UNIVERSITY OF ALBERTA

THE EFFECTS OF A WESTERN DIET ON STROKE SEVERITY AND FUNCTIONAL OUTCOME FOLLOWING GLOBAL ISCHEMIA IN RATS

BY

ANASTASIA PASCHALES ARVANITIDIS

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE

DEPARTMENT OF PSYCHOLOGY

©ANASTASIA PASCHALES ARVANITIDIS
FALL 2009
EDMONTON, ALBERTA

PERMISSION IS HEREBY GRANTED TO THE UNIVERSITY OF ALBERTA LIBRARIES TO REPRODUCE SINGLE COPIES OF THIS THESIS AND TO LEND OR SELL SUCH COPIES FOR PRIVATE, SCHOLARLY OR SCIENTIFIC RESEARCH PURPOSES ONLY. WHERE THE THESIS IS CONVERTED TO, OR OTHERWISE MADE AVAILABLE IN DIGITAL FORM, THE UNIVERSITY OF ALBERTA WILL ADVISE POTENTIAL USERS OF THE THESIS OF THESE TERMS.

THE AUTHOR RESERVES ALL OTHER PUBLICATION AND OTHER RIGHTS IN ASSOCIATION WITH THE COPYRIGHT IN THE THESIS AND, EXCEPT AS HEREIN BEFORE PROVIDED, NEITHER THE THESIS NOR ANY SUBSTANTIAL PORTION THEREOF MAY BE PRINTED OR OTHERWISE REPRODUCED IN ANY MATERIAL FORM WHATSOEVER WITHOUT THE AUTHOR'S PRIOR WRITTEN PERMISSION.

Examining Committee

Fred Colbourne, Psychology/Neuroscience

Dallas Treit, Psychology

Chris Sturdy, Psychology

Kathryn Todd, Neuroscience

DEDICATION

I would like to dedicate this thesis to my father, who has always been my guiding light, strength, and inspiration.

ABSTRACT

A diet high in saturated fat and similar in composition to western diets (WD) has been shown to exacerbate injury following traumatic brain injury. Thus, we investigated the effects of a WD on cell death and functional outcome following global ischemia. First we assessed the effects of a 60-day WD regimen on temperature, activity and glucose levels in normal rats (Experiment 1). Second, we evaluated the influence of a 60-day WD regimen on hippocampal CA1 injury and learning and memory impairments following global ischemia in rats (Experiment 2). Briefly, results from experiment 1 revealed no differences in glucose or temperature profiles between animals fed the WD and CD; however, WD animals were significantly less active than CD animals. Eight minutes of ischemia in experiment 2 induced severe hippocampal CA1 cell loss (~90%) and severe learning and memory impairments relative to unoperated controls. However, the WD did not exacerbate CA1 injury or behavioural deficits. These findings suggest that a 60-day WD regimen does not significantly influence recovery following global ischemia. However, previous literature on saturated fat and neuronal plasticity suggest that our feeding protocol was not long enough.

Thus, the second portion of my thesis examined the effects of a 120-day WD regimen on stroke severity and cognition following global ischemia. We hypothesized that a prolonged WD would aggravate hippocampal CA1 cell death and spatial learning and memory deficits as compared to animals fed a CD. Briefly, I found that the surgical protocol used to induce a global ischemic insult, in this case, did not produce consistent damage across all animals. Plausible reasons for this surgical variability and future directions are discussed.

ACKNOWLEDGEMENT

I would like to thank my family, especially my grandparents, for their love and support throughout the course of my thesis. I would also like to thank the members of my committee (Drs. Dallas Treit and Chris Sturdy) for their comments and suggestions. I am indebted to Sylvia Zafiriu (aka "my ruv"), who has been there from pre-beginning to end with her unconditional friendship and much needed comic relief. I am also grateful to Hang Huynh, Janelle Pakan, Angela Nguyen, Reginia Yan, and Angela Auriat, who have all made grad school enjoyable and memorable. Lastly, I would like to thank Dr. Frederick Colbourne for his guidance and supervision throughout the entire thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-VO – two-vessel occlusion

