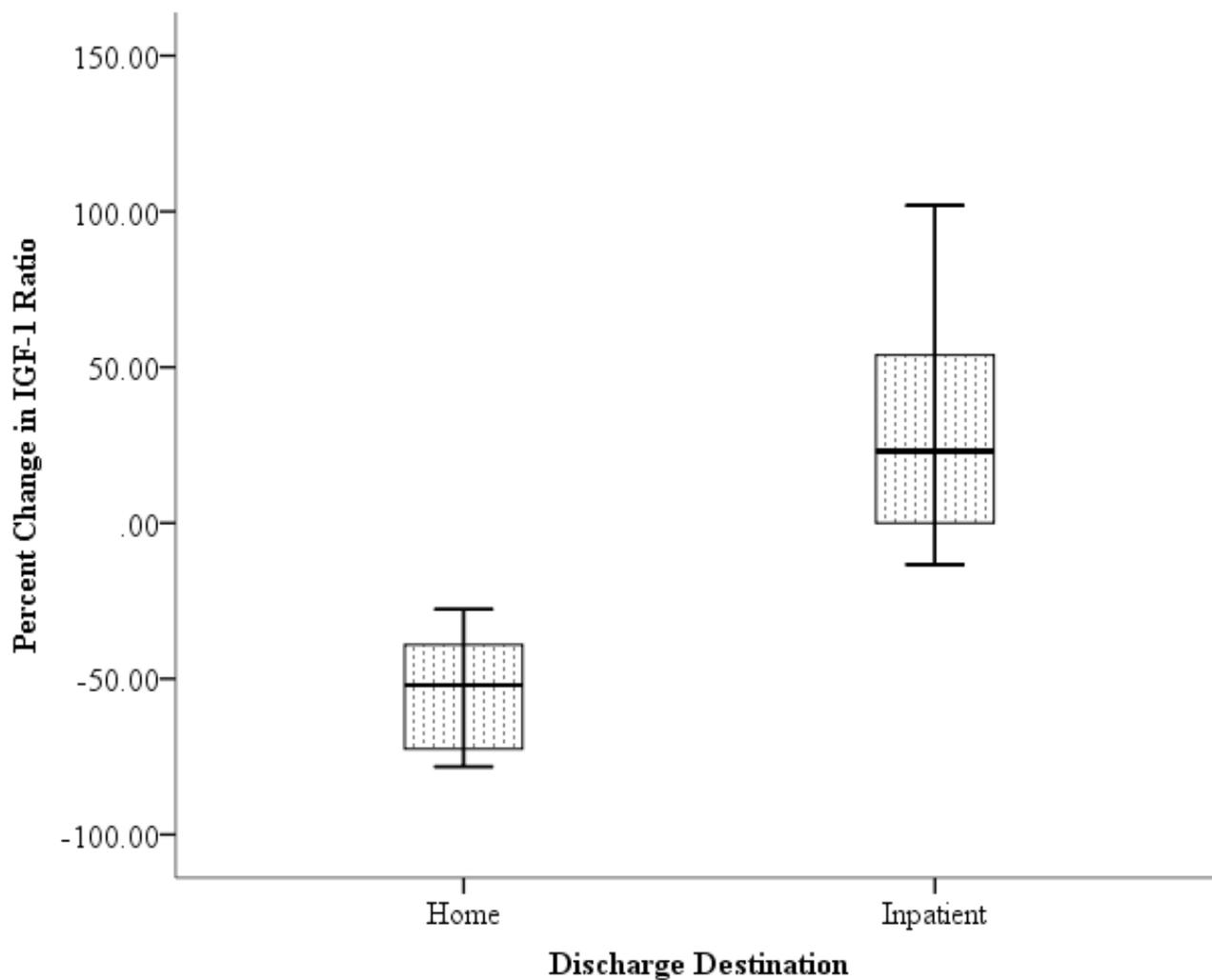
Figure 5.5. Change in IGF-1 Ratio and Discharge Destination

Figure 5.5 Individuals who discharged home from the hospital had decreases in IGF-1 Ratio during the first week of stroke, while individuals who discharged to inpatient facilities had increases in IGF-1 Ratio ($p = .014$).

5.5. Discussion

Our hypothesis that an increase in IGF-1 and IGF-1 ratio during the first week of stroke would result in fewer days spent in the hospital, lower mRS scores (less functional independence) at one month, and discharging home was not supported. In fact, our results showed the opposite in some measures of IGF-1: a decrease in IGF-1 and/or IGF-1 ratio during the first week of stroke was related to positive outcomes (i.e. discharging home and lower mRS at one month). Although the results of the current study were not as expected, the findings have important implications for stroke recovery in humans and raise additional questions for which future work should be based.

5.5.1. *Insulin-Like Growth Factor-1*

In humans, studies have been performed for which their results indicate that high baseline measurements of IGF-1 are related to better stroke recovery and greater survival rates. Aberg and colleagues measured IGF-1 levels from the serum of stroke patients between 1 and 19 days post-stroke (Aberg et al., 2011). Baseline serum IGF-1 levels in individuals with acute stroke were significantly associated with improvements in the mRS and Scandinavian Stroke Scale tested at 3 and 24 months post stroke. However, the widespread variation of sampling may have impeded their ability to draw conclusions. Individuals who had their blood sampled from Day 0-2 showed statistically different IGF-1 levels compared to those who had their blood sampled on Days 9-19. This indicates that IGF-1 levels during the first two weeks of stroke are not stable (show either significant decreases or increases) and that regulation of IGF-1 has the potential to differ from person to person. No studies have examined how this response, during the first few weeks of stroke, relates to recovery or stroke outcomes. The current study seeks to fill this gap

and provides novel, preliminary evidence to suggest that decreases in IGF-1 and IGF-1 ratio during the first week of stroke may be beneficial to recovery.

