9-2016

Neuropsychological Effects of Pomegranate Supplementation Following Ischemic Stroke

John A. Bellone

LOMA LINDA UNIVERSITY
School of Behavioral Health
in conjunction with the
Faculty of Graduate Studies

Neuropsychological Effects of Pomegranate Supplementation Following Ischemic Stroke

by

John A. Bellone

A Dissertation submitted in partial satisfaction of
the requirements for the degree
Doctor of Philosophy in Clinical Psychology

September 2016

Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

_____, Co-Chairperson
Richard E. Hartman, Professor of Psychology

_____, Co-Chairperson
Travis G. Fogel, Assistant Professor of Physical Medicine and Rehabilitation

_____
Michael J. Gilewski, Associate Professor of Physical Medicine and Rehabilitation

_____
Holly E.R. Morrell, Assistant Professor of Psychology

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Dr. Rich Hartman for all his contributions to my academic development and work on this project. Among many things, Rich is my research mentor and provided numerous opportunities to sharpen my research skills and neuroscience knowledge. He provided training regarding research methodology and statistical analysis, and reviewed the many drafts and presentations involved with this project, to name just a few of his many contributions. His advice and direction were instrumental to my growth as a scientist. Additionally, his prior research laid the foundation on which this project was conceived.

Dr. Travis Fogel was another key individual responsible for the completion of this project and my development both on a professional and personal level. I consider Dr. Fogel to be my clinical mentor, and the one who ignited my passion to pursue a career in neuropsychology. His time, support, and efforts both in regards to my training and to the project are greatly appreciated.

Completing any complex project is impossible without a team of competent, committed professionals, and this project had a particularly stellar clinical team. Dr. Paolo Jorge (resident physician) conducted all screening and consenting of patients, and was involved in documentation. Dr. Jorge was supervised by Dr. Mary Kim (attending physician), who submitted all neuropsychology and pharmacy orders, as well as provided invaluable guidance during the design and implementation phases of the project. Jeff Murray (psychology doctoral student supervised by Dr. Fogel) conducted all the neuropsychological testing, even if it meant coming in over the weekend or his day off, and was my point person for managing data onsite. Dr. Desiree Wallace was the

pharmacy coordinator and supervised the pharmacy staff, ensuring the accuracy of subject randomization and treatment administration. Last, the nursing staff played the vital role of delivering treatment to patients. Each of these individuals and teams were responsible, available, and incredibly competent at their job, and I am truly indebted to them for their hard work and dedication to this project.

I would also like to thank Drs. Mike Gilewski and Holly Morrell for their time spent reviewing my manuscript, attending my presentations, and providing feedback that has positively impacted the project. Dr. Morrell's guidance regarding the data analysis and interpretation phase was also instrumental and greatly appreciated. Additionally, the scoring software and self-rating sheets provided by Dr. Kyrstle Barrera, as well as all her advice over the years, were invaluable. Last, I thank Raquel Bellone for creating Figure 4.

For correspondence or questions, please contact John at jbellone@llu.edu.

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ABBREVIATIONS

AD	Alzheimer's Disease
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BDI	Beck Depression Inventory
BDNF	Brain-derived Neurotrophic Factor
BTA	Brief Test of Attention
CI	Confidence Interval
CNS	Central Nervous System
CONSORT	Consolidated Standards of Reporting Trials
CTC	Clinical Trials Center
GRAS	Generally Recognized as Safe
IRB	Institutional Review Board
INR	International Normalized Ratio
LLUECH	Loma Linda University East Campus Hospital
LTP	Long-term Potentiation
MANOVA	Multivariate Analysis of Variance
MMSE	Mini-mental State Examination
PP(s)	Pomegranate Polyphenol(s)
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
SD	Standard Deviation
STAI	State-trait Anxiety Inventory

TOPF	Test of Premorbid Functioning
TMT	Trail Making Test
tPA	Tissue Plasminogen Activator

ABSTRACT OF THE DISSERTATION

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by

John A. Bellone

Doctor of Philosophy, Graduate Program in Clinical Psychology
Loma Linda University, September 2016
Drs. Richard E. Hartman & Travis G. Fogel, Chairpersons

Polyphenols are compounds found in fruits and vegetables that have antioxidant and anti-inflammatory properties. Mounting evidence suggests that dietary polyphenol intake can reduce the detrimental effects of various disease processes, and pomegranates have frequently been examined because of their particularly high polyphenol content. Since stroke induces both oxidative stress and inflammation and is currently the leading cause of long-term disability in the U.S., we sought to determine whether dietary supplementation with polyphenols could enhance cognitive recovery in individuals who had suffered an ischemic stroke. We administered polyphenols via 2 POMx pills containing polyphenols derived from pomegranates equivalent to the content of approximately 8 ounces of pomegranate juice, or placebo pills (capsules containing no polyphenol ingredients), every day for one week to inpatients who were in the acute post-stroke phase. Neuropsychological testing pre- and post-treatment was used to determine whether there were any changes in cognitive functioning as a result of pomegranate supplementation. Results trended toward subtle improvements in cognitive abilities in pomegranate-treated subjects compared to placebo-controlled subjects. Findings from this randomized, placebo-controlled, double-blind clinical trial suggest that pomegranate

polyphenols may be effective at enhancing the recovery of cognitive functioning after ischemic stroke, although studies with larger sample sizes and longer treatment durations are needed to make any conclusions regarding these potential effects.

CHAPTER ONE

INTRODUCTION

Dietary supplementation with polyphenol-rich foods and beverages has received much attention from consumers and manufacturers over the past several years as a way to promote overall health, and has increasingly been investigated for its utility in preventing or improving a variety of disease states. Foods rich in polyphenols include various types of fruits (e.g., strawberries, blueberries), vegetables (e.g., broccoli, red onions), legumes (e.g., lentils, fava beans), nuts (e.g., walnuts, pistachios), seeds (e.g., pumpkin and sunflower seeds), herbs (e.g., rosemary, sage), and spices (e.g., curry, cinnamon). Pomegranates (*Punica granatum*) contain particularly large amounts of polyphenols compared with other foods, with estimates of roughly 3 times the antioxidant activity of red wine and green tea (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000). Their high phenol content has made them a target for studies investigating the health-promoting qualities of polyphenols.

Pomegranate polyphenols (PPs) have been touted for their antioxidant and anti-inflammatory properties, and have been successfully shown to reduce the detrimental effects of many different disease processes. For example, animal and clinical studies have demonstrated the efficacy of PPs in treating hypertension (Aviram & Dornfeld, 2001), diabetes (T. H. W. Huang et al., 2005), depression-like behavior (Mori-Okamoto, Otawara-Hamamoto, Yamato, & Yoshimura, 2004), neonatal hypoxia-ischemia (Loren, Seeram, Schulman, & Holtzman, 2005; West, Atzeva, & Holtzman, 2007), prostate, breast, skin, and lung cancer (Afaq, Zaid, Khan, Dreher, & Mukhtar, 2009; G. N. Khan et

al., 2009; N. Khan, Afaq, Kweon, Kim, & Mukhtar, 2007; Paller et al., 2012), atherosclerosis (Aviram et al., 2000; de Nigris et al., 2005), coronary heart disease (Sumner et al., 2005), hyperlipidemia (Esmaillzadeh, Tahbaz, Gaieni, Alavi-Majd, & Azadbakht, 2004), microbial infections (Braga et al., 2005), rheumatoid arthritis (Balbir-Gurman, Fuhrman, Braun-Moscovici, Markovits, & Aviram, 2011), and even erectile dysfunction (Azadzoi, Schulman, Aviram, & Siroky, 2005; Forest, Padma-Nathan, & Liker, 2007). Dr. Hartman (committee co-chair) and colleagues have also had success in using pomegranate juice to ameliorate deficits in animal models of Alzheimer's disease (Hartman et al., 2006) and exposure to proton radiation (Dulcich & Hartman, 2013).

Although the beneficial effects of PPs have been well established for the prevention of various disease processes, and cognitive and emotional improvements are consistently shown in animal models, the effects of such compounds on cognitive functioning in humans are less established. However, other polyphenol-laden foods and beverages, such as curry and green tea, may improve cognitive and emotional functioning in humans (Kuriyama et al., 2006; Ng et al., 2006). Furthermore, two recent studies have shown promising findings using PPs, suggesting that they can improve memory functioning in a clinical population following cardiac surgery (Ropacki, Patel, & Hartman, 2013) and for individuals with mild memory complaints (Bookheimer et al., 2013). These results indicate the need for further investigation into the effects of PPs on cognitive and emotional functioning in other types of disease processes.

One disease state that leads to a particularly large societal burden and frequently results in considerable cognitive dysfunction is stroke. An ischemic stroke is a cerebrovascular event that reduces or blocks the flow of blood (and thus also oxygen and

nutrients) to the brain, often resulting in temporary and/or permanent cellular damage. This damage occurs via many pathological processes following the event, although the main mechanisms include oxidative stress (El Kossi & Zakhary, 2000), inflammation (J. Huang, Upadhyay, & Tamargo, 2006), excitotoxicity (Castillo, Dávalos, & Noya, 1997), and apoptosis (Du, Hu, Csernansky, Hsu, & Choi, 1996). For survivors, severe, debilitating cognitive deficits and emotional disturbances often remain, and long-term morbidity is the norm in this population (Go et al., 2014).

Approximately 800,000 Americans experience a new or recurrent stroke each year (Go et al., 2014), with a large percentage of survivors experiencing extensive cognitive deficits (M. D. Patel, Coshall, Rudd, & Wolfe, 2002; Tatemichi et al., 1994). The estimated cost of stroke to the U.S. in 2010 was 36.5 billion dollars, with the total medical costs projected to triple in the next two decades (Go et al., 2014). Although there are hundreds of thousands of survivors each year (actual estimate is 670,500 people), very few effective treatments are available to prevent or reduce the long-term debilitating effects. Furthermore, although early initiation of medical and rehabilitation services, as well as rehabilitation in an interdisciplinary setting, drastically improves functional outcome (Cifu & Stewart, 1999; Salter, Hartley, & Foley, 2006; Paolucci et al., 2000), some degree of cognitive and adaptive deficits often remain following rehabilitation, and it has been estimated that only one-third of survivors receive such services (CDC, 2007).

Because PPs have demonstrated effectiveness in the treatment of pathological processes similar to those seen following stroke (e.g., oxidative stress, inflammation, excitotoxicity, and apoptosis), and because of their effectiveness at ameliorating various

other disease states, we hypothesized that PP intake would be an effective method of enhancing cognitive and emotional recovery following a stroke. To our knowledge, this is the first study assessing the efficacy of PPs in a clinical stroke population, and one of few studies to use neuropsychological assessment methods to describe the progression of cognitive functioning following PP administration.

Specific Aims and Hypotheses

The present study was designed to assess the cognitive and emotional effects of pomegranate polyphenols (PPs) on patients who had suffered a recent ischemic stroke, as well as identify specific domains that may be differentially impacted.

Aim 1: To Determine Whether PPs Improve Global Cognitive and/or Emotional Functioning in Individuals who have Experienced an Ischemic Stroke

Data suggest that stroke results in long-lasting cognitive impairment, likely due to oxidative stress, inflammation, excitotoxicity, apoptosis, and numerous other deleterious processes (see Chapter 2). Polyphenols can ameliorate each of these processes. Mood symptoms are also prevalent after stroke as a result of both physical and psychosocial factors, and polyphenol intake has been shown to decrease these symptoms. We hypothesized that PPs would be beneficial when administered shortly after a stroke, and would bolster cognitive recovery and decrease mood symptoms that are often experienced by stroke survivors. Some positive change was also expected for the placebo group, since spontaneous recovery is common following stroke, but we hypothesized that

gains made by pomegranate-treated patients would exceed those made by placebo-controlled patients.

Specific Hypothesis 1

Stroke patients who are administered PPs would have a higher degree of positive change (i.e., would perform better) on a post-treatment neuropsychological evaluation assessing global cognitive functioning (relative to their baseline functioning) than placebo controls. More specifically, their RBANS Total Scale Index score and MMSE-2 score (see Chapter 3 for description) would show more positive change than scores for controls. Figure 1 depicts these hypothesized findings.

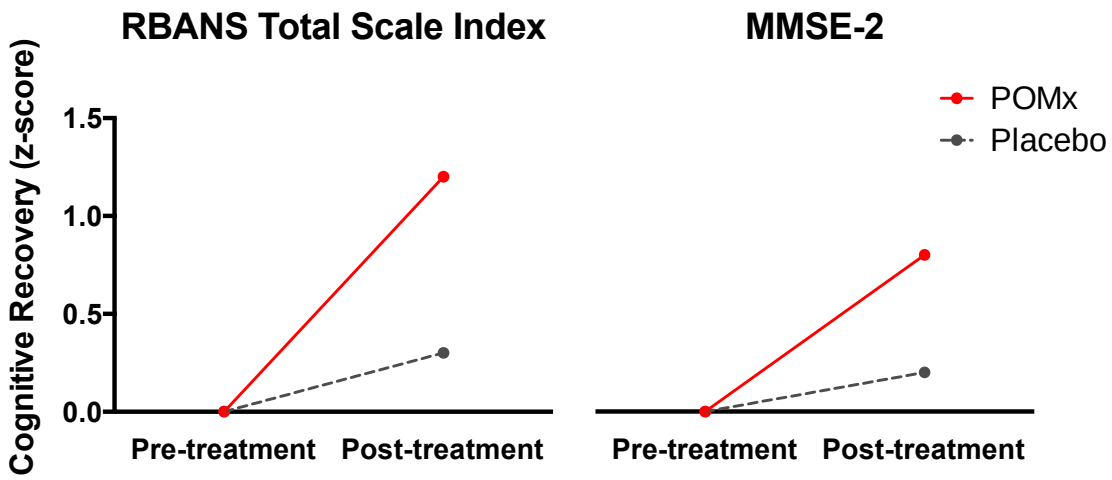

Figure 1. Hypothesized effects of PPs on overall neuropsychological performance. RBANS: Repeatable Battery for the Assessment of Neuropsychological Status. MMSE-2: Mini-Mental State Examination – Second Edition

Specific Hypothesis 2

Stroke patients who took PPs would have a larger decrease in symptoms of

depression and anxiety on a post-treatment assessment (relative to their baseline functioning) than placebo controls. More specifically, pomegranate-treated patients would endorse fewer depressive symptoms on the Beck Depression Inventory – Second Edition (BDI-II) and fewer anxiety symptoms on the State-Trait Anxiety Inventory (STAI; see Chapter 3 for description) relative to controls on post-treatment assessment. Figure 2 depicts these hypothesized findings.

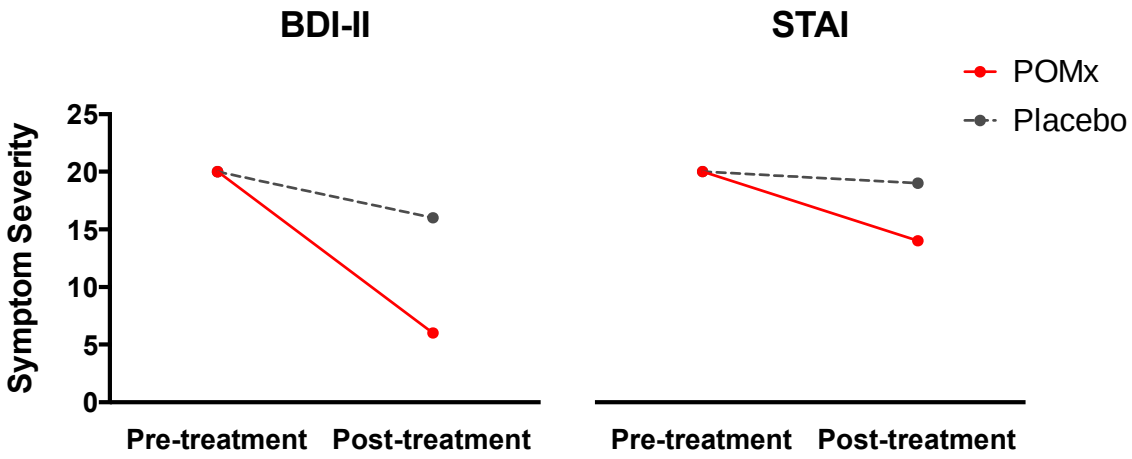

Figure 2. Hypothesized effects of PPs on emotional functioning.
BDI-II: Beck Depression Inventory – Second Edition
STAI: State-Trait Anxiety Inventory

Aim 2: To Determine Whether PPs Differentially Affect Cognitive Domains

Although the profile of cognitive deficits seen following stroke is often highly variable and largely depends on the lesion location (see Chapter 2), decrements may show patterns of variation by cognitive domain. We hypothesized that domains most affected by stroke would be most improved by PPs, since they would leave the most room for improvement.

Specific Hypothesis

Treatment with PPs would affect cognitive domains differently, where subjects that receive PPs would have larger improvements in the domains that changed most over time (see Figure 3).

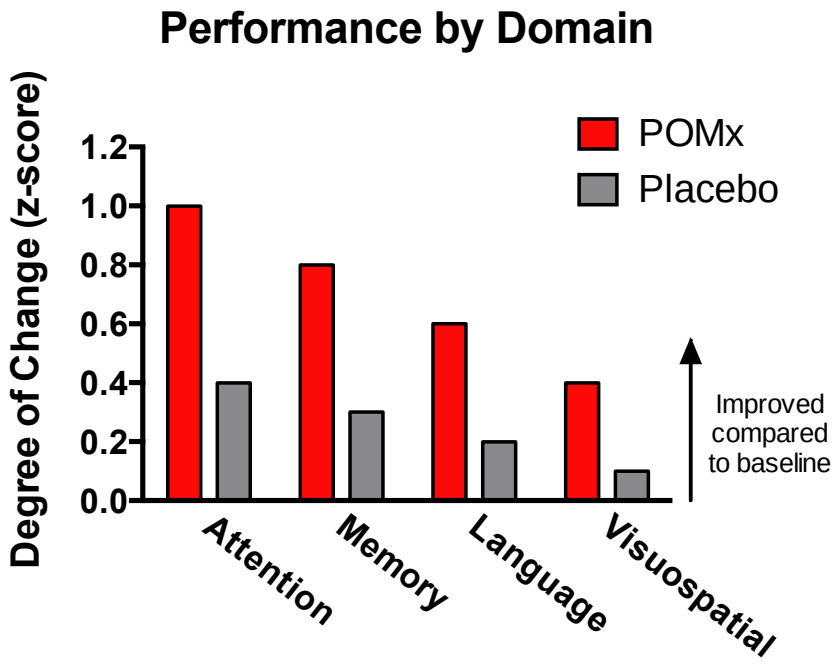

Figure 3. Hypothesized effects of PPs on neuropsychological performance with varying degrees of change per domain.

CHAPTER TWO

REVIEW OF THE LITERATURE

Polyphenols

Claims for the benefits of consuming fruits and vegetables have tended to precede hypotheses as to the mechanism of their effects. Many have suggested that these foods contain compounds that have effects independent of that of known nutrients (Arts & Hollman, 2005; Biesalski, 2007; Liu, 2003; Sun, Chu, Wu, & Liu, 2002). Polyphenols, a subclass of phytochemicals that are abundant in a variety of plants and have many unique qualities, are a promising candidate.

The term "polyphenols" refers to the large family of phenol structural units formed by attaching one or more hydroxyl group (i.e., oxygen atom connected by a covalent bond to a hydrogen atom) to one or more aromatic phenyl rings (see Figure 4 for a depiction of common polyphenol structures). These bioactive compounds are found in most plant families and are an integral part of a plant's physiology (e.g., provide pigmentation). They are involved in development and reproduction, prevent decay, and provide protection from predators, pathogens, and ultraviolet radiation (Bravo, 1998; Hart & Hillis, 1974). Polyphenols can be divided into over 10 major classes, each with numerous divisions and subdivisions. For example, the flavonoids category can be divided into over 8000 different types of polyphenols (Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011). Humans mainly consume polyphenols in the phenolic acid and flavonoid classes, and, to a lesser extent, lignans and stilbenes. Due to the large number of diverse compounds present in different foods, it is difficult to evaluate the effectiveness of individual phenols. To review the phenolic breakdown of many different

types of foods, please visit the following website: http://www.phenol-explorer.eu/ (Neveu et al., 2010).