4-VO – four-vessel occlusion

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid

ATP – adenosine triphosphate

BDNF – brain-derived Neurotrophic factor

BP – blood pressure

Ca^{2+} – calcium

Cl^- – chlorine,

CD – control diet

ctHb – hemoglobin

iNOS – nitric oxide synthase

IL-1β – interleukin-1β

ISC – ischemia

K^+ – potassium

MABP – Mean arterial blood pressure

MWM – Morris Water Maze

Na^+ – sodium,

NMDA – *N*-methyl-D-aspartate

NO-ISC – non-ischemic

PEM – protein energy malnutrition

TNF-α – tumoral necrosis factor

T_s – skull temperature

WD – western diet

CHAPTER 1

INTRODUCTION

1.1 Overview of Strokes

Stroke is the second leading cause of death and the main cause of disability worldwide. With staggering human and economic tolls, stroke imposes a major burden to society. Stroke has a mortality rate of approximately 32%, and leaves more than 50% of survivors with debilitating impairments (Rothwell et al., 2004). Stroke is also a major cause of depression, dementia, epilepsy, and falls. Stroke expenditure for the United States alone was estimated to be $53.6 billion in 2004, and is expected to rise even more with the increasing elderly population (American Heart Association, 2004).

There are two types of strokes: hemorrhagic and ischemic strokes. Hemorrhagic strokes result from the rupture of a blood vessel(s) in the brain (primary intracerebral hemorrhage - ICH) or on the surface of the brain (subarachnoid hemorrhage). Hemorrhagic strokes account for 15% of all strokes (Thrift et al., 1995), and about 30% of thrombo-embolic strokes (stroke resulting from emboli lodging in cerebral arteries) undergo hemorrhagic transformation (Lyden and Zivin, 1993), especially following treatment with anti-coagulants (i.e., tissue plasminogen activator) (Larrue et al., 1997); however, they are often the most lethal and functionally devastating. For instance, injury following ICH has a mortality rate approaching 50% at 30 days (Broderick et al, 1999), and survivors are left with permanent functional impairments. Accounting for the remaining 85% is cerebral ischemia, which results from a sudden interruption of blood flow to the brain (Block, 1998). Cerebral ischemia can occur in two forms: focal or global ischemia. Focal ischemia results when an artery (commonly the middle cerebral artery in humans) is occluded (either by a thrombus or embolus) preventing oxygen and nutrients from supplying that particular area of the brain. Global ischemia on the other hand occurs when blood flow to widespread areas of the central nervous system is severely compromised, such as occurs with

human cases of cardiac arrest. It affects the entire brain, is dense (or complete), and transient (Siesjo et al, 1990). The present thesis will focus on global ischemic strokes.

1.2 GLOBAL ISCHEMIA

Cardiac arrest results in global hypoxic-ischemic injury, which is a significant cause of mortality and severe neurologic disability. It has a mortality rate ranging from 67 to 90% (Sunnerhagen et al., 1996), leaving more than 50% of cardiac arrest survivors with permanent neurological deficits (Herlitz et al., 2000; Pusswald et al., 2000), and another 50% of survivors to experience secondary anoxic brain damage (Pusswald et al., 2000).

Left untreated, the brain may be able to tolerate ischemia for up to 2 to 4 minutes (Krause et al., 1986), after which irreversible neuronal damage ensues. Arrest times exceeding 15 minutes are for the most part associated with poor outcomes (Cummins et al., 1991; Cummins et al., 1987; Earnest et al., 1980; Eisenberg et al., 1980; Bedell et al., 1983). Early activation of both basic and advanced life support, early return of spontaneous circulation (Cummins et al., 1991), and neurological status on the second day following cardiac arrest predicts survivors' hospital discharge rate and quality of life (Earnest et al., 1980). Cardiopulmonary resuscitation successfully restores spontaneous circulation to approximately 70,000 patients per year in the United States alone (Krause et al., 1986), but only 10-20% of these patients show a recovery; the others pass away or persist in a vegetative state (Basseti et al., 1996).

Patients who show some recovery display neuropsychological deficits, which vary with cause of ischemia and duration. For instance, ischemia caused by marked hypotension during cardiopulmonary operations leaves 35% of patients with neuropsychological deficits, whereas ischemia caused by cardiac arrest leaves 50% of patients with neuropsychological deficits (Shaw

et al., 1987). Cardiac arrest results in ischemic cerebral damage of variable extent, causing damage to the cortex and basal ganglia, and leading to persistent and severe physical, cognitive and behavioural impairments (Sauve et al., 1996). For instance, cardiac arrest patients endure moderate to severe neuropsychological deficits even 1 year later (Roine et al., 1993), even with intensive rehabilition (Pusswald et al., 2000).