Experimental studies may provide insight into why the results of the current study were opposite to our original hypotheses. Chang and colleagues showed important evidence of IGF-1's ability to exert neuroprotective benefits and that during this time, brain levels of IGF-1 increase while peripheral levels decrease, suggesting uptake of IGF-1 into the brain from circulation (Chang et al., 2011). IGF-1 levels were measured 14 days after MCAO from the affected motor cortex, striatum, and plasma. IGF-1 was significantly decreased in the plasma, while significantly increased in the motor cortex and striatum. Although we are not able to measure IGF-1 in the brain of humans pre-mortem, the findings of Chang's study are important for the interpretation for the current study in that the decreases in IGF-1 seen in the periphery in humans may underlie increases in IGF-1 in the brain. It is important for future studies in acute stroke to examine the change in both peripheral circulating IGF-1 and CSF levels of IGF-1 in order to determine whether individuals who have decreases in IGF-1 during the first week are more efficient at IGF-1 uptake into the CSF and brain and whether these measures are related to functional stroke outcomes.

5.5.2. *Stroke Outcomes*

Many studies have used stroke outcomes such as LOS in the hospital, mRS, and discharge destination to assess recovery. These outcomes are extremely easy to collect and are many times automatically tracked and calculated by electronic medical record systems. LOS and discharge destination can be used as markers of recovery in the investigations of meaningful relationships to other recovery outcomes and neuroprotective agents.

A study performed by Kuptniratsaikul and colleagues examined motor recovery in 192 individuals with stroke across nine hospitals (Kuptniratsaikul et al., 2016). Motor recovery at 6 and 12 months after stroke was assessed by the Brunnstrom motor recovery stages (BMRS). The BMRS is broken into 6 stages of recovery with each level (1-6) describing progressive improvements in movement patterns in the hand, arm and leg, separately. Their results showed that improvements in the BMRS were associated with a shorter length of stay (Kuptniratsaikul et al., 2016). Another study, which investigated 481 individuals with acute stroke, determined the relationship between Functional Independence Measure (FIM) scores on admission and discharge destination (Roberts et al., 2015). They observed that individuals who discharged to the community, rather than to inpatient rehabilitation, had significantly better FIM scores upon admission (Roberts et al., 2015). Other studies have shown significant relationships between discharge destination and LOS with global disability at three months and having a fall during hospitalization (Wong et al., 2015; Zhang et al., 2015).

These studies indicate that easily obtainable outcome measures such as LOS and discharge destination can give valuable information into stroke recovery and physical function in individuals with stroke. However, their relationship to the response of IGF-1 and IGF-1 ratio after stroke has been understudied in humans. Our results were novel in that we showed that decreases in IGF-1 and IGF-1 were associated with a shorter LOS, better mRS at one-month post-stroke, and discharging home. Large clinical trials examining the relationship between the response of IGF-1 and IGF-1 ratio during the first week and objective functional outcomes can provide important insight into neuroprotection during stroke recovery.

5.5.3. *Limitations*

This study provides novel evidence regarding the potential importance of the percent change in IGF-1 and IGF-1 ratio during the first week of stroke in understanding recovery. However, a limitation of the current study is that circulating concentrations of IGF-1 were not supplemented with the levels of IGF-1 in the cerebral spinal fluid (CSF). Quantifying IGF-1 and IGF-1 ratio in the CSF can provide information regarding levels in the brain. IGF-1 is known to cross the blood-brain barrier (BBB) and measuring it in CSF can be beneficial. An experimental study showed evidence of IGF-1 crossing the BBB by inducing a middle cerebral artery occlusion (MCAO) in rats and administering radio-labeled IGF-1 into periphery immediately after. Within 30 minutes of IGF-1 administration, the majority of IGF-1 proteins were seen to be associated with neurons of the infarcted hemisphere (Liu et al., 2001).

Another limitation of the current study is the small sample size and lack of objective functional measurements. Larger clinical trials need to be performed in order to corroborate these findings, while also assessing functional ambulation and performance of activities of daily living. Although used in previous literature, the outcomes in this study (LOS, mRS, and discharge destination) limit our ability to understand how percent change in IGF-1 and IGF-1 ratio relate to objective measurements of functional recovery such as walking endurance and walking speed. Finally, other factors, unaccounted for in the current study, may influence the outcome of LOS and discharge destination. One study suggests that decisions of discharge destinations may be influenced by not only the condition of the individual with stroke, but also by social, economic, and policy factors (Johnson et al., 2015). However, another suggests that discharge destination is among the strongest of variables to predict global disability at 3 months (Zhang et al., 2015). The current study provides support in favor of undergoing larger, clinical

trials that use objective, measurable assessments of physical function to assess the potential benefits of IGF-1 and IGF-1 ratio.

5.6. Conclusions

Despite the suggestion of current literature regarding high baseline levels of IGF-1, our novel findings suggest that a decrease in IGF-1 and IGF-1 ratio during the first week of stroke may be related to positive, or more favorable, outcomes (i.e. shorter LOS, discharging home vs inpatient facilities, and improved functional independence at one-month post-stroke). When studying IGF-1 and its relationship to stroke recovery in humans, it may be important to consider multiple measurements of IGF-1 instead of single, baseline time points. Moreover, multiple measurements of IGF-1 ratio may also deepen our understanding of how IGF-1 is regulated after stroke and how this relates to functional recovery. The percent change in IGF-1 and IGF-1 ratio may be a meaningful biomarker of stroke recovery at one month. Further work is warranted in a larger clinical trial and in experimental models to determine why decreases in IGF-1 and IGF-1 ratio are beneficial and their relationship to objective measurements of function during the recovery of stroke.