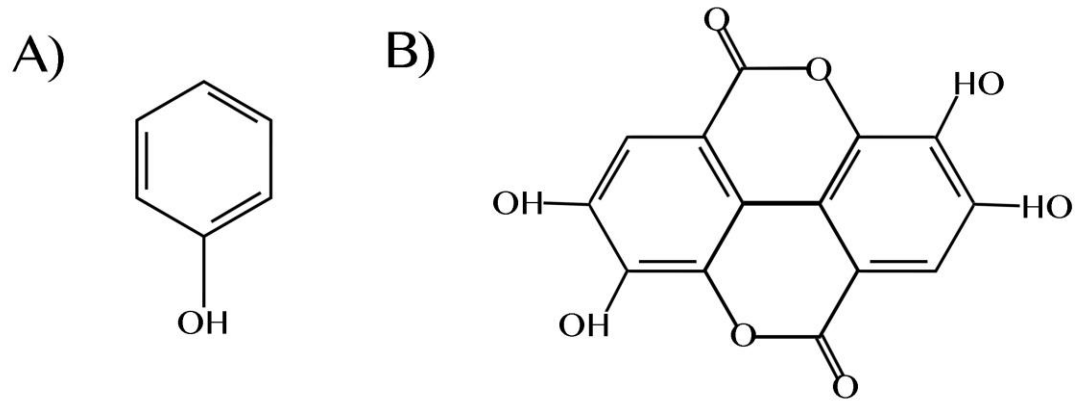

Figure 4. Depiction of A) the simplest phenolic compound, containing one six-member carbon ring and one hydroxyl group, and B) ellagic acid, one polyphenol structure commonly found in pomegranate juice/extract.

There has been an eruption of research on the salutary effects of polyphenols in the past two decades. As aforementioned (see Chapter 1), a variety of polyphenol-rich foods have been demonstrated to prevent or improve pathological processes involved in many disease states, including cognitive and psychiatric symptoms (Gomez-Pinilla & Nguyen, 2012; Z. Huang et al., 2011; Kuriyama et al., 2006; Mori-Okamoto et al., 2004; Xu et al., 2006). Although the main purported mechanisms by which polyphenols relay their benefits are likely through the reduction of oxidative stress and inflammation, they also affect apoptosis, neuroplasticity, and hemodynamics; modulate a broad array of receptors and enzymes (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004); protect against excitotoxicity (Castillo et al., 1997); reduce the probability of infection due to their antiproliferative/antimicrobial effects (Seeram et al., 2005); and have

gastroprotective effects via the modulation of nitrite and nitric oxide (Dykhuizen et al., 1996; Rocha, Gago, Barbosa, & Laranjinha, 2009). Another promising, albeit somewhat counter-intuitive, mechanism may be through hormesis, or pre-conditioning of the biological system with a mildly toxic agent. These mechanisms are further discussed below.

Bioavailability

Bioavailability refers to the amount of ingested polyphenols that is absorbed and becomes available at a site of action. Many factors limit polyphenol bioavailability, such as gastrointestinal degradation, first pass metabolism, poor solubility, insufficient permeability, and instability (Ratnam, Ankola, Bhardwaj, Sahana, & Kumar, 2006). Despite relative similarities, the different varieties of polyphenols can have widely varying pharmacokinetic properties (Manach et al., 2004). Furthermore, the distribution of polyphenol category depends on the type of food, geological location, and method of processing (e.g., culinary preparation methods, such as cooking or peeling the skin from fruits and vegetables can substantially reduce polyphenol content; D'Archivio et al., 2007). Different properties, concentrations, and interactions among polyphenols simultaneously consumed make it extremely difficulty to get an accurate measure of typical bioavailability.

Although not studied extensively, evidence (e.g., antioxidant capacity and plasma/urine concentrations) suggests that a portion of polyphenols get absorbed through the gut epithelium (Young et al., 1999; refer to D'Archivio et al., 2007, Table 1, for a review of the bioavailability of different types of polyphenols and foods). Once

absorbed, the compounds are widely distributed throughout the body (see Lewandowska, Szewczyk, Hrabec, Janecka, & Gorlach, 2013, Figure 1, for a pharmacokinetic schematic of polyphenols). Common metabolites of polyphenols, such as microbiota-derived urolithins, are also circulated throughout the body and have relatively strong antioxidant and anti-inflammatory properties (Bialonska, Kasimsetty, Khan, & Ferreira, 2009; Larrosa et al., 2010).

Despite initial uncertainty regarding the capacity of polyphenols to cross the blood-brain barrier, it is now clear that a variety of polyphenols and their metabolites reach the brain (Datla, Christidou, Widmer, Rooprai, & Dexter, 2001; El Mohsen et al., 2002; Youdim et al., 2003; Y.-J. Zhang et al., 2011). Furthermore, they have been shown to accumulate in concentrations that are sufficiently high to confer neurological benefits (Spencer, 2010; Williams, Spencer, & Rice-Evans, 2004), such as reduced pathology and improved learning and memory ability (Andres-Lacueva et al., 2005; J. Wang et al., 2012). Even if they did not directly modify the central nervous system (CNS), polyphenols could exert their benefits by altering signaling pathways from peripheral organs to the CNS (thus improving cerebral blood flow) and by influencing influx and efflux mechanisms at the blood-brain barrier (Schaffer & Halliwell, 2012).

Mechanisms of Action

Oxidative Stress

Polyphenols have long been known for their antioxidant properties. Like vitamin C, vitamin E, and carotenoids, polyphenols are reducing agents, and protect bodily tissues from oxidative stress (Scalbert & Williamson, 2000). Oxidative stress is a deleterious

state that results from an imbalance in the reactive intermediate forms of O_2, collectively known as reactive oxygen species (ROS; e.g., free radicals and peroxides). Although maintaining a certain level of ROS is crucial to biological systems, an excess, such as that caused by various disease states, can lead to cellular pathology and death (Evans & Cooke, 2004). Unfortunately, the brain has relatively low levels of endogenous antioxidant enzymes and is typically unable counteract these imbalances (Rossi, Mazzitelli, Arciello, Capo, & Rotilio, 2008).

Antioxidants, such as polyphenols, work by trapping and scavenging free radicals (atoms or molecules with a missing electron). Providing an electron to these radicals prevents them from going on to pilfer electrons from other atoms, which could otherwise lead to the oxidation and damage of membrane lipids, proteins, enzymes, carbohydrates, DNA, and RNA (Bandyopadhyay, Das, & Banerjee, 1999). Antioxidants also provide stability to peroxides (unstable compounds with an oxygen-oxygen chemical bond) that split into reactive radicals (Marcus et al., 1998). Furthermore, the rapid donation of a hydrogen atom to radicals interferes with the lipid oxidation process. This antioxidant capacity to inhibit low-density lipoprotein oxidation reduces the accumulation of arterial cholesterol deposits, thereby reducing atherosclerosis (Ismail, Sestili, & Akhtar, 2012; Serafini, Laranjinha, Almeida, & Maiani, 2000). The anti-atherogenic characteristics of polyphenols are also due to their ability to upregulate other antioxidant factors (Khateeb, Gantman, Kreitenberg, Aviram, & Fuhrman, 2010). A similar mechanism (i.e., reduction of ROS) is likely partially responsible for the anti-carcinogenic effects of polyphenols (Ismail et al., 2012).

Although there are many sources of antioxidants in our diets, polyphenols are the most abundant (Scalbert & Williamson, 2000). However, the antioxidant characteristics greatly depend on the specific type of polyphenol (i.e., its chemical structure). For example, flavonoids are more potent antioxidants than other polyphenol classes because of their unique structural elements (Bravo, 1998). They also prevent reactions that would lead to increased levels of ROS (Fuhrman, Lavy, & Aviram, 1995). The antioxidant qualities of polyphenols depend on their rate of absorption, with certain subclasses exhibiting greater effects because they are less soluble and get digested slowly, thus remaining in the digestive tract longer and prolonging their antioxidant activity (Hagerman et al., 1998). Although only a small portion of polyphenols from food are actually absorbed and digested, it has been shown that even very low levels are sufficient to provide antioxidant effects (Serafini, Ghiselli, & Ferro-Luzzi, 1996).

Inflammation

Although the beneficial effects of polyphenols have long been attributed to their ability to reduce oxidative stress, recent attention has shifted to their anti-inflammatory properties. Inflammation is a feature of the complex biological response to noxious stimuli. It can be acute or chronic, and involves a cascade of events that include the body's vascular and immune systems (Schauss, 2013). At the first sign of an injury or infection, pattern recognition receptors on cells release inflammatory mediators that dilate blood vessels and signal the migration of leukocytes (i.e., white blood cells) to the site of injury. Leukocytes play a role in the initiation and maintenance of the inflammatory response, and some of them act as phagocytes, removing cellular debris.

The vasodilation increases blood flow to the area, up-regulating plasma fluid that contains important proteins.

Although the inflammatory response is initially adaptive and promotes healing, its persistence can be destructive and is implemented in many disease processes (e.g., cancer, neurodegenerative diseases, diabetes). The acute phase of the response lasts minutes to hours, until the area of injury is returned to homeostasis. In chronic (or systemic) inflammation, the acute phase persists for weeks, days, or even years, resulting in the increased production of ROS (and thus oxidative stress), enzymes, growth factors, and cytokines that contribute to cell damage and death (Schauss, 2013).

One particular mechanism that propagates the chronic inflammatory response is via a protein complex responsible for DNA transcription, named NF-$_K$B. ROS and other harmful stimuli activate NF-$_K$B, which can rapidly alter gene expression and enhance the immune response (by way of T-cell up-regulation; Gilmore, 2006). Thus, a maladaptive cycle ensues, whereby ROS increase the inflammatory response that, in turn, increases levels of ROS. This leads to the continuous activation of NF-$_K$B and the chronic, deleterious immune/inflammatory response.

A growing body of evidence has demonstrated the effectiveness of polyphenols in reducing the inflammatory process involved in various disease states, although the mechanisms by which it accomplishes this are still largely unknown. One of the main ways polyphenols reduce chronic inflammation is likely by down-regulating the expression of pro-inflammatory biomarkers (e.g., NF-$_K$B) that maintain the immune response (Biesalski, 2007; Gonzalez et al., 2011). For example, one study showed that a variety of polyphenol-containing plant extracts modulate NF-$_K$B and attenuate disease-

related activity (Paur, Austenaa, & Blomhoff, 2008). Another study found that the cycle of ROS-inflammation could be blunted by the polyphenols in turmeric and red wine (Rahman, Biswas, & Kirkham, 2006). Furthermore, it is likely that the antioxidant properties of polyphenols can reduce inflammation via a decrease in ROS and oxidative stress, suggesting that their antioxidant and anti-inflammatory properties are intimately linked.

Apoptosis

Apoptosis is a genetically controlled process of programmed cell death that is essential for proper development and continued homeostasis throughout an organism's life. However, the malfunction of this process (i.e., too much or too little apoptosis) is involved in a range of pathologies, from degenerative diseases to cancer. Polyphenols modulate apoptosis, which adds to their utility as therapeutic agents (Giovannini & Masella, 2012). Although generally touted for their anti-apoptotic qualities that are largely associated with their antioxidant properties (Chao, Hou, Chao, Weng, & Ho, 2009; Chen et al., 2012; Kairisalo et al., 2011), polyphenols can also induce apoptosis. Whether they act as anti-apoptotic or pro-apoptotic agents depends on a variety of factors, such as the concentration, disease type or stage, and cell system (Giovannini & Masella, 2012; Loo, 2003).

The pro-apoptotic qualities of polyphenols make them chemopreventive, and numerous studies have found reductions in cancer cell proliferation and tumor growth following polyphenol administration (N. Khan et al., 2007; Koyama et al., 2010; Seeram et al., 2005). Although the specifics are largely unknown, several potential mechanisms

have been identified to explain these properties. Cancer cells, especially the aggressive, invasive types, rely on consistent amounts of ROS (particularly hydrogen peroxide) and are sensitive to changes in ROS levels (Loo, 2003). Since conditions of moderate oxidative stress increase cancer cell survival potential and proliferation (Arora-Kuruganti, Lucchesi, & Wurster, 1999; Del Bello, Paolicchi, Comporti, Pompella, & Maellaro, 1999), the antioxidant properties of polyphenols may reduce ROS levels to a point that triggers apoptosis (Seeram et al., 2005). In contrast, polyphenols have paradoxically been shown to selectively increase oxidative stress in cancer cells while sparing healthy normal cells (Babich, Pinsky, Muskin, & Zuckerbraun, 2006; Cheng et al., 2010; Feng et al., 2007). Part of these seemingly paradoxical properties may be due to a difference between the chemical and biological definitions of an "antioxidant" (Forman & Ursini, 2011). Specifically, the commonly used biological definition is relatively broad and refers to any process that protects against oxidative stress, regardless of the mechanism.

Neuroplasticity

Different types of fruits and teas have been assessed for their utility in promoting neuroplasticity (i.e., synaptic and structural modifications in the brain). Short-term blueberry supplementation, for example, has been shown to increase different parameters of hippocampal neuronal plasticity in aged rats (Casadesus et al., 2004). A grape polyphenol preparation comprising grape seed extract, Concord purple grape juice extract, and resveratrol also showed promise, rescuing the long-term potentiation (LTP; i.e., the activity-dependent increase in synaptic efficacy) deficits found in a diet-induced animal model of metabolic syndrome (J. Wang et al., 2013). Additionally, one

polyphenol-rich component in green tea (i.e., EGCG) has led to enhanced levels of LTP, both in hippocampal slices from normal mice and in a Down's syndrome mouse model (Xie, Ramakrishna, Wieraszko, & Hwang, 2008). LTP was similarly promoted following administration of an extract from a traditional Chinese herb (i.e., *Polygonum multiflorum*; T. Wang et al., 2011).

Curcumin, a polyphenol-laden spice found in turmeric, was shown to incorporate into neural stem cells and induce neurogenesis (i.e., the birth of new neurons) in the dentate gyrus of the hippocampus (S. Kim et al., 2008; Tiwari et al., 2013). Curcumin also has neuroprotective effects that are likely mediated by its ability to increase brain-derived neurotrophic factor (BDNF), a growth factor involved in initiating several neuroplastic processes (R. Wang et al., 2008; R. Wang et al., 2010). For example, it has protected against the deleterious effects of traumatic brain injury on markers of neuroplasticity (Wu, Ying, & Gomez-Pinilla, 2006) and prevented a stress-induced decrease in BDNF (Xu et al., 2006), as well as increased hippocampal neurogenesis in this stress-induced model (Xu et al., 2007). It is thought that this polyphenol-induced increase in BDNF is one of the mechanisms behind its antidepressant properties (Z. Huang et al., 2011); the other proposed mechanism being increased serotonin and dopamine through the inhibition of monoamine oxidase enzymes (Kulkarni, Dhir, & Akula, 2009; Kulkarni, Bhutani, & Bishnoi, 2008).

Hemodynamic Effects

Several recent studies have suggested that polyphenols have an effect on hemodynamic forces (i.e., the circulation of blood flow). For example, both pomegranate

juice and supplements (i.e., POMx) reduce platelet activation (Mattiello, Trifirò, Jotti, & Pulcinelli, 2009), making blood less likely to clot. Many authors have attributed the resulting increased blood flow as one of the main mechanisms by which polyphenols (especially from pomegranates) confer their cardiovascular health benefits (Cordier & Steenkamp, 2012; Phang, Lazarus, Wood, & Garg, 2011; Stoclet et al., 2004). By preventing or reducing the cerebrovascular compromise that results from atherosclerosis, hypertension, diabetes, and hyperlipidemia, polyphenol-mediated hemodynamic changes would likely reduce rates of numerous adverse events, such as stroke (Ghosh & Scheepens, 2009).

Hormesis

As aforementioned, some types of polyphenols are toxic to predators (e.g., leaf-eating insects) and pathogens and therefore protect plants from harm (Son, Camandola, & Mattson, 2008). This may be the reason why polyphenols tend to be concentrated in vulnerable areas of plants, such as their roots, leaves, and the rind or skin of their fruit (Mattson, 2008a; Mattson & Cheng, 2006). One potential mechanism for the benefits of polyphenols that has been gaining support in recent years is the possibility that human ingestion of polyphenols may initiate a process of increased energy demand, mild level of free radical production, and ion fluxes that "exercises" the cellular stress response and improves its ability to defend against subsequent stressors (Son et al., 2008). This concept is known as "hormesis," or pre-conditioning, and has been postulated to also mediate the health benefits of caloric restriction/intermittent fasting (Mattson, 2008a) and

subtoxic radiation exposure (i.e., "radiation hormesis"; Gori & Münzel, 2011), among other usually harmful agents (Rattan, 2008).

The process involves a biphasic dose-response relationship (i.e., has a J-shaped or U-shaped curve), meaning there is a dose range where polyphenols may have hormetic properties (Chirumbolo, 2011; Mattson, 2008b; Son et al., 2008). Mechanisms of action differ based on polyphenol type and cell variety, but involves the regulation of transcription factors, signaling kinases, and protein expression in an adaptive fashion (Mattson & Cheng, 2006; Mattson, Son, & Camandola, 2007). A related concept is the "xenohormesis hypothesis," which proposes that our cells can "sense" the potential impending stress responses from food sources (i.e., the accumulation of polyphenols) and trigger the hormetic response (Baur & Sinclair, 2006).

Pomegranates

Pomegranates have been consumed since the beginning of recorded history, being seen as a symbol of divine femininity and fertility (Lansky, Shubert, & Neeman, 2000). They were extolled by the Greeks, Egyptians, Babylonians, Jews, Persians, and Chinese for their mystical and medicinal properties (Lansky et al., 2000). It has been documented that many cultures have used the fruit as a treatment for leprosy (Singh, Sharma, & Khare, 1980), snake bites (Jain & Puri, 1984), intestinal worms (Naqvi, Khan, & Vohora, 1991; Wren, 1988), assorted gynecological issues (Singh et al., 1980), burns (Siang, 1983), and diarrhea (Boukef, Souissi, & Balansard, 1982), among others. Although many of the previous notions and applications of the pomegranate fruit are no longer popular,

the full extent of health-promoting qualities of pomegranates is just beginning to be discovered.

Although there are numerous foods that contain large amounts of polyphenols, pomegranates have a particularly large amount and variety of phenol compounds (Gil et al., 2000; Seeram et al., 2008). For example, they contain punicalagins, anthocyanins, and ellagic acid (of the phenolic acids class), as well as various types of tannins (of the flavonoids class). It has also been suggested that the effects of pomegranate juice may be better attributed to the metabolic by-products of its polyphenols by colonic microflora (microbes in the gut), rather than just to the polyphenols themselves (Cerdá, Espín, Parra, Martínez, & Tomás-Barberán, 2004). Furthermore, the effects of individual phenols and their metabolites may be enhanced when combined with other phenols, leading to a synergistic effect (Seeram et al., 2005). Evidence also suggests that the whole fruit (with rind and husk included) is better than just juice from the arils (Gil et al., 2000). This is how commercial pomegranate juice and supplements of the Wonderful variety, such as is being utilized in the present study, are made.