Following cardiac arrest, three typical clinical syndromes occur: amnesic syndrome, severe dementia with very severe physical and intellectual impairment, and moderate dementia with deficits in more than three cognitive domains. Patients with amnesia have preserved shorter-term and working memory but exhibit deficits in orientation, verbal and non-verbal memory, attention, and visuospatial impairments (Pusswald et al., 2000; Zola-Morgan et al., 1986). Patients with moderate to severe post-anoxia dementia show cognitive impairments such as disorientation, mental slowing, amnesia, and lack of awareness. Language and visuospatial abilities are preserved, a clinical profile that differs markedly from the clinical pattern of other dementia illness' (Pusswald et al., 2000). Patients with severe physical deficits require support in walking while others may not be able to walk at all. Severe to moderate intellectual decline also occurs. The most important long-term morbidities of most patients include psychological (i.e., depression), behavioural (i.e., personality changes), social (i.e., withdrawn) and family disturbances (i.e., inadequate social support, decrease of fixed income) (Brooks et al., 1983).

Clinical therapies directed at reducing the extent of neuronal damage by means of pharmacological agents have proven unsuccessful (i.e., barbiturates (Brain Resuscitation Clinical Trial I Study Group, 1986), calcium channel antagonists (Brain Resuscitation Clinical Trial II Study Group, 1991), benzodiazepines, magnesium, and steroids (Jastremski et al., 1989). Mild to moderate induced hypothermia has proven to be the only clinically effective tool for attenuating

ischemic brain damage (Van Der Worp, 2007). Benson and colleagues (1959) were one of the first groups to highlight the potential efficacy of hypothermia. This group of researchers applied lengthy (1-8hr) hypothermia (30°C - 32°C) to 12 cardiac arrest patients following a 1-3 hr delay. Although not statistically significant, 6 of twelve cardiac arrest patients survived in the hypothermia group as compared to 1 of 7 in the untreated control group. In recent years however, hypothermia has been shown to positively and remarkably affect outcome following cerebral ischemia. A recent meta-analysis (Polderman, 2008) found that patients who received hypothermia were more likely to be discharged with no or minimal neurological damage, even when age, gender, time from collapse to return of spontaneous circulation, and technique were all controlled for.

1.3 Risk Factors

Numerous risk factors contribute to the development of ischemia, and while most risk factors have an independent effect, there are important interactions between individual factors, as will be discussed shortly. Non-modifiable risk factors include age, sex, race-ethnicity, low-birth weight, and genetic factors. Age is the single most important risk factor for ischemia, as the risk of ischemic stroke doubles for every successive decade after the age of 55 (Brown et al., 1996; Wolf et al., 1992). Stroke is more prevalent in men than in women (Brown et al., 1996), particularly between the ages of 44 - 85. Between 35 to 44 years of age, and > 85 years of age, women have slightly greater age-specific stroke incidence than their male counterparts (Sacco et al., 1998), owing to factors such as oral contraceptive use and pregnancy, which contribute to the increased risk of stroke in young women (Kittner et al., 1996; Qureshi et al., 1997; Mosca et al., 1997). Ethnicity and race also seem to play a role in risk of ischemic stroke. In general, African

Americans (Broderick et al., 1998; Sacco et al., 1998) and some Hispanic Americans (Gorelick et al., 1998; Howard et al., 1994) have higher ischemic stroke incidence and mortality than European Americans, possibly owing to a higher prevalence of hypertension, obesity and diabetes within the black population (Giles et al., 1995; Gillum et al., 1999). Low-birth rate (Barker et al., 2003; Lackland et al., 2003), and paternal and maternal history of stroke (Kiely et al., 1993) have been associated with an increased stroke risk.