CHAPTER 6

Discussion and Conclusions

6.1. Summary of Findings

The research and data presented in this dissertation was undertaken with the goal of understanding physical and physiological characteristics of the acute phase of stroke recovery. Stroke rehabilitation and recovery during the acute stage is an understudied area of research, specifically in the areas of physical activity, aerobic fitness, and insulin-like growth factor 1 (IGF-1), a known neuronal rescue agent. Our results show that individuals after stroke are extremely sedentary during their acute hospital stay and that increased sedentary time may be related to decreased function after stroke. Further, we demonstrated that we could assess pre-stroke aerobic fitness within 48 hours of hospital admission using a previously established and clinically feasible tool to predict aerobic fitness. This was an important finding and it has clinical utility for healthcare professionals working with individuals after stroke. We found that pre-stroke aerobic fitness was positively correlated with circulating levels of IGF-1. These results provide additional information regarding the benefits of exercise and aerobic fitness on stroke recovery and support that improving aerobic fitness prior to stroke may be beneficial through increases in baseline IGF-1. Finally, we showed that decreased circulating IGF-1 during the first week of stroke may be related to positive outcomes after stroke (i.e. discharging home vs. an inpatient facility and more independence in activities 1-month post stroke). However, the interplay between physical activity, aerobic fitness, and IGF-1 and how these factors are related to objective, functional outcomes after stroke remains unclear. The current set of experiments has provided the framework for future research in this area. This chapter summarizes the findings presented in this body of work and concludes with important clinical implications, limitations of our investigations, and directions for which future studies should be based.

6.1.1. Chapter 2. Use of Accelerometers to Examine Sedentary Time on an Acute Stroke Unit

The amount of physical activity performed by individuals with stroke has been quantified and observed previously in community-dwelling individuals with chronic stroke. Behavioral mapping for physical activity has been conducted during the acute and subacute stage of stroke recovery. However, no studies have objectively quantified physical activity, specifically, using accelerometers during their initial hospital stay. Using accelerometers provides greater understanding of how sedentary time very early after stroke may affect function and recovery post-stroke. Therefore, we undertook the study of quantitatively assessing sedentary time using tri-axial accelerometers during the first several days of stroke in the acute hospital setting. We then determined whether sedentary time was related to functional performance at discharge from the hospital.

Our investigation of 32 individuals showed that individuals with acute stroke spent a large majority of time sedentary during their hospital stay. Sedentary time was positively related to the Physical Performance Test, even when controlling for baseline performance, which suggests that individuals who spent more hospital time sedentary performed worse on functional tasks prior to discharge, regardless of their performance at baseline. These results demonstrate that people recovering from stroke spend most of their hospital stay sedentary. Our results may have important implications for stroke recovery.

6.1.2. Chapter 3. Use of a Non-Exercise Estimate for Pre-Stroke Peak VO₂ during the Acute Stroke Hospital Stay

Previous literature shows that aerobic fitness, or peak VO₂, is diminished in individuals with subacute and chronic stroke. However, information is lacking regarding peak VO₂ levels in individuals very early after stroke. This is partly due to the difficulty in measuring peak VO₂ in

these individuals. Performing maximal exercise tests during this sensitive time is infeasible due to a myriad of reasons such as time constraints, motor limitations, and lack of resources (i.e. expensive equipment, trained personnel, and medical oversight). However, feasible-to-use, non-exercise estimations of aerobic fitness very early after stroke may give way to new and groundbreaking insight into stroke recovery. Chapter 2 focused on using a previously validated non-exercise estimation equation to predict pre-stroke peak VO_2 in individuals with acute stroke during their hospital stay. Further, we set out to examine whether estimated pre-stroke peak VO_2 was related to function at hospital discharge.

Our results suggest that the non-exercise estimation of pre-stroke peak VO_2 is easily administered within 48 hours of hospital admission in individuals with acute stroke. Analysis of the relationship between estimated pre-stroke peak VO_2 and functional performance at discharge revealed no significant relationships when considering the total sample. However, when stratifying the sample by gender, significant relationships were observed in females between estimated pre-stroke peak VO_2 and the Fugl-Meyer Assessment of lower extremity motor function. Females with higher aerobic fitness prior to stroke exhibited better motor function of the lower extremities at discharge from the hospital. However, no significant relationships were observed for men. This could be due to a poor prediction of aerobic fitness in men because of over estimations of self-reported physical activity levels. Therefore, estimations of pre-stroke peak VO_2 are feasible to use in individuals during the acute hospital setting. However, the association between pre-stroke aerobic fitness and functional recovery remains unclear. Nonetheless, the results of this investigation provide important information for future studies to characterize fitness prior to stroke and how it may relate to objective measures of physical function during stroke recovery.

6.1.3. Chapter 4. Estimated Pre-Stroke Peak VO₂ is Related to Circulating IGF-1 Levels during Acute Stroke

Current literature suggests that IGF-1 is neuroprotective after stroke, such that higher levels of IGF-1 early after stroke are associated with positive outcomes (i.e. greater survival rates and greater independence). Further, in healthy individuals, IGF-1 levels can be influenced by physical activity and aerobic fitness. Therefore, to further understand the relationship of aerobic fitness and potential mechanisms of neuroprotection during stroke recovery, we sought to examine a known neuronal rescue agent, IGF-1, and its relationship to estimated pre-stroke peak VO₂ levels in individuals with acute stroke. Individuals completed a questionnaire which estimated pre-stroke peak VO₂ and had blood drawn within 72 hours of hospital admission for determination of IGF-1 levels.

Our results indicate that, in 15 individuals with acute stroke, estimated pre-stroke peak VO₂ is significantly related to circulating IGF-1 levels obtained within 72 hours of hospital admission. Further, individuals with higher than median IGF-1 levels possessed significantly better aerobic fitness prior to their stroke. These results indicate that improving aerobic fitness prior to stroke may be beneficial and provide neuroprotection by increasing baseline IGF-1 levels. These results inspire questions for future studies to investigate whether IGF-1 and aerobic fitness interact to protect and/or maintain physical function during stroke recovery.