As aforementioned, the beneficial effects of pomegranate juice have been well established for dozens of different disease states (see Chapter 1). Pomegranate polyphenols (PPs) are known to be potent antioxidants and anti-inflammatory agents, and it is believed that these are just a couple of the mechanisms by which they confer their health benefits (Gil et al., 2000; Ismail et al., 2012; see above for a discussion regarding other mechanisms). These biological benefits ultimately lead to improvements in cognitive functioning, as has been demonstrated in a number of recent animal studies. For example, one study used pomegranate flowers to improve learning and memory

performance and decrease oxidative stress in diabetic rats (Cambay, Baydas, Tuzcu, & Bal, 2011). Other studies have assessed the effects of PPs on memory and Alzheimer's disease (AD)-like pathology. In a study using transgenic AD (APPsw/TG2576) mice, it was found that mice fed a diet containing 4% pomegranate extract for over one year had improved learning, memory, and locomotor functioning, and decreased anxiety levels, as compared with transgenic AD mice on a normal diet (Subash et al., 2014). Another study used pomegranate seed extract to reduce retention deficits in aged mice (Kumar, Maheshwari, & Singh, 2009).

As previously noted, Dr. Hartman has experience studying the effects of PPs on behavior and cognition. For example, his study was the first to demonstrate the effectiveness of PPs in a transgenic mouse model of AD, where mice that were administered PPs had half the plaque load and showed improved learning abilities compared with transgenic controls (Hartman et al., 2006). His team also studied the effects of PPs on behavior in mice shortly after exposure to a low dose of proton radiation. Although no learning or memory differences were found (likely due to the minimal short-term effects of a low dose of proton radiation), the group administered PPs showed decreased depression-like behavior and improved balance and coordination (Dulcich & Hartman, 2013). It is important to note that there were greater effects on male mice than on female mice, possibly because polyphenols exhibit phytoestrogen activity (Cos et al., 2003). A recent pilot study Dr. Hartman's team conducted in a clinical population undergoing heart surgery showed that PPs can be used to prevent memory retention deficits commonly seen following this type of procedure (Ropacki et al., 2013). Another lab also recently conducted a pilot study on the effects of PPs on

cognition. Their findings showed that, after 4 weeks of administering PPs to a group of older adults with age-associated memory complaints, subjects who received PPs showed a significant improvement on a verbal memory test and had increased fMRI activity during verbal and visual memory tasks as compared with placebo controls (Bookheimer et al., 2013).

Stroke

Stroke is a cerebrovascular accident that results in a disruption of blood flow to the brain and subsequent neurological dysfunction. This disruption can occur in the form of a reduction or complete blockage of blood flow (i.e., ischemia), or an excess of blood flow (i.e., hemorrhage). Because neurons have a high metabolic rate compared with other types of cells, they are particularly susceptible to drastic changes in the level of oxygen and glucose (Attwell & Laughlin, 2001; Laughlin, van Steveninck, & Anderson, 1998). The hypo-perfusion (i.e., decreased blood flow) and nutrient deficiencies often lead to a cascade of events that result in neuronal damage and death in the immediate area and in the penumbra (i.e., area around the lesion that survives the infarct). First, there is a change in membrane potentials and ion concentrations (Martin, Lloyd, & Cowan, 1994). Specifically, the decrease in glucose causes the sodium-potassium pump to stop working due to a lack of energy availability, leading to an excess of sodium influx. As the membrane potential falls toward 0 millivolts the extracellular concentration of excitatory amino acid neurotransmitters, such as glutamate, increase to toxic levels. In a process termed “excitotoxicity” (Olney, 1969), this pathologically high glutamate concentration leads to increased gene transcription and neuronal over-activation that initiates the

apoptotic response. Neuronal apoptosis has been shown to even occur in mild ischemic events (Du et al., 1996).

Many other mechanisms also contribute to the behavioral and cognitive changes commonly observed after stroke. For example, the creation of ROS results from the hemolytic disruption of covalent bonds after ischemic reperfusion and by the release of transition metal ions (Bandyopadhyay et al., 1999). These go on to cause oxidative stress that can result in damage to proteins, lipid membranes, and DNA. The increased ROS levels, activation of intracellular second-messenger systems (from cellular over-activation), presence of cellular debris from necrosis brought about by excitotoxicity and apoptosis, and the hypoxic event itself can lead to the activation of pro-inflammatory transcription factors (i.e., NF-$_K$B) that maintain chronic inflammation (Dirnagl, Iadecola, & Moskowitz, 1999; Sánchez-Moreno et al., 2004). The inflammatory response, although most prominent approximately 5 to 7 days post event, can persist for months (Emsley et al., 2003). As mentioned above, this chronic inflammation leads to increased oxidative stress and apoptosis and continues the pathological cycle.

Cognitive and Psychological Effects

Stroke survivors typically experience numerous cognitive and neurological changes. Although the specific decrements depend largely on the location and size of cerebral infarction (Crafton, Mark, & Cramer, 2003; Ferro, 2001; Hillis et al., 2004), and there is a high degree of variability across individuals (Cramer, 2008a), certain patterns of behavioral deficits are common. In one study, over 70% of patients demonstrated slowed information processing, and more than 40% had impairments in

visuospatial/constructive skills, memory, language functions, and arithmetic ability (Hochstenbach, Mulder, van Limbeek, Donders, & Schoonderwaldt, 1998). Other studies have shown similar impairments in memory, language, attention, and orientation (Tatemichi et al., 1994), as well as deficits in higher-order cognitive abilities, such as abstract thinking, judgment, and comprehension (Galski, Bruno, Zorowitz, & Walker, 1993). Additional common deficits include anosognosia (i.e., lack of awareness of one's impairments), apraxia (i.e., inability to perform learned movements), hemispatial neglect, and hemiparesis (i.e., contralateral physical weakness; Hier, Mondlock, & Caplan, 1983). Dysfunction can also occur in remote brain areas (termed "diaschisis") that relied on connection with the now-damaged region (Y. Kim et al., 2005).

In addition to cognitive effects, mood symptoms are quite common following stroke. One study showed that 40% of stroke survivors developed mild depressive symptoms and 12% developed moderate to severe symptoms, which is much higher than national averages (Nys et al., 2005; see Hackett, Yapa, Parag, & Anderson, 2005) for a systematic review). The severity of depression was strongly correlated with the degree of cognitive impairment, functional impairment, and lesion volume, while having no correlation with lesion location or demographic variables in that study. Moderate to severe depression was closely tied to language, memory, and visual-perceptual impairments. A follow up study showed that unilateral neglect was the greatest risk factor for depressive symptoms after stroke (Nys et al., 2006). Cognitive impairment and functional dependence also predicted a reduction in quality of life. Rates of anxiety disorders in this population are similar to rates of depression and interfere substantially with recovery (Åström, 1996).

Time Course

Many neuroplastic changes occur after a stroke, and some authors have broken the post-stroke period into three "epochs" based on common changes the brain undergoes at different time intervals (Cramer, 2008a). The acute injury phase (i.e., initial hours to days after stroke) is referred to as the first epoch, when edema, inflammation, altered metabolism, and other changes are typically at their greatest. The second epoch lasts for days to a few months post-stroke, and is the time period when most spontaneous recovery occurs. During this period, the non-affected neurons in the area around the infarct send new branches toward the lesion site in an effort to re-organize connections and rescue function (C. Brown, Aminoltejari, Erb, Winship & Murphy, 2009). Increases in BDNF (J. Chen et al., 2005), neurogenesis (Zhang, Zhang & Chopp, 2008), the formation of new synapses (Warraich & Kleim, 2010), and many other molecular and structural changes (Cramer, 2008a) are ongoing during this epoch. Improvements in cognitive abilities typically follow a similar time course as the neuroplastic changes, with most cognitive and adaptive gains made in the first few months post-stroke (Jørgensen et al., 1995; Kwakkel, Kollen, & Twisk, 2006). However, it is important to understand that the rate and extent of functional recovery varies somewhat based on the particular neurological domain (Cramer, Koroshetz, & Finklestein, 2007).

The third epoch typically starts weeks to months after infarction, when neuroplastic changes tend to plateau and smaller, slower improvements are made. Although most of the neurological repair has already taken place, improvements in cognitive and adaptive functioning may continue for years. However, despite the amount of spontaneous recovery and subsequent gains that are typical following stroke, most

individuals have long-lasting cognitive, emotional, and adaptive problems (M. Patel, Coshall, Rudd, & Wolfe, 2003). For example, studies have shown that as few as 15% of stroke survivors return to their cognitive baseline one year post stroke (Desmond, Moroney, Sano, & Stern, 1996; Hofgren, Björkdahl, Esbjörnsson, & Stibrant-Sunnerhagen, 2007), with similarly low rates of gainful employment. Regarding the time course of depression, rates decrease after the first few months post stroke, but then tend to increase within a couple years, mainly due to difficulties with activities of daily living, cognitive limitations, and decreased social connection (Åström, Adolfsson, & Asplund, 1993). Anxiety symptoms tend to remain relatively stable years after stroke (Åström, 1996).

Neuropsychological Testing

There are a variety of measures that are used clinically to assess cognitive and psychological functioning following stroke. Unfortunately, few studies of stroke include any formal cognitive assessment, and many of those that do only include the Mini-Mental State Examination (MMSE). Although measures like the MMSE can be valuable tools, some suggest that tests that provide domain-specific scores, rather than only a global index that lacks sensitivity, may be a better approach (Mysiw, Beegan, & Gatens, 1989). This was the rationale for selecting the many domain-specific tests used in the present study (see Chapter 3 for a description of each test used).

Even brief neuropsychological batteries can provide valuable diagnostic and prognostic information to assist with treatment planning (Larson et al., 2003; Stewart, Gale, & Diamond, 2002), and they have recently been used to construct a cognitive

profile and predict recovery following stroke. One recent study using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998; the primary outcome measure used in the present study) in stroke inpatients showed that the indices (i.e., the domain-specific scores) significantly predicted cognitive ability up to 6 months after testing (Larson et al., 2003). This indicates the utility of neuropsychological measures even in the acute post-stroke phase (i.e., the first epoch), before significant cognitive recovery has begun. A follow up study demonstrated the ability of the RBANS to predict cognitive ability one year after subjects' inpatient stay, with individual indices predicting instrumental activities of daily living (Larson, Kirschner, Bode, Heinemann, & Goodman, 2005).

Current Treatments

There are currently several treatments that are either in use or in the process of being evaluated for stroke survivors, although their effectiveness and practicality are mixed. One widely available treatment used to degrade arterial thrombi (typically blood clots that occlude arteries) is tissue plasminogen activator (tPA), although it has a very brief administration window with many contraindications (Katzan et al., 2004). Research suggests that co-administering an anti-inflammatory agent (potentially PPs) with tPA reduces the probability of the drug leaking across the blood-brain barrier, where it is neurotoxic (L. Zhang et al., 2003). Another promising clinical treatment is induced moderate hypothermia, which is believed to decrease the generation of ROS and attenuate neuronal cell death (Gluckman et al., 2005; Shankaran et al., 2005). However, this technique has mainly been demonstrated effective in animal models of neonatal

ischemia/hypoxia, and is still in clinical trials (van der Worp, Macleod, & Kollmar, 2010).

Another potential avenue for treating stroke is via anti-inflammatory interventions (Perera et al., 2006). Treatments are typically aimed at down-regulating immune cells and inhibiting enzymes responsible for generating toxic mediators. However, it is unlikely that a specific inhibitor of a certain site will have a detectable effect, since the inflammatory response is such a complex, overlapping process (del Zoppo, Becker, & Hallenbeck, 2001; Hallenbeck & Frerichs, 1993). Other interventions have focused on drugs that reduce ROS levels. For example, a free radical-trapping agent named "NXY-059" has shown some effectiveness in treating ischemic stroke (Lees et al., 2006).

There are many other prospective interventions (e.g., use of growth factors, cell-based therapies, electromagnetic stimulation) being tested to improve functional restoration after stroke (see Cramer, 2008b for a review of several therapies), but the majority of them will likely take years before their efficacy is demonstrated, will be very expensive, will have numerous side effects, and may never get through clinical trials.

Polyphenols as a Treatment

Polyphenols, particularly flavonoids, have vasoprotective and antithrombotic qualities that can aid in recovery following stroke (Bravo, 1998; Panickar & Anderson, 2011). As aforementioned, they are also potent antioxidants, reduce inflammation, protect against excitotoxicity, regulate apoptosis, alter hemodynamics, and have metal chelating properties. Furthermore, it is possible that there is relatively large brain bioavailability of polyphenols after stroke, since ischemia can compromise the blood-brain barrier (Borlongan et al., 2004; Brown & Davis, 2002; Latour, Kang, Ezzeddine,

Chalela, & Warach, 2004; Sandoval & Witt, 2008). Additionally, by treating hypertension (Aviram & Dornfeld, 2001), atherosclerosis (de Nigris et al., 2005), diabetes (T. Huang et al., 2005), hyperlipidemia (Aviram et al., 2000), and coronary heart disease (Sumner et al., 2005), polyphenols have a role not only in improving recovery following stroke, but preventing the event altogether.

Although no known study has assessed the effects of PPs following stroke in a clinical population, a few studies have examined the effects in animal models. For example, one study demonstrated that a maternal diet of pomegranate juice protects newborn mouse pups from a prolonged ischemic injury (Loren et al., 2005). The juice not only diminished caspase-3 activation (a protein involved in apoptosis) by 84%, but also decreased tissue loss by over 50%. A follow up study replicated their previous findings, showing that pomegranate juice administered to mothers is neuroprotective to their offspring (West et al., 2007). Other studies have shown that pomegranate extract prevents DNA damage and improves memory in rats subjected to cerebral ischemia (Ahmed, El Morsy, & Ahmed, 2014; Sarkaki & Rezaiei, 2013). The protective influence of consumption of PPs against stroke likely also applies to humans, since increased fruit and vegetable intake in general is associated with decreased risk of ischemic stroke in both men and women (Gillman et al., 1995; Joshipura et al., 1999; Keli, Hertog, Feskens, & Kromhout, 1996).

The ability of PPs to tackle chronic inflammation, oxidative stress, and other deleterious processes in a global, multi-approach manner makes them an exciting prospect for treating stroke. Furthermore, they are very inexpensive, have no side effects in the majority of the population, have few contraindications, and have been shown to be

effective in numerous disease states.

CHAPTER THREE

METHODS

Subjects

Participants were 16 adults who experienced an ischemic stroke and were admitted to the rehabilitation program at Loma Linda University East Campus Hospital (LLUECH) for inpatient care. Half of the participants were randomly assigned to receive a PP supplement (n = 8) and the other half received a placebo (n = 8). The treatment assignment was predetermined and known only by the pharmacy coordinator (Desiree Wallace, Pharm.D., R.Ph), who was not directly involved in patient care. All other clinical staff remained blind to the treatment group, as were the patients. Recruitment extended from June 2015 through March 2016. During that 10-month interval, 183 patients were admitted to LLUECH due to stroke. Each admit was screened for potential study eligibility, and the resident physician (i.e., Paolo Jorge, M.D.) involved in the screening/consenting process met with 48 patients to further assess their eligibility. Twenty two patients (12% of total admits) met all inclusion criteria (see criteria below). Of these 22 patients, 6 declined to participate, with the main stated reason being that they did not want to take any additional medication. The remaining 16 patients (9% of total admits) agreed to participate and signed informed consent documentation. Two participants (1 in each treatment group) did not complete post-treatment testing (see Subject Inclusion Approach subsection below for more details), and were thus excluded from the final analyses. Figure 5 is a flow diagram of the screening/recruitment process and distribution across the two study arms.

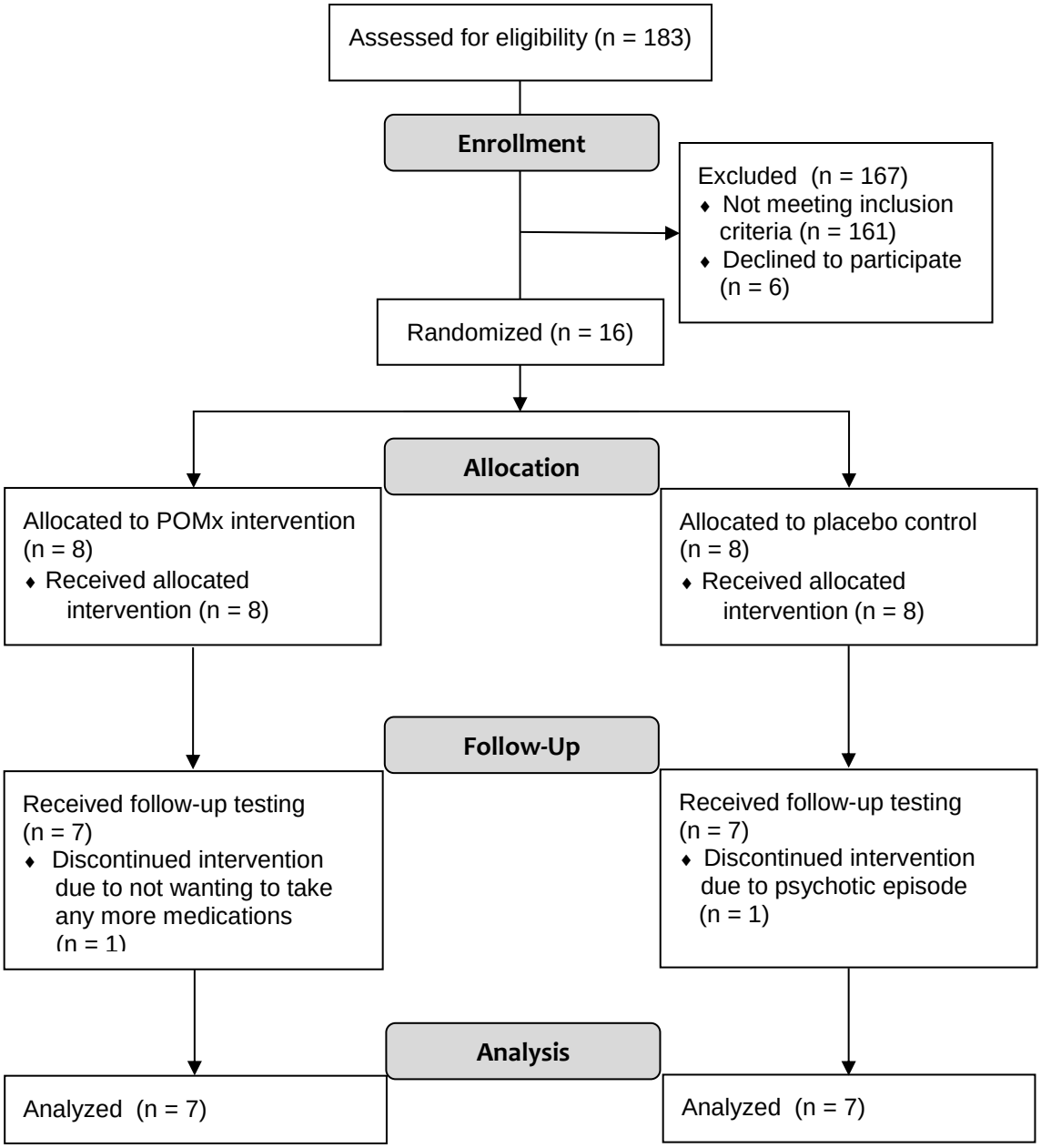

Figure 5. Flow diagram of subject progress through study phases. Figure adapted from CONSORT; http://www.consort-statement.org/consort-statement/flow-diagram

Randomization

Random assignment to treatment group is the gold standard for allocation methodology, since it eliminates sampling bias and allows for causal inferences to be made (Little et al., 2012). We used a form of restricted randomization called "permuted-block randomization" to balance group sizes given the relatively small sample size. A block size of 4 and allocation ratio of 1:1 was chosen to ensure balanced groups. Thus, every group of 4 subjects had an equal number of individuals receiving pomegranate or placebo supplements to ensure that the treatment groups would be roughly equal if we were unable to attain the desired sample size (originally set at 28). As aforementioned, our allocation concealment method (i.e., our procedure for ensuring that treatment allocation was kept masked) was a technique known as "pharmacy-controlled randomization," meaning that only the lead pharmacist knew the specific treatment each subject received.