Hypertension is the most important modifiable risk factor for ischemic stroke. High blood pressure increases the risk of stroke (Lewington et al., 2002), and increases with increasing age (Burt et al., 1995), such that normotensive individuals at 55 years of age have a 90% chance of developing hypertension (Vasan et al., 2002). The relationship between smoking and ischemic stroke is indisputable. The risk of an ischemic stroke doubles with cigarette smoking (Manolio et al., 1996; Rodriguez et al., 2002; Wolf et al., 1991a), likely through thrombus generation in narrowed arteries and atherosclerosis. However, passive cigarette smoking (i.e., exposure to environmental tobacco smoke) also doubles a persons chances of an ischemic stroke (Barnoya et al., 2004; Bonita et al., 1999; You et al., 1999), also through the development of atherosclerosis (Howard et al., 1998). Persons with type 2 diabetes are more susceptible of developing atherosclerosis, hypertension, obesity and abnormal blood lipids, which all serve to increase the risk of an ischemic stroke by as much as 6 fold (American Heart Association, 2004). Atrial fibrillation alone is associated with a 3- to 4-fold increased risk of stroke (Wolf et al., 1991), and is a source of cardiogenic emoboli that increase one's risk of cardiac arrest (Benjamin et al., 1998). Physical activity has been shown to reduce the risk for stroke (Abbott et al., 1994; Fletcher, 1994; Kiely et al., 1994; Gillum et al., 1996). Several aspects of diet have been associated with stroke risk and will be discussed in greater detail in the following section.

1.4 DIET AND STROKE

Nutrition's role in stroke risk and prevention, as well as stroke outcome and recovery, is becoming increasingly more apparent. The consequences of stroke are often incapacitating and irrecoverable; thus stroke prevention is pivotal. Lifestyle habits, which, as compared to surgical or medical therapies, are relatively low risk, low cost, and widely available, are paramount for prevention; even small reductions in risk due to lifestyle changes can have substantial health benefits on a population or public health level. In the United States alone, poor dietary habits are estimated to cost $42 billion annually in medical expenses and lost productivity from chronic diseases (Preventing Obesity and Chronic Diseases Through Good Nutrition and Physical Activity, 2004). Potential mechanisms through which nutrition may influence stroke risk are blood pressure, insulin resistance, inflammation, thrombosis, endothelial function and oxidation (Ding and Mozaffarian, 2006). Diets low in sodium, and high in potassium (Hajjar et al., 2001; Stamler et al., 1991), magnesium (Joffres et al., 1987), calcium (Jorde et al., 2000), and fruit and vegetable consumption (Bazzano et al., 2003) lower blood pressure and consequently reduce stroke risk. Dietary fiber (e.g., fruits, vegetables, legumes), rich in vitamins and antioxidants, is thought to favorably affect serum lipids, glucose levels, insulin sensitivity, and blood pressure (Burke et al., 2001; Pereira and Pins, 2000), and by these means lower stroke risk. However, dietary fiber from cereal has been shown to predict even lower risk of ischemia than dietary fiber from fruits and vegetables (Ascherio et al., 1998; Mozaffarian et al., 2003), but this difference suggests that other dietary fibers related to cereals, rather than fiber content per se, may be influencing stroke risk. Potential candidates include (1) phyotchemicals and nutrients in whole grains and (2) carbohydrate quality (glycemic index and load). The effects of other dietary factors on stroke risk are less clear. For instance, non-dairy calcium had a weaker association

with the inverse relationship between calcium intake and stroke risk than calcium consumed in diary products.

Evidence regarding fat intake is also inconclusive. Many authors (Gillman et al., 1997; McGee et al., 1985; Reed et al., 1988) found higher intakes of saturated fat to lower stroke risk and mortality, while others (He et al., 2003) found no association between types of fat or total fat to be significantly associated with incidence of ischemic strokes. Iso and colleagues (2001) found that polyunsaturated fat predicted lower risk of ischemic stroke, while other prospective cohort studies (Gillam et al., 1997; He et al., 2003; Iso et al., 2001) found no significant association between polyunsaturated fatty acid intake and risk of ischemic stroke. With respect to trans fats, He and colleagues (2003) and Iso and colleagues (2001) found no significant association between trans fat intake and stroke, and are the only two prospective studies to report on the association between trans fat and stroke. Intake of marine monounsaturated fat (e.g., fish) two to four times a week, as compared to less than once a month, has been associated with ~20% lower risk of ischemic stroke (He et al., 2004a).