6.1.4. Chapter 5. Change in IGF-1 Levels and the Ratio of IGF-1 to IGFBP-3 during the First Week of Stroke is Related to Stroke Outcomes

Chapter 6 gives evidence to suggest that aerobic fitness prior to stroke may provide neuroprotection through IGF-1 during post-stroke recovery. Current literature also describes

IGF-1 as having neuronal rescue abilities. Both in animal models and in humans, IGF-1 has provided neuroprotection to individuals with stroke. However, in humans, studies have primarily been employed using questionnaires and broad assessments of outcomes of stroke. Many studies have seen that individuals with high IGF-1 soon after stroke have a greater chance at survival and more independence 3 months later. However, these studies may have provided a limited understand of IGF-1's neuroprotective qualities by not considering how the response of IGF-1 during the first weeks of stroke is important and by only using general questionnaires to assess outcomes. Therefore, Chapter 5 aimed to characterize the response of IGF-1 during the first week of stroke and how it may be related to outcomes (i.e. discharge placement and independence).

Individuals with decreases in IGF-1 during the first week of stroke had more desirable outcomes compared to individuals with increases in IGF-1. The same is also true for the ratio of IGF-1 and IGFBP-3. Individuals with increases in IGF-1 and IGF-1 ratio (defined as IGF-1:IGFBP-3) during the first week had a longer length of stay in the hospital, had less independence and greater stroke severity at one month post-stroke, and went to inpatient facilities instead of directly home when discharged from the hospital. Baseline IGF-1 and IGF-1 ratio were not related to any outcomes and were not significantly different between those who went home or those who went to inpatient facilities. While other studies have shown that high baseline levels of IGf-1 are related to positive outcomes, these results may provide preliminary evidence that the change in IGF-1 and IGF-1 ratio during the first week of stroke are also important to recovery. Further work should be done to investigate the relationship between change in IGF-1 early after stroke and functional recovery.

6.2. Sedentary Time, Estimated Aerobic Fitness, and IGF-1 Levels in Individuals with Acute Stroke

6.2.1. Sample and Methods

A total of 44 individuals admitted to the hospital with a diagnosis of acute stroke were used for the analyses presented in these investigations. Individuals were recruited 24-72 hours of admission to the neuro-progressive or neuro-intensive care units of University of Kansas Hospital. All individuals signed institutionally approved informed consent forms prior to the initiation of data collection.

Chapters 2 and 3 focused on the objective quantification of sedentary time, estimation of pre-stroke aerobic fitness, and their relationship to functional recovery. Individuals were fitted with tri-axial accelerometers, which they wore on their ankles until discharge from the hospital, or for a maximum of 4 days. Additionally, individuals completed questionnaires to assess pre-stroke physical activity (Rapid Assessment of Physical Activity; RAPA) and aerobic fitness (non-exercise estimate of peak VO_2). Finally, individuals completed the following objective assessments of functional performance: Fugl-Meyer Assessment of lower extremity motor function, 6 Minute Walk Test, Timed-Up and Go with dual cognitive task, and the Physical Performance Test. Functional assessments were completed immediately after enrollment and prior to discharges (or 4 days following baseline assessments).

In Chapters 4 and 5, blood draws were performed by hospital staff or study team members, in the morning, and under fasting conditions. Within one hour of collection, samples were centrifuged at 4°C, and 1,900 *ref* for 15 minutes to separate and obtain plasma. Each plasma sample was aliquoted into 3-1.5 mL microcentrifuge polypropylene tubes and stored at -80°C

until assaying. Finally, after all samples were collected, IGF-1 and IGFBP-3 were quantified using ELISA kits from ALPCO® Diagnostics (Salem, NH).

6.2.2. *Statistical Analysis*

Pearson's correlations were performed to assess the relationship between two variables. In some cases a partial correlation was used to control for a confounding variable. T-tests were performed when assessing between group differences. Type 1 error (p-value) was always set at 0.05. IBM® SPSS® Statistics Version 22 (SPSS, Inc., Chicago, IL) was used for all statistical analyses.

6.2.3. *Discussion of Results*

6.2.3.1. Physical Activity in Stroke

Low levels of activity have been documented in those hospitalized for an acute illness other than stroke (Brown et al., 2004; Brown et al.; Lazarus et al., 1991). Inactivity during hospitalization has been associated with functional decline that necessitated nursing home placement even for those who resided in the community prior to hospitalization. Brown and colleagues monitored activity continuously (24 hours/day) using accelerometers and reported that older adults recovering from an acute illness in the hospital spend approximately 83% of their day lying in bed despite being able to walk independently during their hospital stay (Brown et al., 2009).

Observational studies have examined activity during inpatient stroke rehabilitation and have reported high levels of sedentary time (Askim et al., 2014; Bernhardt et al., 2008). When observing activity in 10 minute intervals from 8:00 am to 5:00 pm, patients during in-patient

stroke rehabilitation were seen in bed or sitting 76% of the day and standing or walking 23% of the day (Bernhardt et al., 2008). This evidence suggests that during an inpatient rehabilitation stay, individuals after stroke are spending a large majority of their time engaging in sedentary behavior, which could have a negative impact on functional recovery.

Prior to the current work, no studies have objectively measured physical activity during the hospital stay in the United States. Accelerometers are a reliable and valid method of measuring activity and use very little resources or dedicated work-time out of the schedules of hospital staff. Our results could have profound impact on the future of clinical practice as we found our participants to be extremely sedentary and not choosing to move on their own. We reported that people hospitalized with an acute stroke might spend a higher percentage of time sedentary than what is reported in the literature for an acute medical illness (Brown et al., 2009). Bed rest studies have demonstrated negative vascular adaptations (Bleeker et al., 2004) and loss of muscle mass which decreases muscle strength (Kortebein et al., 2007). Considering patients' low cardiopulmonary fitness levels in acute stroke (Mackay-Lyons, Makrides, 2002), and that the low intensity during rehabilitation is not sufficient for cardiopulmonary health (MacKay-Lyons, Makrides, 2002), efforts to increase physical activity and minimize decline from sedentary behavior should be encouraged. Therefore, using a multidisciplinary team approach to reduce sedentary time such as early mobilization during the acute hospital stay may slow the further decline of cardiopulmonary health and allow people post-stroke to engage more efficiently in rehabilitation.