Inclusion/Exclusion Criteria

Patients were included in the sample if they (1) were admitted to LLUECH immediately following medical stabilization for an ischemic stroke, (2) were between 18-89 years old, (3) spoke English fluently, (4) were not globally aphasic, (5) were not currently taking warfarin (Coumadin), (6) had not suffered an intracerebral hemorrhage in the past 6 months, (7) had not undergone neurosurgery in the past month, (8) were not pregnant, (9) had at least 6 years of education, (10) had no history of traumatic brain injury, (11) had no history of neurologic condition with known cognitive impact (e.g., dementia), (12) did not have active renal or liver disease, (13) had no history of allergy to

pomegranate products, (14) were less than one month post-stroke, (15) had an estimated length of hospital stay that exceeded the study timeline, and (16) attained a score of at least 18/30 on the Mini-Mental State Examination – Second Edition (MMSE-2). See Appendix D for the checklist the resident physician used to assess whether patients met the inclusion criteria.

Procedures

Dr. Jorge, under the supervision of an attending physician (Mary Kim, M.D.) screened newly admitted LLUECH patients for individuals who met study criteria. The physician met with patients who appeared to be potential candidates to provide information regarding the study, ensure they met inclusion criteria (including administering the MMSE-2), review informed consent documentation, and ask if they would like to participate. The physician then filed the signed informed consent documents (see Appendix A, B, and C), provided authorization to nursing and pharmacy, and documented the interaction in the patient's medical record (see Appendix E and F for physician work flow and initial patient visit script).

A trained psychology doctoral student (Jeff Murray) under the supervision of a board-certified neuropsychologist (Travis Fogel, Ph.D., ABPP-CN) administered a brief neuropsychological testing battery to newly consented patients to establish pretreatment baseline cognitive abilities (see Appendix G for neuropsychology procedure). Nursing staff subsequently administered pomegranate or placebo capsules twice per day (9am and 9pm) for the following week, for a total of 14 doses (see Appendix H for the nursing staff information sheet). A post-treatment neuropsychological evaluation was conducted at the

end of the treatment week (refer to Table 1 for a depiction of the timeline and sequence of procedures). Onsite healthcare staff monitored treatment compliance and adverse events. All procedures were conducted in accordance with the Loma Linda University Institutional Review Board (IRB) guidelines and approval.

Table 1. *Timeline and sequence of procedures.*

Day 1	Days 2-8	Day 9
Informed Consent Neuropsychological Baseline Testing	Pomegranate Supplements or Placebo Taken Orally Twice Per Day (14 total doses)	Neuropsychological Post-Treatment Testing

Treatment was delivered within the time window for spontaneous recovery (i.e., during the second epoch), which has been referred to as the "golden period" for initiating restorative therapies following stroke (Cramer, 2008a). Regarding the treatment timeline, many studies have administered PPs for a long period of time, as much as 1 year in animal studies and 1.5 months in clinical populations. The typical length of stay for inpatients at LLUECH has traditionally been under three weeks, thus limiting the treatment duration. Although our original plan was to deliver treatment for two weeks, we opted to change to a one-week treatment protocol after 5 months of very low subject recruitment due to the combination of a low census and short hospital stays. We also did this to increase the probability that each participant would receive the same duration of treatment, rather than risk subjects discharging prior to the completion of the treatment protocol.

Prior pomegranate studies have found effects following relatively short treatment protocols. For example, the aforementioned study that administered pomegranate extract after ischemic injury in rats only administered the treatment for two weeks (Sarkaki & Rezaiei, 2013). Additionally, a recent clinical pilot study showed that one POMx pill per day for two weeks decreased oxidative stress (Hayek, Rosenblat, Volkova, Attias, & Mahamid, 2014). Other studies also utilized a two-week protocol (at a single dose per day) and found positive effects of pomegranate treatment (Ahmed et al., 2014; Al-Jarallah et al., 2013; Asgary, Keshvari, Sahebkar, Hashemi, & Rafieian-Kopaei, 2013; Asgary et al., 2014). One study showed that even one dose benefited diabetic patients (Banihani et al., 2014). Due to these positive findings following brief administration periods, we were optimistic that the protocol chosen for the current study (i.e., a total of 14 doses) would be sufficient to observe effects.

Setting

The rehabilitation program at LLUECH is a CARF (Commission on Accreditation of Rehabilitation Facilities) accredited Stroke Specialty Program. According to CARF International's website (http://www.carf.org/providerProfile.aspx?cid=14678), a Comprehensive Integrated Inpatient Rehabilitation Program must provide coordinated and integrated medical and rehabilitation services 24 hours a day and endorse the active participation and preferences of the person served throughout the entire program. There must be collaboration with interdisciplinary team members, and individual resource needs and predicted outcomes of the person served must drive the appropriate use of the rehabilitation continuum of services. Patients typically get a number of rehabilitative

services from professionals in diverse disciplines, such as physical therapy, occupational therapy, therapeutic recreation, and speech therapy. They also have daily interactions with medical and nursing staff, frequent meetings with a social worker who coordinates discharge planning, the option to meet with chaplain, and access to specialists as needed (e.g., neuropsychology, psychiatry, ophthalmology). Patients are often transferred to LLUECH following stabilization at Loma Linda University Medical Center, which is recognized as a Certified Stroke Center by The Joint Commission.

Pomegranate Polyphenol Treatment

We administered two POMx or placebo capsules (POM Wonderful, CA, USA) to participants per day, one in the morning and one in the evening. Each POMx capsule contained 1g of a concentrated blend of polyphenols derived from 240mL of pomegranate juice (approximately 375mg punicalagins, 93mg anthocyanins, 29mg ellagic acid, and 100mg of other tannins). We chose to use supplements, as opposed to pomegranate juice, because they do not contain sugar (so we could administer them to diabetic patients), are easier to swallow (since many stroke survivors experience dysphagia), and do not have the tart taste some individuals dislike. Additionally, POMx supplements have been shown to have similar levels of polyphenols as compared with pomegranate juice (Seeram, Zhang, et al., 2008). Administering two capsules per day is approximately the equivalent of two cups of pomegranate juice, and has been shown to be a safe and effective dose in other human studies (Balbir-Gurman et al., 2011; Heber et al., 2007; Paller et al., 2012; Seeram, Zhang, et al., 2008). Furthermore, POMx supplements have Generally Recognized as Safe (GRAS) status by the U.S. Food and

Drug Administration. Placebo capsules did not contain pomegranate ingredients. The nursing staff at LLUECH administered the capsules along with the participant's other medications and documented that each patient took each dose. POM Wonderful provided all placebo capsules used for this study (they were re-purposed from a previous study), and we purchased POMx capsules directly from the company. All staff involved in the study denied any conflicting interest with POM Wonderful, and the company did not provide any financial support for the project. Although many other companies sell pomegranate extract products for much cheaper (see Vitacost.com, as one example), POM Wonderful's products have received the most attention from the scientific community. We administered a questionnaire regarding pre-admittance diet to participants to attain an estimate of polyphenol intake prior to being admitted to the hospital.

Risks and Potential Drug Interactions

As aforementioned (see Chapter 2), recent data suggest that pomegranate products have an effect on hemodynamic forces, likely reducing platelet activation (Mattiello et al., 2009). Although these effects lead to cardiovascular health benefits, the decreased risk of blood clots associated with the inhibition of platelet function could potentially result in an increased risk of bleeding. Although there have been no known publications regarding adverse effects based on decreased platelet function, and there were no adverse events reported from our recent study assessing the effects of POMx in a high-risk cardiac surgery population (Ropacki et al., 2013), we opted to exclude patients who had suffered a hemorrhagic stroke or had undergone neurosurgery in the month prior to

hospital admission from the current study as an added precaution. For similar reasons, we also excluded patients who had an intracerebral hemorrhage in the past 6 months or neurosurgery in the past month.

Some authors have expressed a potential for polyphenol-drug interactions, although only a few case studies have been reported. Specifically, pomegranate products have been alleged to have a modulatory effect on response to warfarin (Coumadin), an anticoagulation drug (Komperda, 2009). One recent review article examined the available literature regarding the potential interaction between warfarin and fruit products and concluded that, although evidence is scarce, clinicians are encouraged to inquire about the consumption of pomegranate juice when determining potential causes of international normalized ratio (INR; a measure of clotting tendency of blood) instability (Norwood, Parke, & Rappa, 2014). Another review article stated that, although pharmacokinetic data from in vitro and animal studies suggest the possibility of pomegranate intake affecting subsets of cytochromes P450 (CYP3A4/CYP2C9; enzymes involved in drug metabolism), current evidence suggests that patients can safely consume pomegranate products along with drugs that are substrates for CYP3A4 and CYP2C9 (Srinivas, 2013). However, due to the potential for an interaction between warfarin and POMx, we excluded patients being administered warfarin from the study.

Neuropsychological Testing

We administered a battery of widely used neuropsychological measures at two time-points (pre- and post-treatment). The measures included paper and pencil types of tests that assessed a breadth of cognitive and psychological domains (refer to Table 2 for

a list of test by domain and administration time-point). Although minor practice effects were expected on post-treatment (i.e., Time 2) performance, each group received the same protocol, so these effects have likely been averaged out. Furthermore, alternate versions of measures were used when available (e.g., MMSE-2 and RBANS) to minimize such effects. Each evaluation took approximately one hour to complete. Although the data reported in the Results section are in their raw form unless otherwise specified, we also compared performance after norming the data based on normative data that were either included in a measure's manual or were commonly used among neuropsychologists to determine if standardization altered the results. Most normative data were age matched and some were also education or gender matched (see Table 3 for the characteristics of the normative data used for each measure). We reported RBANS data as standard scores in the Results section because the 6 RBANS Indexes are not available in raw form since they are composites of subtests (see the Statistical Analysis section below for more details).

Table 2. *List of neuropsychological measure by domain and time-point*.

Neuropsychological Measure	Domain Assessed	Time-point
Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)		1 (Form A) & 2 (Form B)
List Learning	Immediate Memory	
Story Memory	Immediate Memory	
Figure Copy	Visuospatial/Constructional Ability	
Line Orientation	Visuospatial/Constructional Ability	
Picture Naming	Language	
Semantic Fluency	Language	
Digit Span	Attention	
Coding	Attention	
List Recall	Delayed Memory - Verbal	
List Recognition	Delayed Memory - Recognition	
Story Recall	Delayed Memory - Verbal	
Figure Recall	Delayed Memory - Visual	
Mini-Mental State Examination – Second Edition (MMSE-2)	General Orientation and Gross Cognitive Functioning	Prior to consent & time 2
Test of Premorbid Functioning (TOPF)	Estimate of Verbal Intelligence	1 only
Trail Making Test (TMT)		1 & 2
Part A	Processing Speed	
Part B	Set Shifting, Executive Functioning	
Brief Test of Attention (BTA)	Attention/Concentration	1 & 2
Line Bisection Test	Visuo-spatial Neglect	1 & 2
Controlled Oral Word Association Test (COWAT)		
FAS	Verbal Fluency, Executive Functioning	1 & 2
Animals	Semantic Fluency	1 & 2
Pre- & Post-Test Rating	Awareness of Functioning	1 & 2
Beck Depression Inventory – Second Edition (BDI-II)	Depression	1 & 2
State-Trait Anxiety Inventory (STAI)	Current and General Anxiety	1 & 2

Table 3. *Characteristics of normative data.*

Test	Age Matched	Education Matched	Gender Matched	Source
RBANS	✓			Manual
MMSE-2	✓	✓		Manual
TOPF	✓			Manual
TMT	✓	✓ (for those age 55+)		(Tombaugh, 2004)
BTA	✓			Manual
COWAT	✓	✓		(Tombaugh et al., 1999)
BDI-II				Manual
STAI	✓		✓	Manual

Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The RBANS was the primary outcome measure used since it provides both a total scale score and scores for 5 different cognitive domains, is relatively brief (approximately 20 minutes total), has alternate forms, and has been frequently used to assess cognitive ability following stroke. Specifically, the test measures immediate memory (with list learning and story memory), visuospatial/constructional ability (with figure copy and line orientation), language (with picture naming and semantic fluency), attention (with digit span and coding), and delayed memory (with list recall, list recognition, story recall, and figure recall). Scores from all subtests are aggregated into a total composite score. Each subtest was scored according to the manual (Randolph, 2012).

The validity and reliability of the RBANS has been established for various disease states, such as traumatic brain injury (McKay, Casey, Wertheimer, & Fichtenberg, 2007), dementia (Garcia, Leahy, Corradi, & Forchetti, 2008), end-stage liver disease (Mooney et al., 2007), schizophrenia (Wilk et al., 2002), and stroke (Larson et al., 2005; Larson et al., 2003; Wilde, 2006), among other populations (Randolph, Tierney, Mohr, & Chase,

1998). The test has strong psychometric properties that are similar to those of other commonly used neuropsychological measures, with the manual reporting average index reliabilities ranging from .75 (visuospatial/construction) to .93 (total index), and a test-retest coefficient of .84 for the total scale (Randolph, 2012). Other sources found similar reliability, with a test-retest stability coefficient of .77 for the total score in healthy participants (Wilk et al., 2002) and .81 in community dwelling older adults (Duff et al., 2005). Practice effects are minimal (Duff et al., 2005) and alternate-form comparison studies show total scale coefficients of .82 between forms A and B (Randolph, 2012). Intercorrelations among indexes range from .29 to .64. Furthermore, the RBANS has been shown effective in distinguishing lesion location (both right versus left hemisphere and cortical versus sub-cortical) following acute ischemic stroke (Wilde, 2010).

Mini-Mental State Examination – Second Edition (MMSE-2)

The MMSE-2 is a brief (about 10 minutes) screening tool that touches upon orientation to time and place, recall, attention/calculation, naming, repetition, comprehension, reading, writing, and drawing, with all the scores from these domains cumulating to a maximum of 30 points. We administered alternate versions of this test at both testing time-points. Although the first edition of the test is more widely used and has demonstrated acceptable validity in detecting impairment in stroke populations (Agrell & Dehlin, 2000), we chose to use the second edition because it has alternate forms and updated norms.

Test of Premorbid Functioning (TOPF)

The TOPF is a word pronunciation task that provides an estimate of educational achievement/general intellectual abilities. It involves pronouncing words that become increasingly difficult and irregular, so that one would only be able to correctly pronounce the word if they had prior exposure to it or similar words. It has been shown to be less susceptible to brain injury or disease processes than other measures (Green et al., 2008; see the Advanced Clinical Solutions manual), and thus can be conceptualized as an estimate of pre-stroke intelligence. There are 70 items on the measure, although the test is discontinued following 5 consecutive errors. The scorer tallies the total number correct out of the attempted items.

Trail Making Test (TMT)

The TMT is one of the oldest and most widely used neuropsychological tools, and consists of two parts: Part A and Part B. Part A involves drawing lines to connect a series of numbers in ascending order and requires visual scanning, psychomotor speed, attention/working memory, sequential processing, and the ability to maintain mental sets, as well as gross visuo-spatial and psychomotor functioning. It is generally categorized under the processing speed or attention domains. Part B is similar to Part A, but adds an alternating component, where the examinee must switch between a number and a letter, in sequential order. This task requires the same abilities as in Part A, with the addition of the ability to rapidly switch mental set and attend to two thought processes. Part B is typically classified as a task of executive functioning due to the set shifting and divided attention requirements. On both Part A and Part B, the examiner calls attention to errors

on the spot and asks the examinee to correct the error. The examiner notes how many seconds it took the examinee to reach the final number for Part A and Part B (providing two separate variables), as well as how many errors occurred on each part. Time continues even if an error is committed. The task is discontinued if a subject takes longer than 5 minutes. In this situation, a score of "301" is assigned. Fewer total seconds indicates better performance.

Brief Test of Attention (BTA)

For the BTA, the examinee listens to a string of numbers and letters and must mentally tally (without the use of their fingers) how many numbers are in a particular trial. They do this for 10 trials and then are given 10 additional trials with the task of tallying how many letters they hear. The task increases in difficulty as the trials progress, and the entire test takes 5-10 minutes to complete. The scorer adds the number of trials correct from all 20 trials to attain a total score.

Line Bisection Test

The Line Bisection Test consists of 20 horizontal lines of varying length and proximity to the center (i.e., some are closer to the left or right sides of the page). The examinee is asked to place a mark where they think the middle of each line is. The scorer measures the degree of deviation from the center of each line and attains the absolute value of the average percentage of deviation across all 20 lines. The scorer also attains the dominant direction of deviation (i.e., whether the examinee misses more to the left or to the right on average across the 20 lines). The value of the largest deviation is imputed

for any omissions. The test is a measure of sensory-perceptual functioning, specifically assessing hemispatial inattention or neglect, which is common following stroke.

Controlled Oral Word Association Test (COWAT)

The COWAT is another very commonly used neuropsychological test and is a measure of controlled verbal fluency that is divided into two parts: phonemic and semantic fluency. The phonemic fluency task involves the examinee naming as many words that begin with a certain letter of the alphabet as he or she can in 1 minute. There are a few rules (i.e., no proper nouns, no numbers, and no words that have the same meaning and only differ by its suffix) and the task is repeated twice more with different letters each time. The scorer tallies the total acceptable words from all 3 trials into one total score. The number of perseverations or intrusions can also be tallied. The semantic fluency task involves providing the examinee a category prompt. For example, the examiner asks the examinee to name as many animals as he or she can in 1 minute. Both parts are commonly included under the "language" sections of neuropsychological reports, and the phonemic fluency score is often also thought to tap into the executive functioning domain.

Pre- and Post-Test Ratings

The examiner asked examinees to rate their concern regarding their cognitive ability both before and after engaging in testing. They drew a line to denote where they fell on a continuum from "not concerned" to "very concerned," and the scorer coded the mark from 1 to 11, respectively, based on a template. Examinees were also asked to rate

how well they thought they performed on the testing, as well as how well they estimate they would have done if they engaged in the testing prior to their stroke. These items were on a continuum from "extremely poorly" to "extremely well," and were coded using the same scale (i.e., 1 to 11, respectively). The four questions were meant to assess the subject's level of insight, or awareness, regarding his or her difficulties both before and after testing. The rating forms were adapted from forms created by Dr. Kyrstle Barrera and used with her permission.

Beck Depression Inventory – Second Edition (BDI-II)

The BDI-II is a widely used self-report questionnaire of depressive symptoms. The examinee is asked to respond to 21 items by endorsing whether or not they experience symptoms of sadness, pessimism, past failure, loss of pleasure, guilty feelings, punishment feelings, self-dislike, self-criticalness, suicidal thoughts or wishes, crying, agitation, loss of interest, indecisiveness, worthlessness, loss of energy, changes in sleeping pattern, irritability, changes in appetite, concentrating difficulty, tiredness or fatigue, and loss of interest in sex. Examinees can also describe the degree of severity of each symptom, as each item ranges from 0-3. The scorer adds the scores for each item to attain a total score, which is interpreted according to the following guidelines: 0-13 = minimal depression, 14-19 = mild depression, 20-28 = moderate depression, 29-63 = severe depression. The attending physician and supervising neuropsychologist on the team were immediately made aware when a subject endorsed suicidal thoughts or wishes, which occurred for 2 subjects at baseline testing but none at post-treatment testing.

State-Trait Anxiety Inventory (STAI)

The STAI is a self-report inventory of anxiety symptoms. The test consists of two parts: 20 questions that assess anxiety level at the time of the examination (i.e., state) and 20 questions that assess the examinee's general level of anxiety (i.e., trait). Items include feeling at ease, feeling upset, feeling self-confident, feeling confused, feeling like a failure, feeling rested, and having disturbing thoughts, among others. Examinees endorse 1 of 4 options on a likert scale, from "not at all" to "very much so."

Data Collection and Storage

Research data (e.g., informed consent documents and neuropsychological measures) were collected onsite by either a resident physician or psychology doctoral student involved in the study and were physically taken to LLUECH's Department of Neuropsychology, where they were stored in hard copy format and kept in a locked office. They were subsequently transported to the psychology department for long-term storage.