While experimental studies suggest that antioxidants may reduce risk of stroke (Giugliano, 2000), clinical trials have not been so clear-cut. Studies using beta-carotene (e.g., vitamin A) supplementation have found a non-significant lower risk (Keli et al., 1996), a trend toward lower risk (Daviglus et al., 1997), a 23% lower risk (Hirvonen et al., 2000), and no effect on overall stroke incidence (Leppala et al., 2000). Conversely, clinical trials using alpha-tocopherol (e.g., vitamin E) supplementation have been consistently associated with a non-significant risk of ischemic stroke (Hirvonen et al., 2000; Keli et al., 1996; Leppala et al., 2000; Yochum et al., 2000). All but one study (Yokoyama et al., 2000) using ascorbic acid (e.g., vitamin C) supplementation have found no significant association with stroke risk (Daviglus et

al., 1997; Keli et al., 1996). The Heart Protection Study Collaborative Group (2002) found no significant association between combined vitamin C, E, and beta-carotene supplementation and risk of cerebral ischemia. Lastly, most studies using flavonoid supplementation have found no significant association with stroke risk (Hirvonen et al., 2000; Sesso et al., 2003; Yochum et al., 1999), with the exception of Keli and colleagues (1996), who found a lower associated risk of stroke with flavonoid supplementation. B vitamins, such as pyridoxine (B_6), Folate (B_9), and Cobalamin (B_{12}) may reduce cardiovascular risk via effect on plasma homocysteine, antioxidant defenses, and endothelial function (Verhaar et al., 2002). Some (Bazzano et al., 2002; He et al., 2004b), but not all (Al-Delaimy et al., 2004) prospective cohort studies have observed inverse associations between B_6, B_{12} and B_9 intake and risk of cerebral ischemia.

Nutrition and its role in stroke outcome has been evaluated in both the clinical and experimental setting. Malnutrition is a common problem among elderly stroke patients (Axelsson et al., 1988, 1989; Gariballa et al., 1998; Unosson et al., 1994), with approximately 16% of stroke patients presenting with protein-energy malnutrition upon admission to the hospital (Paterson and Juurlink, 1996). Commonly, body fat and visceral proteins are the nutritional constituents found to be significantly decreased in patients (Axelsson et al., 1988; Davis et al., 2004; Unosson et al., 1994). Nutritional status (as measured by serum albumin, but less commonly by weight/height measurements, blood tests and anthropometry) declines during hospital stay, with estimates of 26.4% malnutrition at 1 week, 35% malnutrition at 2 weeks, and 49% malnutrition reported by the time of admission to a rehabilitation unit (Finestone et al., 1995). Deterioration of nutritional status following stroke is attributed to physical and mental incapacity, perception and communication problems, depression, eating and swallowing difficulties, and poor nutritional support (Finestone et al., 1995; Gariballa et al., 1998;

Nyswonger and Helmchen, 1992). Finestone and colleagues (1996) found that the rate of functional improvement in a rehabilitation center following stroke was significantly slower and the duration of stay in the unit longer for the malnourished patients. Indeed, several studies found malnutrition to be a predictor of poor outcome following stroke. For instance, post-admission nutrition has been associated with mortality and dependence at 1 month after stroke (Davalos et al., 1996; Davis et al., 2004; Gariballa et al., 1998), and with death and dependence at 6 months after stroke (FOOD Trial Collaboration, 2003). Protein-energy malnutrition, evaluated in an animal model of stroke, produced similar findings. Bobyn and colleagues (2005) showed that protein-energy malnutrition impaired functional outcome as demonstrated by an inability to habituate in an open field test as long as 10 days after ischemia. The ability to habituate to an open field is utilized as a measure of hippocampal function and thus spatial learning and memory. Following global ischemic injury, animals fed a diet deficient in protein displayed greater spatial memory impairments in the open field test than animals fed a control diet. Other nutritional constituents and aspects of stroke outcome have been assessed in various animal models of stroke. Clarke and colleagues (2005) found omega-3 polyunsaturated fatty acids to exacerbated forelimb motor function following hemorrhagic stroke.

It is imperative that concrete nutritional intervention protocols are put in place to improve patient survival and quality of life; nonetheless few clinical studies have investigated whether nutritional intervention in acute stroke patients improves outcome. Nevertheless, the aforementioned section highlights the therapeutic potential of effective nutritional interventions, which may effectively reduce brain damage and improve functional outcome in both animals and humans. For instance, increasing intake of visceral proteins and fats in elderly stroke patients admitted to hospitals can decrease the amount of time spent in the hospital and increase rate of

functional improvement in a rehabilitation center following stroke. However, the effects of dietary constituents on severity and recovery following stroke may be better worked out in controlled animals models, because with humans there are co-morbidities (e.g., hypertension), which make segregating dietary influences from other factors (e.g., to distinguish the effects of diet vs. hyperglycemia) difficult at best. Controlled experimental settings, whereby dietary manipulations in rodents can be assessed, may be key in facilitating the implementation of nutritional intervention programs for humans in the event of cerebral ischemia.