6.2.3.2. Using Non-Exercise Estimation of Peak VO₂ in People with Stroke

Individuals post-stroke are physically inactive, spend small amounts of time walking per day (Roos et al., 2012), and have poor aerobic fitness, defined as peak oxygen consumption (peak VO₂) (Billinger et al., 2012; Gordon et al., 2004; Mackay-Lyons, Makrides, 2002; Macko et al., 1997). Assessing peak VO₂ using a metabolic cart with gas analysis is considered the gold standard (ACSM, 2010). Peak VO₂ may be an indicator of one's ability to participate in varying levels of activity such as exercise and activities of daily living (ADLs), especially in older adults (Billinger et al., 2011; Fleg et al., 2013) and people after stroke (Gordon et al., 2004). However, peak exercise testing is difficult, if not impossible, to conduct during the acute stroke hospital stay particularly with shorter lengths of stay especially in the United States. Therefore, using non-exercise measurements, such as prediction equations, to estimate peak VO₂ could be a simple, low-cost alternative to healthcare providers and especially physical therapists during the acute stroke hospital stay.

Our study was the first to use non-exercise estimation of pre-stroke peak VO₂ in those with acute stroke and to investigate whether pre-stroke peak VO₂ is related to function at discharge from the hospital. Our results showed a weak, non-significant correlation between estimated pre-stroke peak VO₂ and two of our outcome measures at discharge. This could be due to over- or under-estimation of physical activity prior to stroke since this is a subjective assessment. Additionally, the acute stroke period is a sensitive time where an individual may not remember their typical physical activity. It is possible that males and females may account for daily physical activity differently. Therefore, in a post-hoc analysis, we examined whether sex differences exist between estimated pre-stroke VO₂ peak and functional performance at

discharge and found that estimated pre-stroke aerobic fitness was related to function in women, but not men.

Although we did not compare the predicted peak VO_2 to a measured VO_2 , one previous study by McAuley and colleagues found that these measures were correlated in older adults with chronic disease (McAuley et al., 2011). The authors used the same non-exercise estimate equation as we did. They investigated the association of the non-exercise estimation of peak VO_2 measures of aerobic fitness (maximal exercise test and 1-mile timed walk) (McAuley et al., 2011). They found that the measure obtained with the estimation equation was significantly correlated with measures from the maximal exercise test and the 1-mile timed walk. McAuley suggests that their results serve as evidence that the non-exercise estimate equation has utility in other populations, but that future work needs to be done to determine whether relationship exist in other domains. This investigation adds to this literature by describing the relationship of pre-stroke peak VO_2 and measures of function in a small sample of individuals with acute stroke. McAuley highlights that assessing aerobic fitness is important in both healthy and diseased populations and may give deeper insight into other domains of health and acknowledge that exercise testing in older adults and individuals with high cardiovascular risk is difficult because of the risks involved, time constraints, and expenses such as obtaining proper equipment, personnel, and medical oversight. Therefore, we believe that there is a need for a short, non-exercise estimation of VO_2 that is easily obtained in the acute, clinical setting.

6.2.3.3. Non-Exercise Estimation of Peak VO₂ and IGF-1 Levels

Insulin-like growth factor-1 (IGF-1) is known to be neuroprotective after middle cerebral artery occlusion (MCAO) in animals (Chang et al., 2011; Guan et al., 1993; Guan et al., 1996; Zheng et al., 2014) and may even act as a neuronal rescue agent when given through intranasal delivery (Guan et al., 1993; Liu et al., 2001). In animals, when administered through intranasal delivery, core lesion size can be reduced up to 94% (Guan et al., 1993) while also improving functional status compared to vehicle controls (Zheng et al., 2014). In humans, those with high circulating levels of IGF-1 early after stroke have greater survival rates and fewer impairments, tested by the National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS), at 3 and 24 months post-stroke compared to those with low IGF-1 levels (Aberg et al., 2011; De Smedt et al., 2011; Denti et al., 2004). This suggests that IGF-1 may also have a neuroprotective effect in humans.

Aerobic fitness has been directly related to circulating IGF-1 levels in 846 healthy men (Nindl et al., 2011). Blood was collected in the morning after an overnight fast for the quantification of IGF-1. Individuals then underwent a graded maximal exercise test on a cycle ergometer with direct gas analysis to obtain peak VO₂. A repeated squats test was also performed to assess leg strength. Individuals with higher peak VO₂ exhibited higher levels of circulating baseline IGF-1. Further, better aerobic fitness was associated with increased performance on the repeated squats test, indicating a greater functional performance in the lower extremities (Nindl et al., 2011). Nindl's study in healthy men could potentially have very large implications for individuals post-stroke. Aerobic fitness prior to a stroke may not only benefit overall brain health, but also provide some level of neuroprotection via circulating IGF-1, which

has been shown to improve stroke outcomes (Aberg et al., 2011; De Smedt et al., 2011; Denti et al., 2004).

The results of our study showed that estimated pre-stroke peak VO_2 was related to circulating levels of IGF-1 early after stroke, but not to levels of IGFBP-3. Further, we sought to determine whether individuals with higher than median levels of IGF-1 would have greater aerobic fitness (peak VO_2) when compared to individuals with lower than median levels, and found our hypothesis to be supported by our data.

6.3. Clinical Implications

6.3.1. Physical Activity in Acute Stroke

Our results showed that individuals with acute stroke are extremely sedentary and that high sedentary time may be detrimental to function within a few days follow stroke. These results have extremely important clinical implications. Education of hospital staff (i.e. physicians, nurses, therapists) in the importance of physical activity and mobility for individuals during their hospital stay may help to resolve any confusion regarding whether or not to keep post-stroke individuals in bed. Further, education of patients and their families may be extremely important. Oftentimes individuals with stroke may be nervous to move for fear of secondary stroke, falling or injury. Settling these fears and encouraging them to get up and move around may help to change their behavior.