Statistical Analysis

We used *Prism* (version 6.0d for Mac OS X, GraphPad Software Inc.) for all analyses except for the MANOVA, which was conducted with *SPSS* (version 23). We used the following website to attain Cohen's *d*: http://www.uccs.edu/~lbecker/. We corrected for multiple *t*-test comparisons with the Holm-Šídák test (similar to the Bonferroni correction, but slightly less stringent; Holm, 1979; Šidák, 1967). Specifically, this method works by computing *p*-values for each comparison in the experiment,

ranking the values from smallest to largest, and sequentially assessing whether the value is less than alpha (.05) divided by the number of remaining comparisons (see http://www.graphpad.com/guides/prism/6/statistics/ for more information). Only comparisons of interest were analyzed to limit the experiment-wise (i.e., type 1) error probability.

All analyses were two-tailed, and error bars on figures represent ± standard deviation (*SD*) or confidence interval (CI), as specified. A neuropsychology doctoral student (i.e., John Bellone) scored all tests and was blind until after all scoring was completed. We did not perform any interim analyses. We conducted all analyses using a complete case approach (i.e., excluding the 2 subjects without outcome data; see description below), unless otherwise specified. Specific analyses for each aspect of the Results chapter are described below.

Demographics and Stroke Characteristics

Fisher's exact test was used to assess differences in treatment group for categorical demographic and stroke characteristic variables, since this method is particularly appropriate for small sample sizes when the data are in the form of a 2x2 contingency table (e.g., comparing whether there are differences in the number of males or females between the POMx and placebo groups). We used the chi-squared test for categorical data that exceeded a 2x2 contingency table (e.g., assessing differences in the racial breakdown between treatment groups). We used independent samples *t* tests for continuous data.

Baseline Test Results

We evaluated baseline data with independent samples *t*-tests to assess if there were any pre-treatment differences in cognitive or emotional functioning. We then attained an effect size (Cohen's *d*) to assess the magnitude of differences for each parameter.

Pre- and Post-Test Ratings

We evaluated pre- and post-test rating data with independent samples *t*-tests.

Aim 1: Assessing the Impact of PPs on Global Cognitive and/or Emotional Functioning

We used two-way mixed ANOVA to analyze global cognitive scores (i.e., RBANS total scale and MMSE-2) and measures of emotional functioning (i.e., BDI-II and STAI). Treatment group (POMx and placebo) was the between-subjects factor and time (baseline and post-treatment testing) was the within-subjects factor. We were mainly interested in whether there was a significant interaction of treatment and time (i.e., whether the POMx group showed greater improvement from baseline to post-treatment testing than the placebo group). RBANS data were age-normed based on the sample described in the manual (Randolph, 2012), and were analyzed as index scores (also referred to as standard scores), which have a mean of 100 and a standard deviation of 15. Data for the other measures (i.e., MMSE-2, BDI-II, and STAI) were kept as raw scores. We conducted Pearson product-moment correlation analyses between the TOPF and several outcome variables to determine the degree of co-variation. We subsequently

used ANCOVA to assess whether the group difference on the RBANS total scale index score change would be altered when controlling for the TOPF variance.

Aim 2: Assessing for Domain-Specific Response to Treatment

We calculated change scores by subtracting each subject's pre-treatment score from his or her corresponding post-treatment score. Positive scores indicate improved performance and negative scores indicate decline for all but four measures (i.e., TMT, Line Bisection Test, BDI-II, and STAI), which are the reverse. We then used independent samples *t*-tests with these change scores to assess for group differences in change from pre- to post-treatment testing, and also assessed effect size (Cohen's *d*) for each change score. A one-way MANOVA was additionally conducted to determine if there was an effect of treatment on performance across the five RBANS indexes (i.e., whether the most affected cognitive domains were differentially benefited by PP intake). According to the RBANS manual, although it is permissible to interpret subtest scores, the index level is the primary level of interpretation since it has the highest degree of internal consistency and stability (Randolph, 2012). We assessed retention memory by calculating a retention composite change score. We arrived at this composite by creating z scores for each of the three retention change scores (i.e., list, story, and figure retention) based on the sample mean and standard deviation and averaging these z scores.

Subject Inclusion Approach

Eleven subjects (5 in the POMx group and 6 in the placebo group) completed the trial according to our protocol (i.e., 14 total doses). One subject (assigned to the placebo

group) completed a full two-week treatment protocol (i.e., received 28 doses), which was prior to a protocol change from two weeks of treatment to one week due to unanticipated short lengths of stay. Another subject (assigned to the POMx group) was also on track to complete a full two week protocol but was discharged early and thus only received a total of 18 doses. A third subject (assigned to the POMx group) recruited after the protocol change also discharged early and only received 7 doses. Two additional subjects were recruited and completed baseline testing but did not complete follow-up testing. One of those subjects (assigned to the placebo group) voluntarily withdrew from the study reportedly because she "did not want any more chemicals in her body," and the other subject (assigned to the POMx group) experienced auditory hallucinations and a psychiatry consultant recommended the discontinuation of treatment. Notably, this latter subject began experiencing auditory hallucinations several days prior to POMx administration, but the psychiatrist recommended discontinuing it as a precaution since no other medications had recently been added to the patient's regimen. Also of note, a subject that was assigned to the placebo group also experienced hallucinations during his course of treatment.

We followed the guidelines for statistical methods set by the CONSORT (Consolidated Standards of Reporting Trials) Statement (Schulz, Altman, & Moher, 2010; more information available at www.consort-statement.org). The CONSORT Statement (item 12a) suggests that investigators either use an "intention-to-treat" approach or a "complete case" approach. An intention-to-treat approach includes each randomized subject in the final analysis, regardless of whether there were subjects who did not receive or adhere to the allocated treatment or withdrew from the study (the motto

is “once randomized, always analyzed”). Although this method is the best way to fully preserve the benefit of randomization, it may be misleading if there were missing outcomes or non-adherence issues and has been criticized for being overly conservative. The complete case approach only includes subjects who have known outcomes in the final analysis. Since we had a relatively small sample size and one subject from each treatment group missing outcome data, we chose to use a complete case approach and exclude those two subjects from the analyses.

Another option we considered was to follow a strict “per protocol” approach (under the category of “modified intention-to-treat”), whereby the analysis would be restricted to subjects who fulfill the protocol exactly as written (e.g., no deviation in the number of doses received). However, adhering to this criterion would exclude the three subjects who received either more or less than the 14 doses set in the updated protocol, as well as the two subjects who did not complete a post-treatment evaluation. Furthermore, this method has been criticized for compromising the randomization process (Schulz et al., 2010) and is not recommended by the CONSORT Statement. All decisions regarding the subject inclusion approach were made prior to the unmasking of treatment groups to prevent potential bias.

IRB Approval Process

The process of going from project conception to subject recruitment was quite an extensive one. The technical classification of the study was initially unclear, and there was uncertainty as to whether we would need to submit it for “full board” review and meet the additional requirements necessary for that process. In speaking with

administrators from Research Affairs and IRB committee members, we were informed that, since the project was considered a clinical trial, we had to go through the Clinical Trials Center (CTC) before we could submit to the IRB. We had several meetings with CTC staff, and they suggested many changes. Several aspects of the original conceived project (e.g., incorporating blood draw pre- and post-treatment to assess polyphenol metabolites and inflammatory and oxidative stress markers, as well as getting lesion volume data from neurology and including non-stroke groups) were eliminated during this phase for numerous reasons, such as limited funds, lack of support from certain departments, and extra administrative requirements.

Although we originally thought we could provide the supplements to patients ourselves (as was done in Dr. Hartman's previous study), we discovered that since we were working with an inpatient population supplements would be considered "medication," which meant we needed to have physicians submit the medication order, pharmacists dispense the supplements, and nurses administer them to patients. Each of these layers added a level of complexity to the project, and we had to attain the approval of each individual and department that would be involved. This led to further protocol modifications and substantial delays in initiating the study. It would have also added significant expenses, but fortunately everyone was willing to work without compensation (other than eventual authorship), for which we were incredibly grateful. The only exception to this was a minimal pharmacy fee to cover packaging expenses.

After finally receiving almost everyone's approval (the exception was the nursing department, who the CTC said they would follow up with) we submitted the completed protocol to the CTC and received their approval (STAR #: 14109) to submit it to

Research Affairs for IRB review (now 6 months into the process). The only other stipulation from the CTC was that we needed to register with ClinicalTrials.gov (NIH-operated registry of national and international clinical trials), which we did (protocol ID: NCT02442804). Research Affairs confirmed that the protocol would need to go "full board," and we submitted 28 copies of our materials for consideration in the next meeting. After several weeks, we received word of the committee's decision to allow us to re-submit the protocol with several changes (e.g., tightening up the inclusion criteria and updating the informed consent document). We made the requested changes and re-submitted 28 copies in time for the next full board meeting. They informed us that the study was conditionally approved, with the stipulation that we submit a letter of support from the nursing department, which took months to attain. We eventually received and submitted this letter and received final IRB approval (IRB #: 5150122) the beginning of June (8 months into the process). We recruited our first subject the following week.

Advice for Junior Investigators

Despite the many details and set-backs of the project that were unanticipated and, to a large extent, unavoidable, there are many things that would have been helpful to have known at the outset, and several things that are common knowledge but worth re-stating. I would like to pass on the following information to those embarking on a dissertation or other large research project:

- Find out all the IRB requirements by reviewing the university's online information and speaking with administrators. If you are planning a randomized controlled trial then you should familiarize yourself with the guidelines set in the

CONSORT Statement (www.consort-statement.org), and design the study with these guidelines in mind. This process should be started as soon as possible.

- Begin the project by conceiving of the ideal study, one that has a large sample size, several research arms, and everything that journal reviewers would want to see. From there, it is essential to tailor the project with a focus on feasibility, and build in a substantial safety margin to allow for things to go wrong. Although ambition is key to success, an overly ambitious design runs a high risk of failure. To further increase the chances of success, the design should be planned meticulously with every detail laid out, because a flaw or oversight at this stage could have drastic and uncorrectable consequences.
- Be flexible and willing to make revisions on the fly. Things will go wrong; collaborators may drop out or let you down, funding may be discontinued, and numerous administrative responsibilities will be added. Following the above recommendation of leaving plenty of room for error and remaining flexible will provide a buffer against these inevitabilities. Be sure to anticipate long delays in time-line estimations, and, above all, try to remain calm.
- Be selective with the individuals you include in your research/clinical team. Building a responsible and reliable team is a prerequisite for success. Once the team is assembled, make every detail of each person's responsibilities explicitly clear, and frequently meet with everyone and send updates so each team member remains aware of the study's progress.
- Also be selective with the subjects you include in the study. If you are conducting a randomized controlled trial you should strive to use an intention-to-treat

approach (see description above). Thus, it is incredibly important that you ensure each potential recruit meets the strict, clear inclusion/exclusion criteria. Do not assign someone a treatment allocation unless you are sure (within reason) that he or she meets those criteria, especially if you anticipate a small final sample size.

- Consider arguing against suggested changes to the design that could negatively impact the study. For example, we should have considered questioning the IRB committee's suggestion to exclude patients on Coumadin, since there is insufficient evidence that pomegranate products modulate response to the medication and this criterion significantly limited the pool of potential recruits.
- Keep a log/journal of all study details and progress. Not only is this good/necessary scientific practice, but essential for being able to retrace your steps and demonstrate progress.
- Complete all tasks that are your responsibility as soon as you can do them. This is obvious but necessary to re-iterate. There are so many details to attend to in a research study, and being the coordinator means that the majority of those details fall upon you, so make sure any delays are not because there is something you are procrastinating on. Also, do not wait too long for others. If someone hints that they might not want to be involved, or otherwise adds unnecessary delays, seriously consider excluding them or looking for alternatives.
- Foster relationships with advisors and your research team so they want to help you and see you succeed. The worst thing you can do is burn bridges or act in a way that fosters a negative reputation. Work hard and be responsible, available, flexible, and respectful, and you will succeed.

CHAPTER FOUR

RESULTS

Demographics and Stroke Characteristics

The mean age and education of the total sample was about 58 years (*SD* = 13.76) and 14 years (*SD* = 2.11), respectively, and there were no differences between treatment groups (see Table 4). The sample was 71% male and the ethnicity was predominantly White (57%), with 29% being Black and 14% being Hispanic. Every subject had a diagnosis of hypertension, 36% had diabetes mellitus, and 64% had dyslipidemia. The majority reported a history of smoking (64%) and/or any alcohol use (71%). On a health habits questionnaire filled out at the time of baseline testing, subjects on average indicated that their overall diet was "neutral" (on a scale from "not healthy" to "very healthy"), that they consumed fruits and vegetables every day (on a scale from "never" to "every day"), and that they exercised approximately once per week (on a scale from "never" to "every day"). We did not observe any group differences on these parameters. However, the placebo group trended towards outperforming the POMx group on a measure of estimated verbal intelligence (i.e., the TOPF) given during the baseline assessment ($p = .08$).

The average time from stroke onset to treatment initiation was about 13 days (*SD* = 4.68) and the average length of stay at LLUECH was about 19 days (*SD* = 6.61). The placebo group spent more time at LLUECH than the POMx group ($t_{12} = 2.64$, $p < .03$), but the difference was not significant when correcting for multiple comparisons. Lesion laterality (i.e., hemisphere affected) and location were determined by CT and/or MRI findings listed in the subjects' medical records. All subjects in the POMx group suffered

a stroke in the right hemisphere of their brain, and 5 out of the 7 subjects in the placebo group had a right hemisphere stroke. The majority of subjects had a subcortical stroke (57%), with 14% having a stroke in their cortex and 29% having a mix of cortical and subcortical lesions. None of the stroke characteristic variables were significantly different between treatment groups. Refer to Table 5 for a detailed description of each subject's neuroimaging findings and symptoms.

Table 4. *Demographic data and stroke characteristics by treatment group.*

	POMx	Placebo	*p*-value
Age in years (Mean ± *SD*) [range]	56.29 (13.60) [39-73]	58.86 (14.37) [40-77]	.74[a]
Years of education	13.57 (1.81) [12-16]	14.14 (2.48) [12-18]	.63[a]
Male/female	5/2	5/2	1.00[b]
Race (%)			.47[c]
White	43	71	
Black	43	14	
Hispanic	14	14	
IQ estimate*	84.57 (7.96) [76-100]	95.00 (11.70) [85-115]	.08[a]
Lesion laterality, right/left	7/0	5/2	.46[b]
Lesion location (%)**			.22[c]
Cortical	29	0	
Subcortical	57	57	
Mix	14	43	
Time from stroke onset to treatment initiation in days	12.14 (2.41) [9-16]	14.00 (6.30) [8-27]	.48[a]
Length of rehabilitation unit stay in days	15.29 (4.27) [11-23]	23.00 (6.46) [16-32]	.02[a,d]
Diabetes (%)	14	57	.12[b]
Dyslipidemia (%)	57	71	1.00[b]
Hypertension (%)	100	100	1.00[b]

SD = standard deviation
[a] Independent samples *t* test
[b] Fisher's exact test
[c] Chi-squared test
[d] Not significant when correcting for multiple comparisons
*IQ estimate is based on TOPF score. It is compared to normative data and is a standard score (mean = 100, *SD* = 15)
**According to Physical Medicine and Rehabilitation History and Physical Note for each subject

Table 5. *Imaging findings and symptoms for each subject.*

Subject #	Imaging Findings*	Symptoms*	Treatment Group
2	8mm R thalamic region (MCA) infarcts	L hemiparesis, dysarthria, ataxia	POMx
3	R striatum and posterior R frontal lobe (MCA) infarct with probable thrombotic origin; complete occlusion of R ICA and high-grade stenosis of proximal L ICA; narrowing of the origins of the bilateral vertebral arteries	L hemiparesis, left neglect, dysarthria	Placebo
5	R anterior pontine infarct (likely thrombotic); diffuse mid to moderate cerebral volume loss; intracranial atherosclerosis with narrowing of basilar artery	L hemiparesis, dysarthria, dysphagia	POMx
6	R ICA stroke with R MCA distribution affected, involving R frontal and temporal lobes; complete occlusion of R ICA; also small posterior infarct	L hemiparesis, dysarthria	POMx
7	Complete occlusion of R ICA and partial occlusion of mid M1 segment of R MCA with diminished flow to distal M1 and M2 segments, involving R temporal lobe, insular cortex, basal ganglia region, corona radiata, and anterior thalamus	L hemiparesis, dysarthria, dysphagia	Placebo
8	L MCA stroke involving L putamen and mesial temporal lobe	R hemiparesis, dysarthria	Placebo
9	Multiple infarcts in R PCA distribution involving exclusively the R occipital lobe; multiple old small lacunar infarcts in bilateral basal ganglia and thalami; etiology likely atheroembolic and hypertensive	L neglect	POMx
10	R mid pons and posterior cortical aspect of L occipital lobe involvement	L hemiparesis, double vision, hearing loss, mild dysarthria	POMx
11	R pons infarct (8x7mm); critical stenosis of R ICA (90-99%);	L hemiparesis, dysphagia, dysarthria; initial NIHSS was 6	Placebo
12	R PICA occlusion involving R cerebellum and medulla	R ataxia, double vision, vertigo; NIHSS	Placebo

		was 5	
13	L central pons infarct; old R basal ganglia lacunar infarct; mild bilateral ICA plaque buildup	R hemiparesis, dysphagia, dysarthria	Placebo
14	multiple foci of small infarcts in posterior limb of R internal capsule (subthalamic)	L hemiparesis	POMx
15	R basal ganglia infarct extending to the body of the R caudate nucleus; etiology likely atheroembolic or cardioembolic; 50% stenosis in proximal R ICA	L hemiparesis	Placebo
16	R posterior limb internal capsule infarcts extending 16mm in length; smaller 5mm subacute infarct of L posterior limb internal capsule; mild white matter infarction or gliosis at the cerebrum	L hemiparesis; NIHSS was 9	POMx

R = right; L = left; MCA = middle cerebral artery; ACA = anterior cerebral artery;
ICA = internal carotid artery; PCA = posterior cerebral artery;
PICA = posterior inferior cerebellar artery; NIHSS = National Institutes of Health Stroke Scale
*According to Physical Medicine and Rehabilitation History and Physical Note for each subject
Note: subjects 1 (Placebo) and 4 (POMx) were excluded since they are missing outcome data.

Baseline Test Results

We compared baseline data on each outcome measure to determine whether there were any pre-treatment group differences. The placebo group outperformed the POMx group on most tests, including each RBANS index (see Table 6). The RBANS total scale score showed the greatest difference ($t_{12} = 2.24$, $p < .05$), but this difference was not significant when correcting for multiple comparisons. The placebo group also reported fewer symptoms of depression and anxiety relative to the POMx group, but these differences were not significantly different. The POMx group outperformed the placebo group on Animals (semantic fluency portion of the COWAT; $t_{12} = 2.69$, $p < .02$), but this difference did not reach significance when correcting for multiple comparisons.