1.5 Pathology of Global Ischemia

Global cerebral ischemia leads to a cascade of pathophysiological processes. First is a fall in phosphorylation potential. The brain's energy supply, adenosine triphosphate [ATP], is directly dependent on continuous cerebral perfusion. ATP is used to maintain transmembrane sensitive structures such as the Na^+- K^+ ATPase and Ca^{2+} ATPase pumps, and various neurotransmitter reuptake systems (Erecinska et al., 1989). A loss of glucose and oxygen during cardiac arrest causes anaerobic glycolysis, leading to a decrease in energy stores. This results in membrane depolarization with an ensuing loss of ion homeostasis; potassium (K^+) exits the cell, while sodium (Na^+), chlorine (Cl^-), calcium (Ca^{2+}) enter the cell, and water is forced in the cell by osmotic forces. Membrane depolarization and its associated Ca^{2+} influx trigger excessive amounts of glutamate to be released from the presynaptic terminal, and with the inhibition of glutamate reuptake mechanisms, extracellular glutamate massively increases (Benveniste et al., 1984; Globus et al., 1991). Glutamate activates α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA), Kainate, and *N*-methyl-D-aspartate (NMDA) receptors (Seeburg et al., 1993). AMPA receptors activate Na^+ ion channels allowing sodium influx. Activation of the

NMDA receptor in turn leads to an enormous and sustained intracellular rise in Ca^{2+} (Mody and MacDonald, 1995), which in turn stimulates Ca^{2+} release from intracellular stores (endoplasmic reticulum) into the cytosol (Farooqui et al., 1994). With Ca^{2+}-ATPase failure, Ca^{2+} accumulates within the cell, eventually activating various enzymes (i.e., proteases, phospholipases, endonucleases and nitric oxide synthase [iNOS]), which damages the cell membrane, nucleus and other organelles (Kristian and Siesjo, 1998). For instance, sustained activation of phospholipases results in the release of free fatty acids (i.e., arachidonic acid from phospholipase A_2) from membrane phospholipids (Farooqui et al., 1994). Arachidonic acid in turn triggers uncontrolled arachidonic acid metabolism to produce free radicals (Grace, 1994). Indeed, rises in intracellular Ca^{2+} contribute to the production of free radicals in two ways. First, high Ca^{2+} levels lead to mitochondrial Ca^{2+} cycling, which increases mitochondrial production of free radicals (Richter and Kass, 1991). Second, Ca^{2+} converts xanthine dehydrogenase to xanthine oxidase, resulting in increased free radical production (Sussman and Bulkley, 1990).

During postischemic reperfusion, the high-energy ATP load recovers rapidly and approaches normal levels within 15 minutes of normalization of blood flow (Siesjo and Ljunggren, 1973). Despite the normalization of cerebral blood flow and energy stores, reoxygenation by reperfusion has the potential to cause molecular damage through a second series of reactions. The absence of oxygen and nutrients from blood creates a condition in which the restoration of circulation leads to secondary changes that can potentially lead to molecular damage (i.e., inflammation and oxidative damage through the induction of oxidative stress). For instance, cell structure degradation is relatively restricted in the absence of oxygen, as is the case with polyribosomes, whose protein synthesis is seized during complete ischemia; with recirculation however, polyribosomes disaggregate (Kleihues et al., 1975).

Inflammation is a key player in the pathophysiological cascade of ischemic neuronal damage. Following global ischemia, neutrophil leukocytes infiltrate the regions of selective neuronal damage (Clarke et al., 1995), and microglia and astrocytes are activated, which last up to 4 weeks (Morioka et al., 1992; Petito et al., 1990). Reactive oxygen species (ROS) generated during the excitotoxic phase contribute to the deterioration of the blood-brain-barrier (BBB) (Yang and Betz, 1994), which leads to vasogenic edema (Pappius, 1989). Vasogenic edema is then exacerbated by invading leukocytes (Shiga et al., 1991), and the edema in turn causes compression of and damage to tissue. The inflammatory cells synthesize and secrete proinflmmatory cytokines (interleukin (IL)-1β and tumoral necrosis factor (TNF)-α), and activate the isoform of iNOS, all of which can be neurotoxic. Very early after global ischemia IL-1β and TNF-α mRNA and proteins are upregulated within the hippocampus (Saito et al., 1996; Sairanen et al., 1997; Uno et al., 1997). Furthermore, beginning 3 days after global ischemia and persisting for up to 1 month, iNOS is expressed in reactive astrocytes within the hippocampal CA1 sector (Endoh et al., 1994). Anti-inflammatory drugs, anti-inflammatory cytokines and substances which either decrease the synthesis of proinflammatory cytokines or inhibit the activity of iNOS markedly reduce neuronal damage in response to global ischemia (Block et al., 1997; Coimbra et al., 1996; Heinrich-Noack et al., 1996; Kohno et al., 1996).