Additionally, there may be a need utilizing “mobility aids”. These individuals could be responsible for safely moving patients from their bed to their chair or helping them walk the hallways. It is no secret that nurses and physicians are incredibly busy with their long list of duties and adding mobility aids will help give dedicated attention to this issue. Mobility aids can

also help to encourage individuals to get up if they do not have family member or loved ones who regularly visit. Mobility aids can talk to those that do have family members present about helping to encourage their post-stroke loved one to get up and move. These important clinical implications from the results of this study may help to stop perpetuating the sedentary behavior before these individuals reach the community.

6.3.2. Using Non-Exercise Estimations of Peak VO₂ to Assess Fitness

As Chapters 3 and 4 describe, non-exercise estimations of peak VO₂ are very easy and feasible to administer to individuals who are in the very early phases of stroke. These chapter show that the Jurca equation may even be related to functional recovery and a hormone known to be neuroprotective. Often times, exercise specialists and physical therapists use information such as peak VO₂ to prescribe physical activity and exercise in rehabilitation of disease. The results seen in these two chapters are important because physicians and physical therapist can easily use equations such as these to assess fitness very early after stroke and even prescribe exercise interventions later in recovery. After further research, non-exercise equations may potentially even be able to use to predict outcomes of stroke recovery. Finally, peak VO₂ estimation equations could also be used as an education tool for patients in order to teach them about aerobic fitness and physical activity.

6.3.3. Fitness and IGF-1

As mentioned, IGF-1 is a neuroprotective hormone and high levels early after stroke are associated with positive outcomes one month and three months following stroke. In Chapter 4, IGF-1 levels are shown to be related to pre-stroke fitness levels. This is important for clinical

practice in that it provides support for physicians to encourage their patients to be physically active and maintain their aerobic capacity. Encouraging individuals, specifically ones with risk factors for stroke, to be more physically active or prescribing exercise as medicine, may increase baseline IGF-1 levels prior to incidents such as stroke. Although current literature does not describe IGF-1 levels prior to stroke or know if pre-stroke IGF-1 levels are associated with recovery, increasing baseline levels could potentially provide neuroprotection in case of cerebral injury.

Chapter 5 discusses the potential relationship between IGF-1 response during the first week of stroke and short-term outcomes. This information could be used in the future by clinicians to predict recovery and/or use as a timeline for exercise prescription and or interventions related to increasing the uptake of IGF-1 into the brain.

6.4. Limitations

The authors acknowledge that this collection of investigations was not performed without pitfalls and limitations. Specific limitations have been outlined in individual chapters. However, several broad limitations of the discussed studies are outlined in the following section. The authors made valiant attempts to avoid limitations where possible. However, with all clinical research, some limitations unforeseeable and unavoidable. Other limitations not described may still be relevant to these investigations.

6.4.1. Subject Characteristics and Sample Size

There are many confounding variables to take into account when considering the size and characteristics of our sample. However, we tried to control for many factors, while also

maintaining feasibility of the investigations. For the examination of physical activity levels on the acute stroke unit, we excluded all individuals who were prescribed physician-ordered bed rest as to not bias the sample toward sedentary behavior. Individuals who were assigned bed-rest were typically individuals who had either received intravenous tissue plasminogen activator (tPA) or individuals who participated in interventional medicine (i.e. clot retrievals, stents, coils, or balloons). Individuals who received tPA had a mandatory 24 hours of bed-rest, while individuals who underwent surgical interventions usually had even longer periods of physician-ordered bedrest. Often times, after individuals were off their prescribed bed-rest period, they were either discharged home or outside the enrollment time-period and no longer eligible for the study.

We did not control for race, ethnicity, socioeconomic status, educational level or intelligence. However, we did not exclude individuals from our studies based on these factors. Overall, we had a very demographically diverse sample that closely reflects our goal enrollment and the population of the surrounding area (49% male, 59% Caucasian, 12% African American, 2% Asian, 89% Non-Hispanic) taken from the United States Census Bureau. The individuals enrolled into our studies were: 53% male and 47% female, 72% Caucasian, 17% African American, 2% Asian, 2% Pacific Islander, and 7% “other”, and 93% non-Hispanic and 7% Hispanic.

Although our sample is demographically diverse, it was relatively small and a larger sample size may reveal further significant results on secondary or less sensitive outcome measures. However, for the purposes of our studies, the necessary sample size was calculated and enrolment achieved for each project. Therefore, we were confident in the number of individuals enrolled for these projects.

6.4.2. *Confounds of Lesion Size and Location*

Lesion size was obtained and calculated from the diagnostic, structural MRI performed upon admission for every enrolled individual. A radiology resident apart of the study team calculated lesion volumes using standardize neuroimaging techniques with an ellipsoidal assumption of lesion shape. Limitations of measuring lesion location include the ellipsoidal assumption of shape. Incorrectly assuming an ellipsoidal shape could yield incorrect estimations of lesion volume. However, these are standard practices used in previously published research, giving us confidence in using this method. Further, quality of MRI (i.e. strength of the magnet, movement of participant, etc) could also affect lesion measurement. Finally, in many cases, individuals were not experiencing a first time stroke. Overlap of old infarcts may diminish confidence in lesion measurement if the borders are hard to define. However, trained radiologists have the ability to discriminate between acute, subacute and chronic lesions and we are confident in ours being able to do so.