Table 6. *Baseline neuropsychological performance by group.*

	Mean (*SD*)		*p*-value	Cohen's *d**
	POMx	Placebo		
RBANS (Indexes)				
Immediate Memory	81.00 (13.47)	88.71 (10.80)	.26	.63
	63.71 (9.32)	73.00 (10.20)	.10	.95
Visuospatial/Constructional				
Language	85.71 (6.40)	88.86 (6.20)	.37	.50
Attention	62.71 (14.57)	78.29 (12.62)	.05	1.14
Delayed Memory	81.00 (16.78)	85.86 (13.95)	.57	.31
Total Scale	67.71 (10.63)	78.00 (5.89)	.04[a]	1.20
MMSE-2	23.00 (2.97)	25.14 (2.19)	.16	.82
TMT**				
Part A	106.30 (100.60)	91.00 (77.97)	.76	.17
Part B	195.70 (102.40)	215.30 (107.30)	.73	.19
BTA	12.14 (4.06)	11.29 (5.99)	.76	.17
Line Bisection Test	9.13 (7.40)	7.37 (6.47)	.65	.25
COWAT				
FAS	26.00 (8.56)	27.14 (3.13)	.75	.18
Animals	16.29 (4.07)	11.57 (2.23)	.02[a]	1.44
BDI-II***	14.86 (12.65)	10.14 (9.67)	.45	.42
STAI***				
State	44.57 (18.12)	34.43 (10.29)	.22	.69
Trait	41.43 (10.95)	32.86 (11.91)	.19	.75

RBANS = Repeatable Batter for the Assessment of Neuropsychological Status;
MMSE-2 = Mini-Mental Status Examination – Second Edition; TMT = Trail Making Test;
BTA = Brief Test of Attention; COWAT = Controlled Oral Word Association Test;
BDI-II = Beck Depression Inventory – Second Edition; STAI = State-Trait Anxiety Inventory
[a]Not statistically significant when correcting for multiple comparisons
*general guidelines: .2 = small effect; .5 = medium effect; .8 = large effect
**lower raw score indicates better performance
***higher scores indicates more mood symptoms

Pre- and Post-Test Ratings

Subjects in the placebo group were more concerned regarding their cognitive abilities than those in the POMx group prior to baseline testing ($t_{12} = 3.57$, $p < .01$). The data also trended in the same direction regarding concern after baseline testing ($p = .06$). There were no differences in perceived performance on testing or estimated pre-stroke performance at this baseline assessment (although the latter approached significance, with the placebo group rating their hypothetical pre-stroke performance lower than the POMx group; $p = .05$).

Subjects in the placebo group were again more concerned regarding their cognitive abilities than those in the POMx group prior to post-treatment testing ($t_{12} = 2.77$, $p < .02$). They were also more concerned regarding their cognitive abilities after post-treatment testing ($t_{12} = 2.24$, $p < .05$). Additionally, the placebo group estimated their hypothetical pre-stroke performance to be lower than the POMx group at this post-treatment testing time-point ($t_{12} = 2.31$, $p < .04$). However, none of these differences were significant after correcting for multiple comparisons. We did not observe any significant differences or trends in the change scores of the four ratings.

Aim 1

The primary aim of the study was to assess whether treatment with PPs improved global cognitive and emotional functioning following stroke. To accomplish this, we utilized the total scale index score on the RBANS, since this score incorporates the subjects' performance on all five cognitive domains assessed. Results of the two-way mixed ANOVA indicated that scores improved significantly over time ($F_{1,12} = 5.35$, $p < .04$). We did not observe a significant main effect of treatment. Although the POMx

group's scores trended toward greater improvement over time relative to the placebo group's scores (interaction of time and treatment, $p = .14$), we did not observe any significant differences (see Figure 6). Figure 7 shows the RBANS total scale index score change for each subject. The lower end of the 95% CI for the POMx group is above baseline, suggesting improvement, whereas the CI for the placebo group is within the baseline range, indicating a lack of improvement.

We also assessed whether the results would differ if we only included subjects who had a subcortical stroke, since 4 in each group had this type of stroke. The data trended in the same direction (i.e., the POMx group improving more than the placebo group) but there were no significant differences. Although the TOPF (Test of Premorbid Functioning; IQ estimate) baseline standard score did not significantly correlate with the RBANS total scale index change score (see Figure 8), we conducted an ANCOVA using the TOPF score as the covariate since the groups trended toward different performance at baseline ($p = .08$). This analysis showed that the POMx group improved significantly more than the placebo group ($F_{1,11} = 5.37$, $p < .05$; see Figure 9).

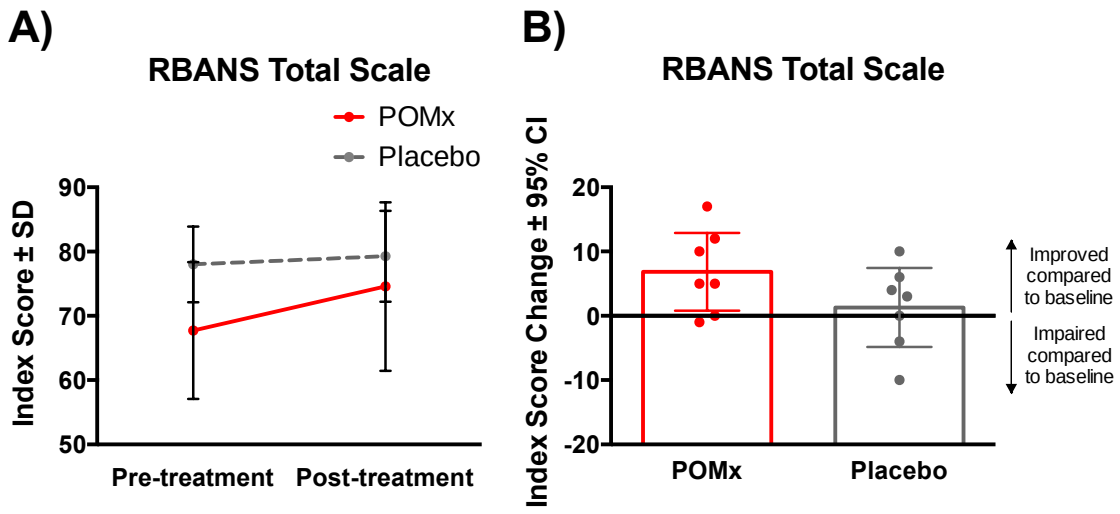

Figure 6. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) total scale score A) over time and B) change (post-testing minus pre-testing score).

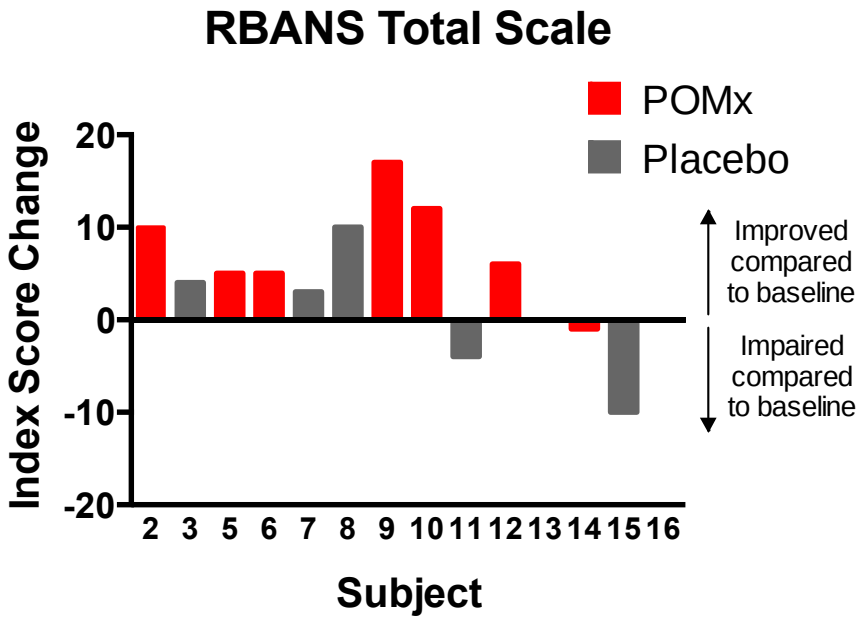

Figure 7. RBANS total scale score change for each subject.
Note: Subjects 13 (Placebo) and 16 (POMx) had a change score of 0, and subjects 1 (Placebo) and 4 (POMx) are missing outcome data.

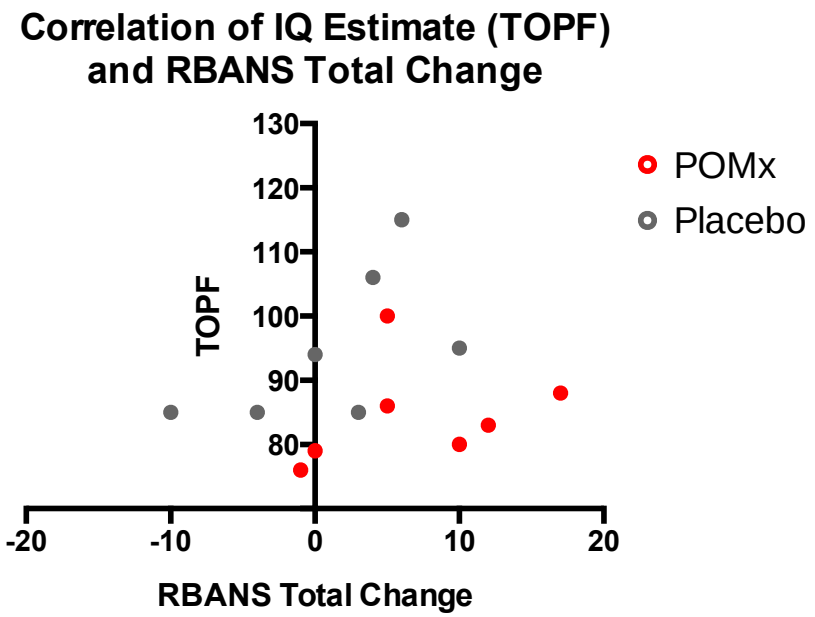

Figure 8. Correlation between RBANS total scale score change and Test of Premorbid Functioning (TOPF) baseline score.

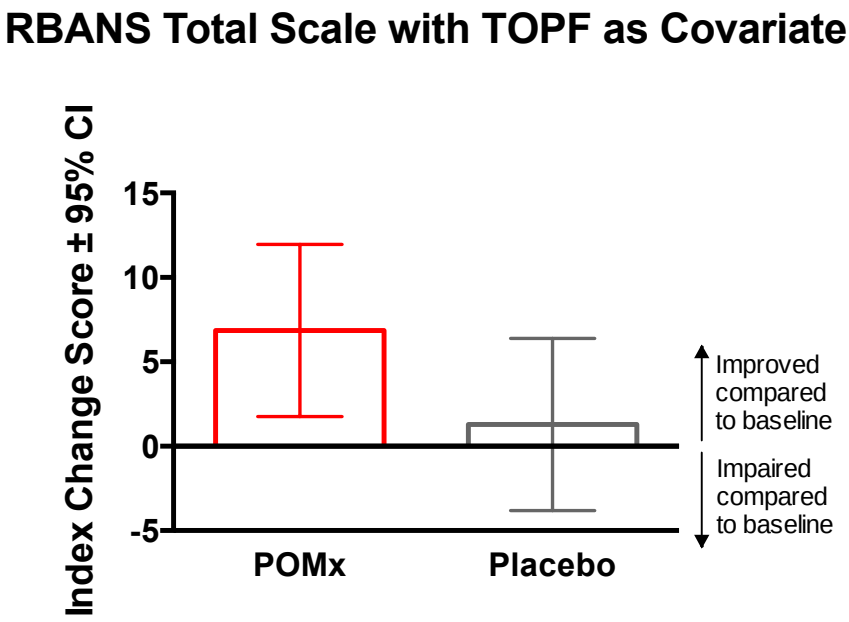

Figure 9. RBANS total scale score change by group with TOPF baseline score as a covariate.

We also examined MMSE-2 score changes from pre- to post-treatment since the MMSE-2 is a widely used screening measure of global cognitive functioning, but found no significant difference (see Figure 10).

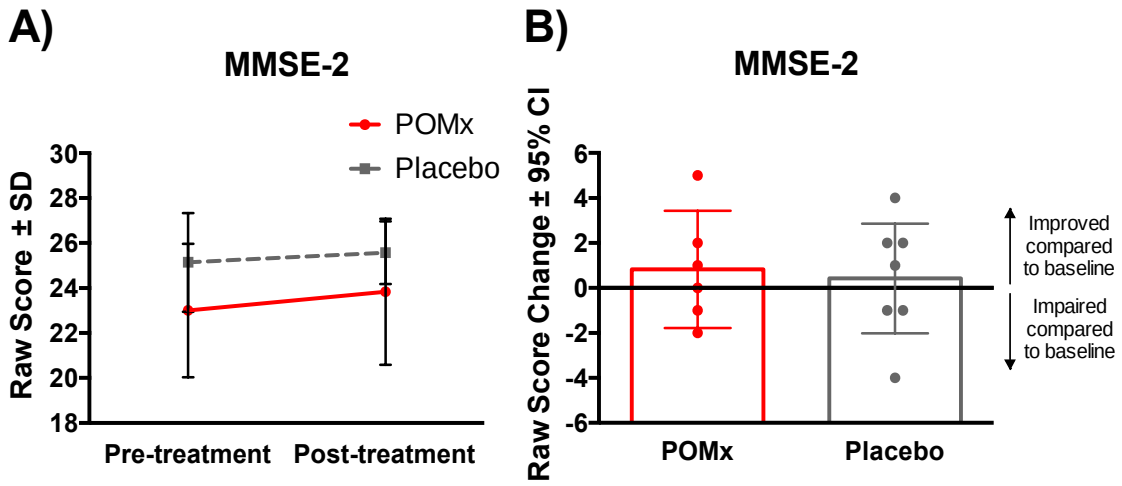

Figure 10. Mini-Mental State Examination-2 (MMSE-2) score A) over time and B) change.

We then analyzed measures of emotional functioning (refer to Chapter 3 for a description of each test). No differences were observed on the BDI-II, a self-report inventory of depression symptoms (see Figure 11). There was a high degree of variability within the POMx group, with scores ranging from 2 to 50 on the post-treatment assessment. No significant differences were seen on either part of the STAI, a self-report inventory of anxiety symptoms (see Figures 12 and 13). Lower scores indicate fewer reported symptoms on each figure. All reported analyses and figures were done using the complete case approach. However, we repeated all Aim 1 analyses using intention-to-treat (with the baseline observation carried forward imputation method) and

per protocol approaches (described in Chapter 3), just to ensure they did not lead to different findings, and observed the same trends as the complete case approach.

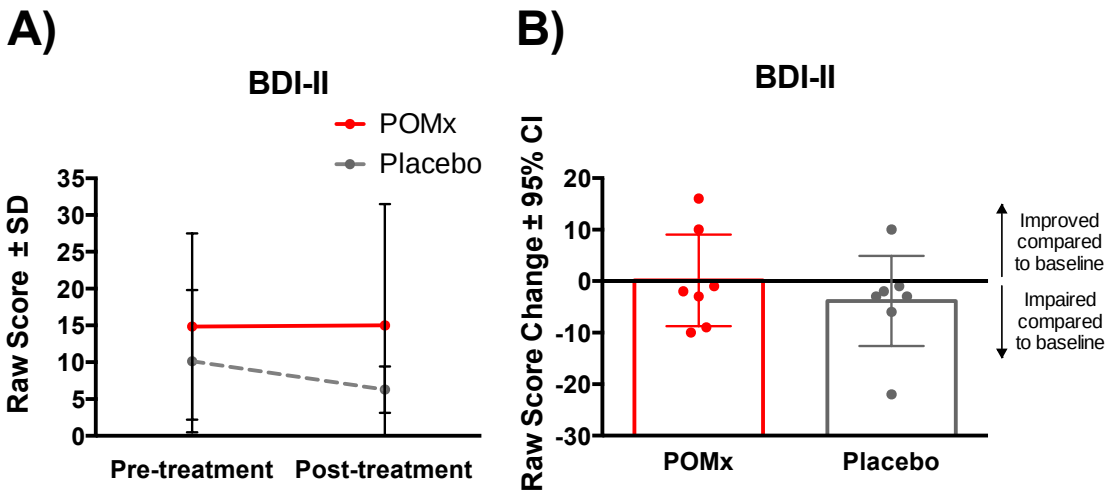

Figure 11. Beck Depression Inventory-II (BDI-II) score A) over time and B) change.

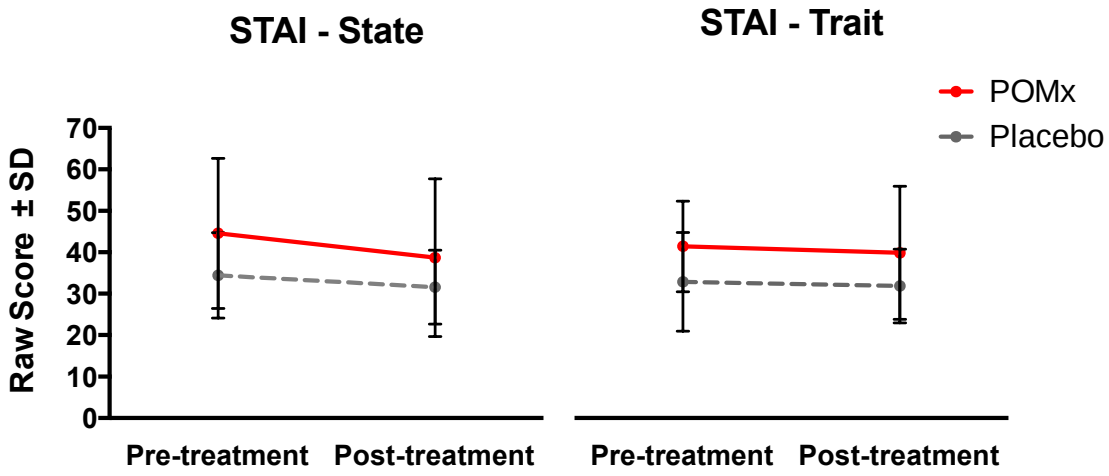

Figure 12. STAI score over time by group.

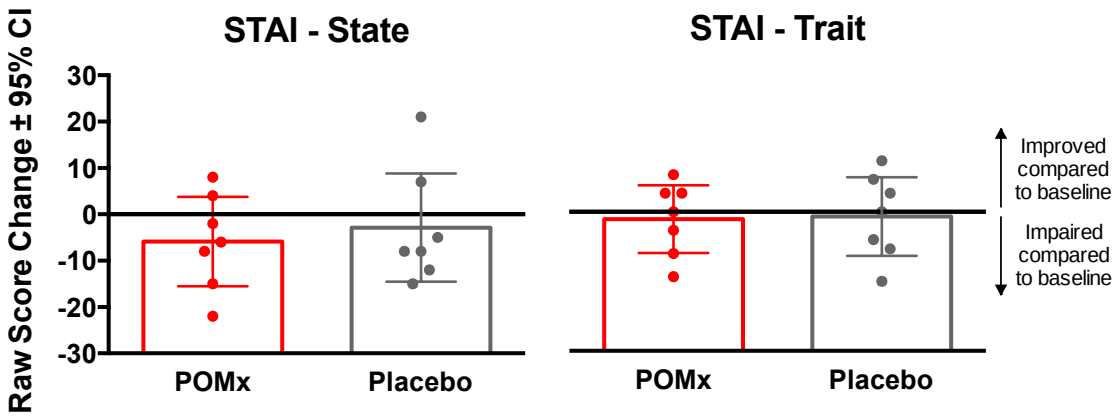

Figure 13. STAI change score by group.

Aim 2

The goal of Aim 2 was to determine whether there were any cognitive domain-specific differences in treatment response. We hypothesized that treatment would affect cognitive domains differently, where subjects that received PPs would have larger improvements in the domains that were most affected by stroke. On the RBANS, we saw trends towards the POMx group having a greater degree of change than the placebo group in the visuospatial/constructional (p = .09) and language (p = .11) domains (see Figure 14). The lower end of the 95% CI for the POMx group is above baseline on the Language domain, suggesting improvement, whereas the CI for the placebo group is within the baseline range, indicating a lack of improvement. There was no overall effect of treatment when conducting a MANOVA with all five RBANS index change scores, although the effect size was relatively large (Wilks' Lambda = .54, $F_{5,8}$ = 1.39, partial eta-squared = .47). Furthermore, we did not see a significant difference when calculating a retention composite change score based on the three RBANS retention scores (i.e., list,

story, and figure retention), although the POMx group trended toward better performance (p = .09).

We then examined other measures (refer to Table 7) and found that the BTA (Brief Test of Attention) showed the largest improvement in the POMx group compared to the placebo group (t_{12} = 2.99, p < .02; see Figure 15). However, after correcting for multiple comparisons, this difference was not significant. Based on this finding, we tested the change in the Digit Span subtest of the RBANS (part of the Attention Index), since it is widely thought to be a measure of simple attention, but did not find a significant group difference.