Diet has been shown to influence some of the aforementioned mechanisms, and by doing so, has been shown to potentiate or ameliorate brain injury. Hyperglycemia has been shown to increase intra- and extracellular acidosis (Myers, 1979; Siesjö, 1981, 1984), edema, and postischemic seizures (Siesjö, 1988, 1985). For instance, animals made hyperglycemic prior to forebrain ischemia show more rapidly maturing lesions, which are often pan-necrotic in nature, and extend to additional structures, such as the cingulated cortex, thalamus, and substantia nagra

(Inamura et al., 1987; Pulsinell et al., 1982; Smith et al., 1988). Protein-energy malnutrition and high-fat diets have been shown to increase levels of oxidative stress in the hippocampus following ischemia (Bobyn et al., 2005), and B-group vitamin supplementation given immediately post-stroke has been suggested to mitigate oxidative damage and inflammation (Ullegaddi et al., 2004). Furthermore, protein-energy malnutrition is thought to increase the inflammatory response of the brain to global ischemia (Bobyn et al., 2005). In addition, chronic pretreatment with blueberry, spinach, or spirulina for 4-weeks has been shown to attenuate ischemic brain injury in rats by reducing ischemic/reperfusion-induced apoptosis and cerebral infarction (Wang et al., 2005).

Distinct neuronal populations have very different vulnerabilities to global ischemia. Following brief periods of global ischemia (~8 min), severe damage (~60%-100% necrosis) is seen throughout hippocampal CA4 sector cells, the subiculum, and anterior hippocampal CA1 sector cells. In addition, the dorsolateral striatum, lateral reticular nucleus of the thalamus, and mid neocortical layers (layers III-V) are selectively damaged. Infarcts are not seen in the neocortical region; however, infarctions often can be seen in the thalamus, substantia nigra, pars reticularis, amygdala, entorhinal cortex, and septal nuclei. With prolonged ischemia (~20 min), neocortical infarction evolves between the anterior and middle cerebral arteries, tapering posteriorly, and cell death extends outside the aforementioned selectively vulnerable regions, such as to hippocampal CA3 cells, glia and even endothelial cells (Pulsinelli, 1982; Smith et al., 1984). Hippocampal CA1 neurons are the most widely studied; they are one of the most vulnerable population of cells to global ischemic injury, are relatively easy to quantify (e.g., large and arranged in a laminar fashion), and are also the slowest to die (e.g., cell death can continue for months (Colbourne et al., 1999; Kirino, 1982; Pulsinelli et al., 1982a)).

1.6 Animal Models of Global Ischemia

Before therapies go to clinical trials, they must first be assessed in animals and deemed safe and effective. Thus, appropriate animal models of global ischemia, in which the pathophysiology mimics the human condition, are essential. Indeed, three widely used rodent models of global ischemia were established in the early 1980's, which approximate the pattern of injury seen in the human condition rather well: the four-vessel occlusion (4-VO) in the rat, the two-vessel occlusion (2-VO) combined with hypotension in the rat, and the 2-VO in the gerbil.

The 4-VO involves permanent coagulation of the vertebral arteries (which has no deleterious effects) and temporarily (anywhere between 10-30 min) occluding the two common carotid arteries (Pulsinelli et al., 1982a). Blood flow is reliably <3% of control values in the hippocampus, striatum, and neocortex. The 2-VO in gerbils involves occluding both common carotid arteries, which is sufficient to induce a global cerebral ischemia because there are no posterior communicating arteries in gerbils (i.e., an incomplete circle of Willis). Changes in blood flow are similar to those in the rat models; blood flow in the hippocampus is ~4% of control values and <1% in the cortex (Kirino, 1982). The vessel occlusion models are often termed "incomplete ischemia" because of the residual blood flow. Indeed, "complete" models of global ischemia have been established where blood flow to the entire brain is zero or <1%, achieved by neck-cuff (Ljunggren et al., 1974), cardiac arrest (Ekholm et al., 1992), or by ligating or compressing all arteries stemming from the heart (Kawai et al., 1992).