Additionally, general locations of lesions were obtained from the MRI report associated with the admitting MRI. In some cases, statistical analyses were controlled for lesion volume. However, no analyses were controlled for lesion location, which could be a potential confounding factor. Location of lesions often have an effect on what type of impairments individuals with stroke experience (i.e. motor, speech, vision, coordination). Therefore, it is important to cautiously interpret our results involving functional outcome measures. Unfortunately, with the wide variety of lesion locations our participants experienced, our sample size was not large enough to account for this variable. However, few studies of this nature consider lesion location due to the infeasibility of recruiting a normally distributed and appropriately large sample of individuals.

6.4.3. *Comorbidities and Medications*

Often people who experience stroke are not without other physical and/or health limitations. For example, 16 individuals in our sample also had Type 1 or Type 2 diabetes. Comorbidities such as these can have affected the outcomes investigated. However, when studying individuals with stroke, it is infeasible to exclude for all potential comorbidities. Individuals with arthritis, back or other joint pain may have had a difficult time completing functional assessments due to their musculoskeletal pain instead of their stroke impairments. However, individuals were continuously asked about their pain and comfort during physical function testing. When there were complaints of pain, this was recorded in their file and they were allowed to rest until they felt like they could continue without additional distress. Individuals with stroke impairments that included deficits in vision or comprehension may have had a hard time seeing or understanding the task before them. However, for studies that involved functional assessments, individuals were not enrolled unless they were able to comprehend activities and information presented to them in the consent form. If comprehension was questioned, the study team consulted with the physicians and nursing staff and determined if the consenting process should continue.

For studies involving blood draw(s), diabetes and liver function, potential confounding factors, were not used as excluding factors. However, levels of blood glucose, HbA1c, and liver enzymes were obtained from the medical record and could be used for post-hoc analyses. No individuals enrolled had levels of liver enzymes out of normal ranges. All past medical history was obtained and recorded in participant files. This included, but was not limited to, other significant medical conditions, medications, and surgical histories. In addition, blood pressure medications, administration of tPA, and diabetes medication could have affected circulating

blood protein levels. However, if medically able to do so, participants were asked to refrain from taking their medications beginning midnight before each blood draw. Finally, individuals could not be within 2 hours of receiving tPA when providing a blood sample.

6.4.4. Assessments of Physical Function

A series of physical function assessments were used in Chapters 2 and 3. Assessments included the Lower Extremity Fugl-Meyer Assessment, Physical Performance Test, 6 Minute Walk Test, Timed-Up and Go, and Four Square Step Test. These assessments were performed in serial and typically during an hour to two-hour period. It is possible that individuals became fatigue during this time. However, individuals were allowed to rest between tasks and between assessments for as long as they felt necessary. Additionally, for assessments performed in the corridor outside of the hospital room, individuals were often wheeled out in a wheelchair to limit fatigable activity unrelated to the assessments. If a wheelchair was unavailable or unwarranted, a chair was placed near the testing area, but as close to the room as possible so that walking distance was minimized and that participants could rest while explanation of the assessment was given. Further, it was not uncommon for individuals to complain of a headache. Performing assessments of physical function was avoided during times of headache so that individuals could perform their best. There were several occasions where performing assessments during headaches was unavoidable. However, headache and pain was noted in the file where appropriate.

Distractions during functional testing were limited. All assessments performed in the participants' rooms were done so with the door closed. Nurses were alerted that testing was being performed so that they could limit the amount of people entering the room during that

time. Hospital staff was educated by the study team to not distract and speak with the participants during testing in the corridor outside participants' rooms. Orange cones were placed around the testing area to alert people to go around. Subjects were instructed to limit conversation with both the study team and other individuals walking by. In many cases, family members or loved ones were present and wanted to watch testing. Study team members allowed family members and loved ones to stay in the room or come into the corridor as long as they did not talk the participant during testing. It is possible that in the presence of family/loved ones, individuals either had more or less motivation to perform well. They may feel more pressure to perform well if others are watching or less motivated to participate if they are wanting to be with their visitor instead. Finally, motivation of the participant may vary day to day depending on the amount of sleep they acquired the night before, the amount of activity they already did during that day, and/or how they were feeling physically and emotionally at that moment.

6.4.5. Assessment of Physical Activity

As with any wearable device, there are several potential limitations. Individuals were instructed to remain wearing the tri-axial accelerometers unless they really needed to remove them for comfort or otherwise. These devices are water-resistant in 1 meter of water for up to 30 minutes, so participants were informed that removal during showers or bathing was not necessary. An individual from the study team checked on participants daily to make sure they were feeling comfortable wearing the accelerometers and to ask whether they had removed the device during the last 24 hours. Occasionally, a sock or gauze was placed between the skin and the accelerometer to provide additional comfort. If any reports of device removal were made, a note was put in the file and that time frame was deleted from a copy of the electronic file.

Further, it is possible that participants failed to report device removal due to failure of remembering or nervousness of admitting. In one case, accelerometers were removed for an extended period of time. Therefore, that participant was not used in data collection.

Although we found our participants to be extremely sedentary, there may still have been an effect of knowing that they are being “watched”. If one knows they are being monitored for physical activity, it is likely that they will perform greater amounts of activity than what they normally would have otherwise. In addition to this, individuals with family members or visitors present may have been encouraged to move more than individuals who did not have visitors present. Finally, because of the nature of tri-axial accelerometers, it is possible that activity may have been artificially inflated due to transportation of participants by either hospital bed or wheelchair. The accelerometers would not have recognized this acceleration as mobility due to outside forces. However, in the proprietary software, it is possible to break down activity by each plane of movement and identify at what times acceleration was rapidly occurring in only one plane (i.e. being pushing forward in a wheelchair, moving up or down in an elevator).

6.4.6. Assessment of Non-Exercise Estimation

Several limitations could be attributed to the Jurca non-exercise estimation of pre-stroke peak VO_2 . For example, one component of the equation is the self-selected measure of physical activity. It is possible that individuals post-stroke could have a hard time remembering or reporting the amount of activity they performed in a typical week prior to their stroke, resulting in an over- or under-estimation of peak VO_2 . Further, individuals may be embarrassed by their true amount of activity and over-estimate it in order to compensate.