We observed the second largest change from pre- to post-treatment testing on the Animals portion of the COWAT, but it was in the opposite direction of the hypothesized effect, with the placebo group improving more than the POMx group in their ability to rapidly name animals (t_{12} = 2.52, p < .03; see Figure 16); they named approximately 3 more animals on average than at their baseline testing, compared to the POMx group that named about the same number of animals as their prior performance. This difference was not significant when correcting for multiple comparisons. The finding led us to test for potential differences on the Semantic Fluency subtest of the RBANS (part of the Language Index), since it assesses the same domain (i.e., involves naming fruits and vegetables at baseline testing and naming animals found in a zoo at post-treatment testing). No significant differences were found but there was a trend in the opposite direction, with the POMx group improving more than the placebo group (p = .10; see Figure 17).

As with Aim 1, all reported analyses and figures were done using the complete case approach. However, we repeated all Aim 2 analyses using intention-to-treat (with the baseline observation carried forward imputation method for the 2 subjects with missing outcome data) and per protocol approaches, just to ensure they did not lead to drastically different findings, and observed the same trends as the complete case approach. Furthermore, as mentioned in the Neuropsychological Testing section of Chapter 3, we also compared performance after norming the data to determine if standardization altered the results, and found that the two methods (i.e., using raw data versus standardizing the data) produced comparable results.

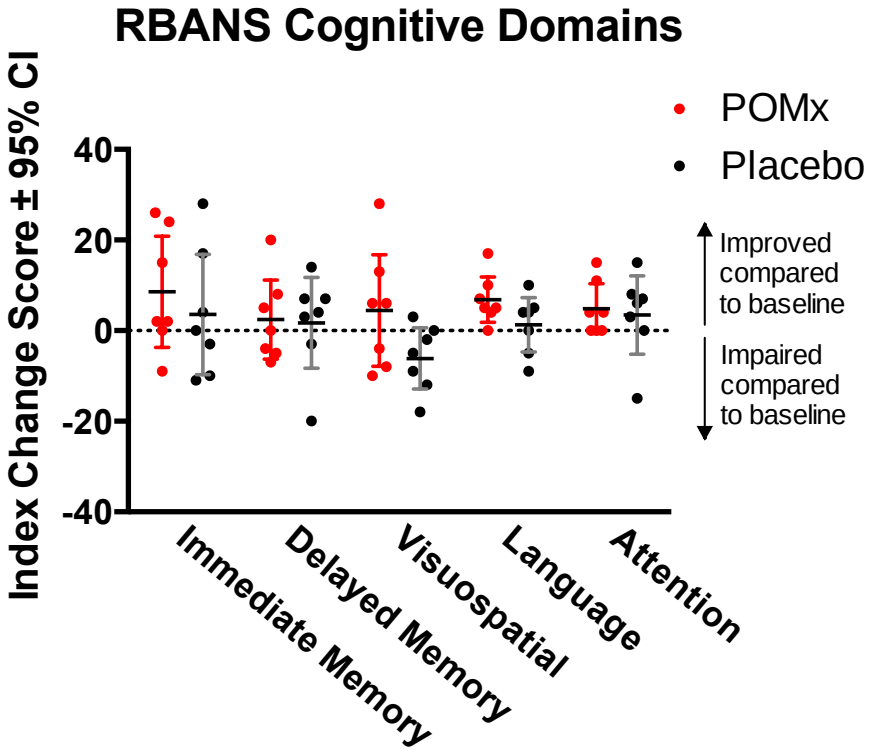

Figure 14. RBANS index change score for each domain by group.

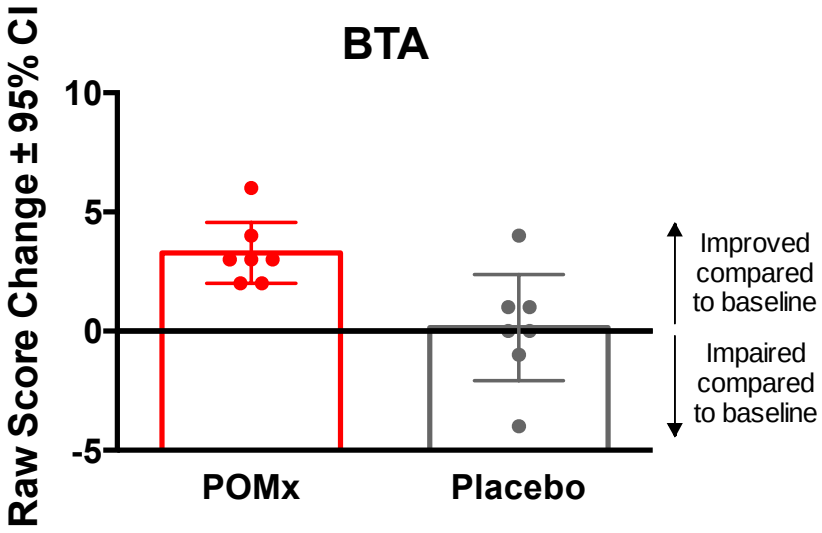

Figure 15. Brief Test of Attention raw score change.

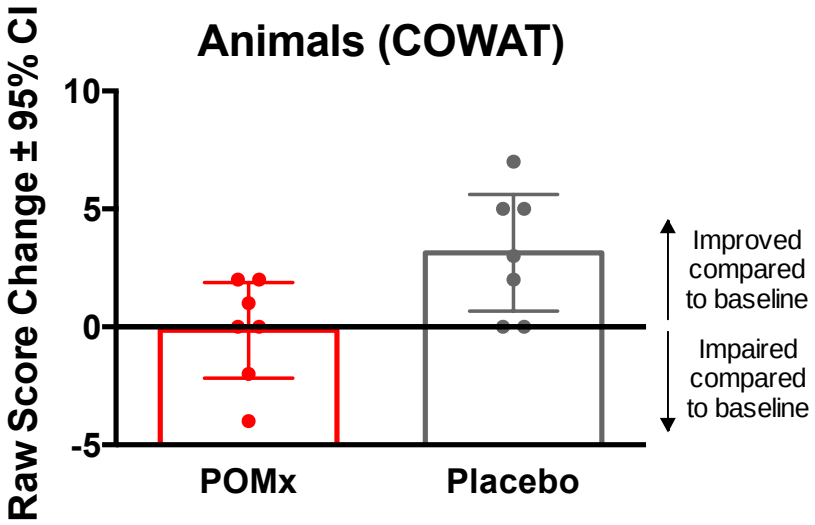

Figure 16. Animals (semantic fluency part of the COWAT) raw score change.

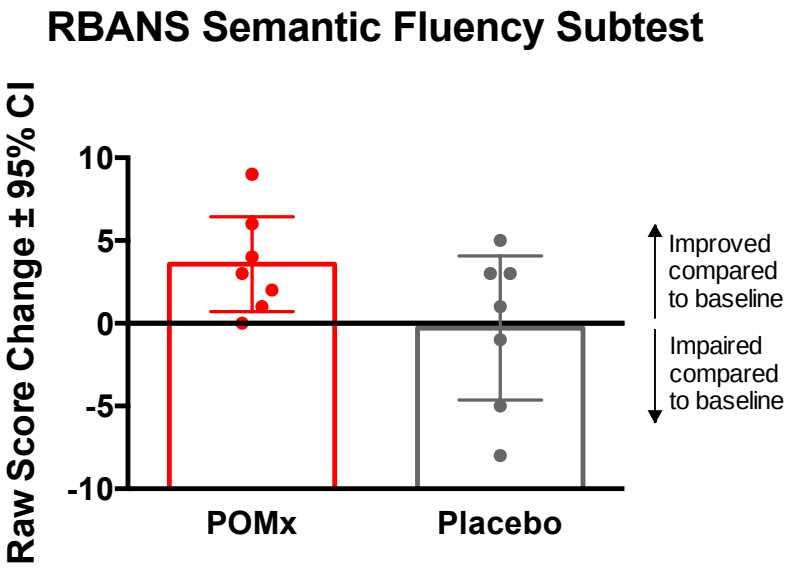

Figure 17. RBANS Semantic Fluency subtest score change.

Table 7. *Change scores for cognitive and emotional outcome measures by group.*

	Mean (*SD*)		*p*-value	Cohen's *d**
	POMx	Placebo		
RBANS (Indexes)				
Immediate Memory	8.57 (13.24)	3.57 (14.32)	.51	.36
	4.43 (13.34)	-6.14 (7.34)	.09	.98
Visuospatial/Constructional				
Language	6.86 (5.40)	1.29 (6.47)	.11	.93
Attention	4.86 (5.96)	3.43 (9.36)	.74	.18
Delayed Memory	2.43 (9.47)	1.71 (10.86)	.90	.07
Total Scale	6.86 (6.52)	1.29 (6.65)	.14	.85
MMSE-2	0.83 (2.48)	0.43 (2.64)	.78	.16
TMT**				
Part A	-18.14 (18.80)	-19.86 (33.35)	.91	.06
Part B	-20.00 (21.03)	-39.00 (39.64)	.28	.60
BTA	3.29 (1.38)	0.14 (2.41)	.01[a]	1.60
Line Bisection Test	-0.91 (3.79)	-3.71 (4.69)	.24	.66
COWAT				
FAS	1.29 (2.43)	2.14 (3.67)	.62	.27
Animals	-0.14 (2.19)	3.14 (2.67)	.03[a]	1.34
BDI-II***	0.14 (9.58)	-3.86 (9.48)	.45	.42
STAI***				
State	-5.86 (10.43)	-2.86 (12.62)	.64	.26
Trait	-1.57 (7.87)	-1.00 (9.17)	.90	.07

RBANS = Repeatable Batter for the Assessment of Neuropsychological Status;
MMSE-2 = Mini-Mental Status Examination – Second Edition; TMT = Trail Making Test;
BTA = Brief Test of Attention; COWAT = Controlled Oral Word Association Test;
BDI-II = Beck Depression Inventory – Second Edition; STAI = State-Trait Anxiety Inventory
*general guidelines: .2 = small effect; .5 = medium effect; .8 = large effect
**lower raw score indicates better performance
***higher scores indicates more mood symptoms
[a]Not statistically significant when correcting for multiple comparisons

CHAPTER FIVE

CONCLUSION

Discussion/Implications of the Findings

In the present study, we recruited 16 subjects who recently suffered an ischemic stroke and tested their cognitive functioning and mood symptoms before and after one week of pomegranate supplement (i.e., POMx) or placebo intake. Subjects were randomly assigned to treatment groups and both they and their clinical team were blind to the treatment allocation. Few studies have assessed the effects of PPs on cognitive and emotional functioning, and no known clinical study has examined their efficacy in enhancing neuropsychological recovery following a stroke. Overall, the results trended toward subtle improvements in cognitive abilities in pomegranate-treated subjects compared to placebo-controlled subjects, but no differences or trends were observed regarding emotional functioning.

Although our randomization protocol yielded groups that were demographically quite similar, it was surprising to observe that the groups differed at baseline (i.e., pre-treatment) on a number of measures (albeit not significantly), including higher estimated intellectual functioning of the placebo group compared to the POMx group. The placebo group also reported fewer mood symptoms at baseline testing. This could have potentially improved our chances of finding effects of POMx since the POMx group had more room to improve, but it could have also hurt our chances since individuals with lower intellectual abilities may tend not to improve as much in general, or may have a floor effect on testing (i.e., perform below the limit of our instruments and thus preclude the observance of true differences).

We observed several trends when comparing groups on post-treatment performance while accounting for pre-treatment performance. The main outcome measure we used was the RBANS total scale score, which is robust since it incorporates scores from the five RBANS indexes. The POMx group significantly improved over time while the placebo group did not, and these effects were likely driven by trends in the visuospatial/constructional and language domains, since these were more improved in the POMx group relative to the placebo group. Furthermore, the POMx group improved significantly more than the placebo group when controlling for baseline intellectual functioning, as measured by the Test of Premorbid Functioning (TOPF). These findings corroborate the results of other studies using pomegranate products to ameliorate cognitive deficits in clinical populations of various pathologies (Bookheimer et al., 2013; Ropacki et al., 2013). However, it is important to note that both groups performed poorly relative to the age-matched normative sample (i.e., the sample the RBANS was normed on; post-treatment total scale score: POMx = 4th percentile, placebo = 6th percentile), which is consistent with other studies showing that cognitive deficits are prevalent following stroke (Hofgren et al., 2007; M. Patel et al., 2003).

Outside of the RBANS, differences fell below the $p < .05$ level on two cognitive measures, although these differences were not significant when correcting for multiple comparisons with the Holm-Šídák method. Furthermore, these differences were in disparate directions. Specifically, the POMx group showed the largest improvement on the BTA (Brief Test of Attention), whereas the placebo group showed the largest improvement on rapid animal naming. It is difficult to make any interpretive claims regarding these effects, especially because outcomes from similar measures did not

converge with either of the findings. It is possible that the Animals finding is due to regression toward the mean, since the placebo group trended toward poorer performance than the POMx group on the baseline testing. Additionally, there was another semantic memory task that also involved rapid animal naming, and the difference trended in the opposite direction (i.e., the POMx group outperforming the placebo group). Regression toward the mean was likely not an explanation for the difference observed on the BTA since both groups were similar at baseline. However, since these differences were not significant after correcting for multiple comparisons, they should be considered as only trends.

Being that the present study is a pilot trial and that there is little guidance from the literature as to what to expect regarding the effects of PPs on cognitive and emotional functioning in a clinical population, the findings are meant to guide future research. Despite being substantially underpowered, we observed several interesting trends and differences in the data that suggest that a larger sample size or other design changes (see Limitations section below) may have uncovered larger effects.

Limitations

We acknowledge several limitations in the present study:

1. The sample size was relatively small, with 16 total subjects recruited and 14 analyzed. This was meant as a pilot trial, since no known study has examined the neuropsychological effects following polyphenol administration in a clinical stroke population, and we expected to be somewhat underpowered. Although recruitment was open for 10 months, the patient census was unusually low and there were many stroke patients who did not meet our strict inclusion criteria. We

were more interested in carefully selecting appropriate candidates for recruitment and ending up with a relatively homogenous sample than recruiting as many subjects as possible. This allowed us to limit many potential confounding factors (e.g., cognitive decline due to a neurodegenerative process).

2. Many other studies using PPs had longer treatment durations than the one-week period in the present study. We originally attempted a two-week treatment protocol (28 doses), but relatively short rehabilitation lengths of stay did not make that duration feasible, so we opted to change it to a one-week treatment protocol (14 doses). It is likely that our chances of finding significant effects would have improved substantially with longer treatment periods.
3. Many prior animal and human studies assessing the effectiveness of PPs in various disease states (e.g., AD, radiation exposure, cardiac surgery, stroke) had begun PP administration before insult, which may have primed the body to defend against the pathological effects after injury. In the present study, it was not possible to begin treatment administration until after a stroke had occurred (days to weeks after the event). Although others have seen behavioral effects when PPs were administered postischemic injury (Sarkaki & Rezaiei, 2013), and our treatment initiation was still well within the therapeutic window (Cramer, 2008a; Emsley et al., 2003), it is likely that our chances of finding differences would have been optimized if treatment was initiated before the insult.
4. There was some heterogeneity in the sample, especially regarding stroke location. Although comparing post-treatment functioning to baseline functioning attenuates the relevance of inter-subject differences, and true randomization is the best way

to control for heterogeneity, it is possible that this could have led to variability in the domains affected by stroke, the recovery trajectory, and/or the response to PP administration.

5. Our sample may not be considered generalizable to all people who have suffered a stroke, since this was a highly selected (i.e., long exclusion criteria list) sample of patients receiving intensive inpatient rehabilitation services, which likely contributed to their functional gains.

Future Directions

The present findings lead to many more questions regarding the effects of pomegranate supplementation after stroke. Since this study was the first to examine neuropsychological outcomes following pomegranate treatment in a clinical stroke population, replication studies are needed to ensure the validity of the present findings. Obvious improvements for these hypothetical future studies would be larger sample sizes with longer treatment durations (e.g., weeks to months) and later assessments (e.g., 1 year post-stroke). Additionally, subsequent studies could use different doses of POMx or other pomegranate products, as well as other measures to test different behavioral constructs. Jeff Murray, the psychology doctoral student who tested subjects in the present study, is currently examining another construct, FIM (Functional Independence Measure) scores, as part of his doctoral project. The FIM system is routinely employed at LLUECH and other rehabilitation centers, and is used to assess a patient's motor functioning/mobility, ability to engage in activities of daily living, social interaction, and problem solving ability, among other abilities. Furthermore, the measure has been validated in an acute stroke population (Hsueh, Lin, Jeng, & Hsieh, 2002). Jeff is

currently in the process of comparing the FIM scores attained at post-treatment from LLUECH with their baseline scores to assess whether the POMx group improved in relation to the placebo group.

There are several other avenues to explore. For example, it would be extremely informative to assess various biomarkers, such as inflammation (e.g., via C-reactive protein, white blood cell count, TNF α, leukocyte count, or fibrinogen) and ROS (e.g., via 8-isoprostane, lipid peroxide, nitric oxide/nitrite, superoxide dismutase, or 8-hydroxy-2-deoxyguanosine), to better understand the potential mechanisms mediating any relationship between PP intake and improvements in cognitive/emotional functioning. It would also be beneficial to test for pomegranate metabolites (e.g., via trolox equivalent antioxidative capacity or urolithin A-glucuronide) to confirm increased antioxidant concentrations in the pomegranate group (as was accomplished in Bookheimer et al., 2013), especially since the precise bioavailability of PPs is still unclear. Attaining lesion volume data (e.g., via MRI or CT scans) both before and after treatment would also help clarify the neurological effects of PPs.

Another option is to recruit individuals who are at substantial risk for suffering a stroke (e.g., older adults with hypertension, dyslipidemia, and diabetes mellitus) and assign them to either take pomegranate products or a placebo. This could help to improve our understanding of the effects of PPs on cerebrovascular risk factors, as well as aid in discovering whether PPs are protective for individuals who later go on to have a stroke. This design would have the added benefit of better modeling most animal studies, where treatment is usually initiated prior to injury/stroke.

Concluding Remarks

Given the growing popularity of dietary manipulations and increased polyphenol intake, it is important to ensure the safety of these agents and identify potential therapeutic approaches. We hope the present study sparks more research that improves our understanding of how dietary interventions may be used to enhance cognitive recovery after a very common, often debilitating, cerebrovascular event, as well as promotes healthy lifestyle changes in those who have cerebrovascular risk factors. We also hope that subsequent studies will continue to assess the effectiveness of polyphenols and other dietary interventions among stroke survivors, potentially introducing inexpensive and safe treatments that lead to improved cognitive functioning, better quality of life, and a reduced financial burden on hospitals and communities.

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APPENDIX A

INFORMED CONSENT DOCUMENT

LOMA LINDA UNIVERSITY

School of Behavioral Health
Department of Psychology

11130 Anderson Street
Loma Linda, California
(909) 558-7116
Fax (909) 558-0171

TITLE: **THE EFFECTS OF POMEGRANATE POLYPHENOLS ON NEUROPSYCHOLOGICAL FUNCTIONING FOLLOWING ISCHEMIC STROKE: A PILOT STUDY**

SPONSOR: **Department Funded**

PRINCIPAL INVESTIGATOR:

Richard Hartman, Ph.D.
Loma Linda University
Department of Psychology
School of Behavior Health
Loma Linda, CA 92350
Telephone Number: [omitted]

1. WHY ARE WE DOING THIS STUDY?

We want to conduct this study to examine whether dietary supplementation with an antioxidant (pomegranate extract) can help promote healthy cognitive functioning (i.e., thinking ability, such as memory or attention) as a component of recovery after stroke.

You are invited to participate in this research study because you are a patient at the Rehabilitation Institute, recently suffered a stroke, and meet our criteria for involvement.

Approximately 28 subjects will participate in this study, all of which will be subjects at Loma Linda University (LLU).

2. HOW WILL YOU BE INVOLVED?

Participation in this study involves the following:

- You will be assigned to one of the groups by chance, using something like the flip of a coin, to determine if you will take the antioxidant capsule or a placebo

capsule (i.e., sugar pill). Neither you, the clinician, nor your nursing staff will know which one you are receiving.

- You will be given a series of tests of your cognitive skills and a few questionnaires of your mood and health habits. One of these paper and pencil test sessions will be conducted right after you sign this informed consent agreement and the other will be given in approximately two weeks. These tests should take less than one hour. Testing sessions will take place in a comfortable, quiet room in the hospital and you can take breaks as needed.

- You will take 2 of the antioxidant capsules or 2 of the placebo capsules daily (one in the morning, one in the evening), which your nurse will give you along with your other medications, for 7 consecutive days during your stay at the hospital.

If you agree to participate, you will be responsible for taking 2 brief sessions of cognitive testing and taking 2 extra supplements, one in the morning and one at night.

3. WHAT ARE THE REASONABLY FORESEEABLE RISKS OR DISCOMFORTS YOU MIGHT HAVE?

The committee at LLU that reviews human studies (Institutional Review Board) has determined that participating in this study exposes you to moderate risk.

Although research suggests that allergic reactions to this antioxidant (or the fruit from which it is derived) and interactions with medications are uncommon, it is possible that some individuals may experience an allergic reaction or potential interactions between the supplement and the medicines they are taking. Also, it has been reported that this antioxidant has effects similar to aspirin and causes blood to clot less easily, which could increase the risk of bleeding. To minimize these risks, anyone who has had a hemorrhagic stroke in the past 6 months, is taking Coumadin, or had brain surgery in the past month will not be considered for participation. Please contact medical staff immediately if you notice any negative reactions that are likely associated with the study. If such concerns arise during the course of your participation, you may be asked to discontinue your participation in the study. If you have any concerns regarding your health that arise during the course of your participation, you should contact your doctor and nurse immediately.

A possible discomfort resulting from your participation is temporary fatigue or frustration during the testing sessions. To ease discomfort, you will be allowed to take breaks as needed. Also, some participants may not like the taste of the antioxidant capsule or the placebo capsule. Participants who are unable to tolerate the capsules may decline further participation in the study at any time.

4. WILL THERE BE ANY BENEFIT TO YOU OR OTHERS?

It is possible that you may not receive any benefit from this study. However, it is also possible that you may experience the benefits of the antioxidant supplement on overall health and/or cognitive functioning (that is, cognitive decline may be prevented or cognitive functioning may be improved).

In addition, the information learned from this study will benefit others in the future. The results of this study may improve our understanding of cognitive functioning and factors that may affect cognitive functioning in persons who suffer a stroke, as well as in individuals who are at risk of having a stroke. Results of this study may also improve our understanding of the role of diet on cognition and brain health, and lead to improved treatments for individuals with stroke and other related injuries. The data collected from this study may also be published in scholarly journals.

5. WHAT ARE YOUR RIGHTS AS A SUBJECT?

Participation in this study is voluntary. Your decision whether or not to participate or withdraw at any time from the study will not affect your ongoing medical care/relationship with your health care team and will not involve any penalty or loss of benefits to which you are otherwise entitled. You may get a second opinion about your decision to be in the study from another doctor at your own cost.

Likewise, your study doctor or the study staff may withdraw you from the study for any reason without your agreement or may stop the study entirely.

If you decide to withdraw from the study, you must notify the study doctor or study staff immediately at [omitted].

6. WILL YOU BE INFORMED OF SIGNIFICANT NEW FINDINGS?

During the study, we may learn new things about the risks and benefits of the study. If such information might affect the willingness of individuals to be in the study, we will share this information with you. Should your condition become worse, should side effects become severe, or should new scientific developments occur indicating that participating in this study is no longer in your best interest, then your study participation may be stopped and other options would be discussed.

7. WHAT OTHER CHOICES DO YOU HAVE?

You may consult a nutritionist if you have questions about any dietary or nutritional needs. You may also request a neuropsychological evaluation if you have concerns regarding your cognitive functioning (i.e., thinking ability).

8. HOW WILL INFORMATION ABOUT YOU BE KEPT CONFIDENTIAL?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will not be identified by name in any publications describing the results of this study, nor in the government registration of this study. Your personal information will be available only to those directly involved in the study or assessment procedures. You will be given an identification number upon entry into the study that will be used to identify your test results. Your neuropsychologist, who is a researcher in this study, may also use the results to best tailor your care while at the hospital. All personal information will be kept in a locked office in the Department of Neuropsychology, and all test results will be kept on a password protected, secure computer in a separate locked office. Your rights regarding permission to use your health information are described on the attached "Authorization for Use of Protected Health Information" form. This informed consent form will also be input into your medical record.

9. WHAT COSTS ARE INVOLVED?

There is no cost to you for participating in this study. The study/sponsor will pay for services, supplies, procedures, and care that are not a part of your routine medical care. This includes the costs of the pomegranate supplements and pharmacy dispensing fees.

You and/or your health insurance must pay for the services, supplies, procedures, and care required for routine medical care. You will be responsible for any co-payments and/or deductibles as required by your insurance.

10. WILL YOU BE PAID TO PARTICIPATE IN THIS STUDY?

You will not be paid to participate in this research study. However, you will not incur any additional costs as a result of your participation.

11. WILL STUDY STAFF RECEIVE PAYMENT?

The study is funded by the LLU Department of Psychology and study staff will not receive payment for their role in the study.

12. WHO DO YOU CALL IF YOU ARE INJURED AS A RESULT OF BEING IN THIS STUDY?

Your study doctors will be monitoring your condition throughout the study, and precautions will be taken to minimize the risks to you from participating. If you are injured or become ill while taking part in this study, please do the following:

- Notify your doctor and nurse as soon as you can.

Appropriate medical treatment will be made available to you. However, you and your insurance company will be billed at the usual charge for the treatment of any research-related injuries, illnesses, or complications. You might still be asked to pay whatever your insurance does not pay.

Also, no funds have been set aside nor any plans made to compensate you for time lost for work, disability, pain, or other discomforts resulting from your participation in this research.

By participating in the study, you do not give up any of your legal rights.

13. WHO DO YOU CALL IF YOU HAVE QUESTIONS?

If you wish to contact an impartial third party not associated with this study regarding any questions about your rights or to report a complaint you may have about the study, you may contact the Office of Patient Relations, Loma Linda University Medical Center, Loma Linda, CA 92354, phone (909) 558-4647, e-mail patientrelations@llu.edu for information and assistance.

14. SUBJECT'S STATEMENT OF CONSENT

- I have read the contents of the consent form, which is in English, a language that I read and understand. I have listened to the verbal explanation given by the investigator.
- My questions concerning this study have been answered to my satisfaction.
- I have received a copy of the California Experimental Subject's Bill of Rights and have had these rights explained to me.
- Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors from their responsibilities.
- I may call Rich Hartman, Ph.D., during routine office hours at [omitted] if I have additional questions or concerns.
- I understand that if I am enrolled in an inpatient study, my primary care physician may be notified of my participation for proper coordination of care.
- I hereby give voluntary consent to participate in this study.

I understand I will be given a copy of this consent form after signing it.

Signature of Subject

Printed Name of Subject

Date

AM / PM

Time

If subject is physically unable to sign:

Subject is unable to sign because _____.

Printed name of Subject

I attest that the above named subject has indicated their consent to participate in this study.

Signature of Witness

Printed Name of Witness

Date

AM / PM

Time

15. INVESTIGATOR'S STATEMENT

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied – that the subject has been provided with a copy of the California Experimental Subject's Bill of Rights, that I have discussed the research project with the subject and that I have explained to him or her in non-technical terms all of the information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the subject to ask questions and that all questions asked were answered. I understand that it is my responsibility to notify the subject's primary care physician of study participation, as needed, for proper coordination of care. I will provide the subject or the legally authorized representative with a signed and dated copy of this consent form.

Signature of Investigator

Printed Name of Investigator

Date

AM / PM

Time

APPENDIX B

AUTHORIZATION FOR USE OF PROTECTED HEALTH INFORMATION

INSTITUTIONAL REVIEW BOARD
Authorization for Use of Protected Health Information (PHI)

Per 45 CFR §164.508(b)

RESEARCH PROTECTION PROGRAMS
LOMA LINDA UNIVERSITY | Office of the Vice President of Research Affairs
24887 Taylor Street, Suite 202 Loma Linda, CA 92350
(909) 558-4531 (voice) / (909) 558-0131 (fax)/e-mail: irb@llu.edu

TITLE OF STUDY:	The Effects of Pomegranate Polyphenols on Neuropsychological Functioning Following Ischemic Stroke: A Pilot Study
PRINCIPAL INVESTIGATOR:	Richard Hartman, Ph.D.
Others who will use, collect, or share PHI:	Sub-investigators: Travis G. Fogel, Ph.D., ABPP Mary Kim, M.D. Students/Personnel: John A. Bellone, M.A. Jeffrey Murray, B.A. Paolo Jorge, M.D.

The study named above may be performed only by using personal information relating to your health. National and international data protection regulations give you the right to control the use of your medical information. Therefore, by signing this form, you specifically authorize your medical information to be used or shared as described below.

The following personal information, considered "Protected Health Information" (PHI) is needed to conduct this study and may include, but is not limited to: medical records and charts, results of blood tests, results of neuropsychological tests.

The individual(s) listed above will use or share this PHI in the course of this study with the Institutional Review Board (IRB) and the Office of Research Affairs of Loma Linda University.

The main reason for sharing this information is to be able to conduct the study as described earlier in the consent form. In addition, it is shared to ensure that the study meets legal, institutional, and accreditation standards. Information may also be shared to report adverse events or situations that may help prevent placing other individuals at risk.

All reasonable efforts will be used to protect the confidentiality of your PHI, which may be shared with others to support this study, to carry out their responsibilities, to conduct public health reporting and to comply with the law as applicable. Those who receive the PHI may share with others if they are required by law, and they may share it with others who may not be required to follow national and international "protected health information" (PHI) regulations such as the federal privacy rule.

Subject to any legal limitations, you have the right to access any protected health information created during this study. You may request this information from the Principal Investigator named above but it will only become available after the study analyses are complete.

-The authorization expires upon the conclusion of this research study.

You may change your mind about this authorization at any time. If this happens, you must withdraw your permission in writing. Beginning on the date you withdraw your permission, no new personal health information will be used for this study. However, study personnel may continue to use the health information that was provided before you withdrew your permission. If you sign this form and enter the study, but later change your mind and withdraw your permission, you will be removed from the study at that time. To withdraw your permission, please contact the Principal Investigator or study personnel at [omitted].

You may refuse to sign this authorization. Refusing to sign will not affect the present or future care you receive at this institution and will not cause any penalty or loss of benefits to which you are entitled. However, if you do not sign this authorization form, you will not be able to take part in the study for which you are being considered. You will receive a copy of this signed and dated authorization prior to your participation in this study.

I agree that my personal health information may be used for the study purposes described in this form.

Signature of Patient	Date

Signature of Investigator Obtaining Authorization	Date

APPENDIX C

EXPERIMENTAL RESEARCH SUBJECTS BILL OF RIGHTS

California law, under Health & Safety Code §24172, requires that any person asked to take part as a subject in research involving a medical experiment, or any person asked to consent to such participation on behalf of another, is entitled to receive the following list of rights written in a language in which the person is fluent. This list includes the right to:

. Be informed of the nature and purpose of the experiment.

. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.

. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.

. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.

. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.

. Be informed of the avenues of medical treatment, if any, available to the subject after the experiment if complications should arise.

. Be given an opportunity to ask any questions concerning the experiment or the procedures involved.

. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.

. Be given a copy of the signed and dated written consent form.

. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion, or undue influence on the subject's decision.

APPENDIX D

INCLUSION CRITERIA CHECKLIST

The potential participant:	[checkmark if statement is true]
Is 18-89 years old	_____
Speaks English fluently	_____
Is not globally aphasic	_____
Is not on warfarin (Coumadin)	_____
Has not suffered an intracerebral hemorrhage in past 6 months	_____
Has not had neurosurgery in the past month	_____
Is not pregnant	_____
Has at least 6 years of education (i.e., completed the 6^{th} grade)	_____
Does not have a history of traumatic brain injury	_____
Does not have a neurologic condition with known cognitive impact (e.g., dementia)	_____
Does not have active renal disease	_____
Does not have active liver disease	_____
Does not have a history of allergy to pomegranate products	_____

If all items are check marked, the patient is eligible for study participation.

Thank you!

APPENDIX E

WORK FLOW FOR RESIDENT

1. Screen new admissions for eligibility by checking the unit lists each day.

2. If a newly admitted patient is eligible (i.e., admitted due to an ischemic stroke), visit with patient and follow Initial Patient Visit Script (see next page).

3. After meeting with the patient, review medical record to confirm inclusion criteria is met (i.e., that the patient is not on Coumadin, does not have active renal or liver disease, etc.).

4. If patient still meets inclusion criteria, contact Dr. Kim to request an order for neuropsychology. Also email the team [omitted], notifying them that a particular patient has met criteria for study inclusion and has consented.

5. Fax the completed (just top portion) Nursing Authorization form to 44039. Form is included at the end of this packet.

6. Make a copy of the informed consent document and give it to the East Campus pharmacy (near unit 1100).

7. Place all the original documents under Dr. Fogel's office door (room 109 near the South Entrance).

8. Change patient's status to research active in LLEAP (specific instructions included at end of this packet).

9. Add visit specifics (dates and whether person consented, declined, or did not meet criteria) to excel sheet.

Who to contact: If you have any questions or concerns, please contact John Bellone, M.A., at jbellone@llu.edu.

Thank you!

APPENDIX F

INITIAL PATIENT VISIT SCRIPT

1) **"Hi Mr./Ms. _____, I'm Dr. Jorge, I'm part of your medical team. Do you know why you're in the hospital?** [if not, say **"well, you had a stroke, which means that blood wasn't getting to a part of your brain for a period of time"**] **Sometimes after a stroke people can experience changes in their thinking ability, like in memory, attention, language. The reason why I'm meeting with you today is because you've been chosen to participate in a research study going on here at the hospital with stroke patients. We're interested in finding ways to help people improve their thinking skills, since there aren't many treatments available for that. Are you interested in hearing more about the study?"**

2) If yes, say **"ok, great, but first I have to ask you a few questions and give you a quick screening measure to make sure you qualify…it'll take just a couple minutes"**; go over Inclusion Criteria Checklist with the patient.

3) If they appear to meet the criteria, administer the MMSE (skip down to #4). If they don't meet criteria, say **"I'm sorry, but based on this information you do not meet criteria for participating in this study. Please ask your doctor for a referral to neuropsychology if you would like to receive cognitive testing. You can also ask your doctor for a referral to a nutritionist if you would like."**

4) If they attain a total score of 18 or above on the MMSE, go to #5. If they attain a total score below 18, read the script listed in #3 and discontinue.

5) Hand them one copy of the California Experimental Subject's Bill of Rights, saying **"This document explains your rights as a potential participant."** Then read the page to them and have them sign your copy, indicating that they understand their rights.

6) Hand them one copy the informed consent document, saying **"Now I'll tell you about the study, what we would ask you to do, and what the potential risks and benefits are."** Then read it to them and provide opportunities for them to ask any questions they may have. If they agree to participate, have them sign/date the bottom and initial/date each page on your form (tell them the correct date if they don't know); then you sign your form.

7) Give them a copy of the Authorization for Use of Protected Health Information form, saying **"This is the last form I have for you. It has to do with how your medical information can be used."** Then read it to them and have them sign yours, and you sign yours.

8) Say **"I'll go make a copy of these forms so you can keep them. I'll be right back."**

9) When you return, say **"Here are your copies. Thank you so much for your involvement in this. A member of the neuropsychology team will meet with you within the next couple days to do some brief testing. If, in the meantime, you have any questions or concerns, please let your doctor and nurses know or call the number on that form I gave you. Thanks."**

APPENDIX G

STUDY PROCEDURE FOR NEUROPSYCHOLOGY

1) Paolo Jorge (Dr. Kim's resident) will email Dr. Fogel and Jeff to inform that a particular patient has been admitted who meets the study's inclusion criteria, and will place the original consent forms under Dr. Fogel's office door. He will also contact Dr. Kim to have her place an order for NP to see the patient for research purposes.

2) NP should schedule Jeff for a 1-hour appointment with the patient at the earliest convenience to complete the baseline NP battery.

3) Print an NP time 1 battery at one of the computers on 1500 or 1100 and administer to the patient. Put only the subject number (e.g., subject 1) on the record form, not any protected health information.

4) Fax the informed consent document, Authorization for Use of PHI document, and CA Experimental Subject's Bill of Rights form (from the packet Paolo Jorge placed under Dr. Fogel's door) using one of the fax machines near a nursing station to [omitted].

5) Using the copying machine in room 117, scan/email the NP packet (not scored) to John at jbellone@llu.edu. Also, scan/email the 3 signed consenting documents and MMSE (from the packet Paolo Jorge placed under Dr. Fogel's door) in a separate email to John at jbellone@llu.edu.

6) Put the completed NP packet in the file cabinet in room 111. If the office is unavailable, place it in Dr. Fogel's box near the reception desk in the lobby.

7) Email Dr. Kim to inform her the NP time 1 testing is completed so she can put in the order for pharmacy to begin treatment administration.

8) Schedule Jeff for a 1-hour appointment with the patient 16 days after the time 1 testing, unless you discover the patient is being discharged early, in which case administer the time 2 battery prior to discharge.

9) The time 2 battery and script can be printed on unit 1500 or 1100.

10) After completing time 2 administration, use the copying machine in room 117 to scan/email the packet (again, not scored) to John (jbellone@llu.edu), and place the forms in the file cabinet in room 111.

Thank you!

APPENDIX H

STUDY INFORMATION SHEET FOR NURSING STAFF

What we are doing: We are examining whether dietary supplementation with an antioxidant (pomegranate extract) can help promote healthy cognitive functioning (i.e., thinking ability, such as memory or attention) as a component of recovery after stroke.

Which patients are eligible: In order for patients to participate, they must have suffered a recent ischemic stroke, be an inpatient at LLUMC Rehabilitation Institute, be between ages 18-89, speak English fluently, have at least 6 years of education, be able to speak and understand language, have no history of allergy to pomegranates, have not had a cerebral hemorrhage in the past 6 months, not be on warfarin (Coumadin), have not undergone brain surgery in the past month, have no history of traumatic brain injury, have no history of neurodegenerative disease or neurologic condition with known cognitive impact, and have no active renal disease or liver disease.

What patients are being asked to do: We will randomly assign patients to either receive an antioxidant supplement or a placebo capsule, and they will be administered this treatment twice per day for two weeks during their hospital stay. Neither the patient nor anyone at the hospital will know which pill the patient is receiving. The neuropsychology department will be conducting cognitive testing (about 1 hour of paper and pencil types of tests) before and after the two weeks of treatment to see if there is any improvement in thinking skills.

What the potential risks are: Although there are no documented cases of negative effects of pomegranate products, there have been reports that this antioxidant has effects similar to aspirin and causes blood to clot less easily, which could increase the risk of bleeding. Also, it is possible that some individuals may experience an allergic reaction or potential interactions between the supplement and the medicines they are taking. We have taken extra measures to reduce these risks, and do not anticipate anything of this nature happening to the study participants.

What your role is: Everything should be taken care of by study staff, which includes the neuropsychology department, pharmacy department, and select physicians. Nursing staff's role will be to administer the pill provided by the pharmacy (either the antioxidant or placebo) with the patient's 9am and 9pm medications. Please treat the patient like any other patient on the unit, but keep an eye out for any potential negative effects of the treatment (discussed above). If anything like that occurs, please contact a physician immediately and call one of the numbers listed below to inform study staff of the incident. Also, please do not attempt to discover which treatment the patient is receiving, and try not to let family members see the capsule's appearance.

Who to contact: If you have any questions or concerns, please call either John Bellone, M.A., at [omitted] or Rich Hartman, Ph.D., at [omitted].