This estimation equation has not yet been validated specifically in stroke. However, cross-validation has occurred in older adults who possess 1-6 cardiovascular risk factors, giving us confidence to use this measure in individuals with stroke. Additionally, it was previously unknown whether resting heart rate remained unchanged from pre- to post-stroke. However, our sample subset showed that resting heart rate was not significantly different from recorded resting heart rate of an outpatient visit prior to stroke to resting heart rate recording during the hospital stay for acute stroke. Finally, our results showed that this equation may not predict peak VO_2 very well in men and therefore, future studies should investigate the use of the Jurca equation in individuals with stroke further.

6.4.7. *Quantification of IGF-1 and IGFBP-3*

Finally, there are several potential limitations of blood collection and analysis. First, circulating levels of IGF-1 and IGFBP-3 are known to be affected by time of day and nutritional status. Therefore, samples collected at different times or under different lengths after meals may be variable in protein levels. However, these variations were limited by enforcing a very strict sampling period in our participants. All samples were obtained during the hours of 7:30 am and 9:30 am. Further, individuals were told to fast from food and drink (with the exception of water) beginning at midnight before sampling. Medications were also held when medically able to do so. Further, hydration of participants may have an effect on the ability to draw blood. In very few cases, we were only able to draw a small amount of blood. The length of time from draw to being frozen may have an effect on protein viability. However, all samples were placed on ice immediately after being drawn. Samples were transported to the laboratory for processing and

frozen within one hour of collection. All samples were processed in the same manner with the same equipment.

Variations in assaying could introduce additional error. Kits for protein concentration outline the number of allowable freeze/thaw cycles. No samples were frozen and thawed more than the recommended times and most samples were only thawed once. Further, errors in assaying due to inexperience and lack of skill may lead to inaccurate results. However, all assays were performed with a standard curve of an r of .98 or greater. Internal controls provided by kits were used for all assays and were well within acceptable ranges. Finally, concentrations of proteins could be influenced by physical activity level of individuals immediately prior to blood sampling, age, gender, body mass index (BMI), and diet.

6.5. Future Directions

The investigations undertaken by this body of work have important implications for future studies. The following section discusses the future directions that are important to the field of research as outlined by the results presented previously.

6.5.1. Physical Activity in Acute Stroke

In Chapter 2 it was shown that individuals admitted to the hospital with acute stroke engage in very little physical activity. Further, the amount of sedentary behavior may be related to function on discharge from the hospital, regardless of functional performance at admission. These results warrant future studies to examine how physical activity during the hospital stay relate to long term follow up of function. That is to say, do people who spend less time sedentary perform better on functional assessments 6 months or 1 year after discharge from the

hospital? Further, do individuals who perform more physical activity during the hospital stay continue performing more activity as post-stroke individuals living in the community compared to post-stroke individuals who performed less activity in the hospital? Finally, is there a minimum amount of activity needed in order to see significant benefits?

6.5.2. IGF-1 and Objective Measures of Physical Function

Chapters 4 and 5 discuss IGF-1, a neuroprotective hormone, and its relationship to aerobic fitness and short-term stroke outcomes. Current literature describes early levels of IGF-1 and how it relates to survival and independence later in recovery. This study adds to the field of knowledge by describing IGF-1's relationship to estimated fitness, IGF-1's response during the first week of stroke, and how IGF-1's response is related to short-term outcomes. Future studies should examine how IGF-1 levels and IGF-1 response early after stroke are related to objective measures of function during stroke recovery. This will provide more in-depth detail on how IGF-1 can affect physical function both in the upper and lower extremities. Using assessments such as the ones performed in Chapters 2 and 3 to assess IGF-1's relationship to function can give important insight into neuroprotection and recovery.

6.5.3. Exercise Intervention Effects on IGF-1 Levels and Recovery

Exercise has shown to increase levels of IGF-1 and its uptake into the brain from the periphery in healthy humans and animals with experimental stroke. In addition to this, the promising results from this collection of work that outlines the relationship of IGF-1 to estimated pre-stroke fitness raise many unanswered questions. Future studies can investigate whether IGF-1 can be increased from exercise interventions in individuals with stroke and if so, what

frequency, intensity, time, and type of exercise elicits most beneficial changes in IGF-1 levels. In addition, future research should study how IGF-1 increases due to physical activity and exercise related to improvements in function during stroke recovery.

6.5.4. Intranasal Administration of IGF-1

Animal models of stroke show that administration of IGF-1 after insult through intranasal delivery provides benefits to lesion size and function. IGF-1 has been administered in healthy adults to study how it affects exercise performance. However, administration of IGF-1 has not been used in individuals who experienced stroke. Large clinical trials using IGF-1 as a rescue agent should determine if there are also beneficial effects in humans. If neuroprotective effects are seen through intranasal delivery, dosing and time of administration should also be studied.

6.6. Conclusions

In conclusion, this body of work describes physical activity and sedentary levels of individuals with acute stroke while they are in the hospital and how activity relates to functional status at discharge. Further, discussion of using non-exercise peak VO_2 in the hospital setting determined that measures such as these are practical and easy to use and may potentially have a relationship to both functional recovery and neuroprotection. Our results showed that individuals with acute stroke who had higher pre-stroke fitness levels had higher levels of IGF-1 compared to individuals with acute stroke with lower pre-stroke fitness levels. Finally, this collection of studies revealed that decreases in IGF-1 levels are related to positive short-term outcome during stroke recovery. This is the first investigation to study how the response of IGF-1 during the first week relates to outcomes and warrants future research. The presented work is

novel and significant in that it provides objective measures of activity and neuroprotection very early after stroke and new evidence for the use of easy-to-administer assessments of fitness.

These studies set important groundwork for additional research to provide greater detail accounts of the interaction of fitness, physical activity, IGF-1 levels, and functional recovery.

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