Although the three main models are technically not global, a large part of the forebrain is quite uniformly affected, and the histopathology, blood flow and metabolism thoroughly characterized (Araki et al., 1992; Kirino, 1982; Pulsinelli et al., 1982a,b; Smith et al., 1984). Ischemic durations of 3 minutes and 10 minutes in the gerbil and rat, respectively, cause

selective neuronal damage in the CA1 sector of the hippocampus (Pulsinelli et al., 1982a; Kirino, 1982), which develops within 48-72 hr following ischemia (Kirino 1982). Twenty minutes of ischemia in rats leads to almost complete neuronal damage in the CA1 sector, and increased to 20 – 30 minutes causes neuronal damage in the dorsolateral striatum of medium seized spiny striatal projection neurons (Chesselet et al., 1990; Pulsinelli et al., 1982a) and layers 3, 5 and 6 of the cortex (Pulsinelli et al., 1982a).

The majority of global ischemic experiments have used rodents, although cats, rabbits, dogs and non-human primates have also been used. Several reasons support using rodents in global ischemic research. First, the anatomy between higher species, human included, and rodents does not differ markedly. Additionally, there is a higher social and ethical acceptance with rodents, lower costs, and a rather high homogeneity owing to inbreeding.

1.7 BEHAVIOURAL DEFICITS

Many treatments appeared to provide neuroprotection following cerebral ischemia when histological endpoints were used (i.e., hypothermia, neuroprotective drugs); however, with the exception of hypothermia, these same treatments proved considerably less efficacious when functional endpoints were incorporated. Indeed, many stroke treatments showed considerable benefits in the experimental setting but failed to show such benefits in the clinical setting, owing partly to the over reliance on histological measures. Functional assessments help detect covert injury, can better predict histological outcome at earlier time points than histological data itself, and is the most important clinical endpoint. Experimental stroke studies must therefore assess behavioral outcome and its relation to histological outcome to optimize validity. To do so, a

comprehensive histological and functional assessment at long survival should be employed (Corbett and Nurse, 1998).

A number of different tests are used to assess functional outcome following global cerebral ischemia. The Morris Water Maze (MWM), radial arm maze, T-maze, and their respective permutations, are often used to assess working (memory for information that is specific to the trial – a form of short-term memory) and reference memory (memory for spatial orientation and location). While each of these tests has components of both working and reference memory, the degree to which these tasks rely on working or reference memory in order to be successful varies. For instance, standard versions of the water maze and T-maze are used to assess spatial orientation (reference memory) and working memory, respectively.

The MWM in particular has become one of the most frequently used laboratory tools in behavioural neuroscience to study spatial learning and memory. In 1982, Morris, Garrud, Rawlings, and O'Keefe found that hippocampal, but not superficial cortical lesions, resulted in profound and tenacious deficits in rats' ability to locate a hidden platform, independent of motor, motivational and reinforcement factors. Since then, studies have also implicated the medial-frontal cortex (Kolb et al., 1982; Sutherland et al., 1982, 1983), parietal cortex (Dimattia and Kesner, 1985), and total cortex (Whishaw and Kolb, 1984) with respect to reference memory in the MWM. Morris water maze performance has also been shown to be influenced by various factors. For instance, nutrition has been found to alter MWM performance. Coscina and colleagues (1986) found that rats fed a diet rich in polyunsaturated fat, but not a saturated fat or control diet, demonstrated a faster rate of learning in the standard reference-memory procedure of the MWM. Other features of the MWM make it superior to other more traditional procedures such as the T-maze or radial-arm maze (Hagan et al., 1986; Panakhova et al., 1984; Sutherland &

Dyck, 1984). For one, the MWM task only requires a few trials to master, as it is relatively simple to solve. However, in situations where behavioural impairments may not be as pronounced (e.g., smaller stroke), permutations to the MWM task can be applied that make it more difficult (e.g., moving the platform every 2 days as opposed to keeping the platform in the same location throughout testing). This can serve to make the task more sensitive to detecting small but very real treatment effects, as opposed to finding that all rats recover and there are no deficits as the task was too easy. Second, there are several independent measures of spatial learning and memory (e.g., latency, path length, directionality), and can all be easily recorded. Third, the demands placed upon the spatial localization system are much stronger than in a T-maze for example; and so the task cannot be solved efficiently by using non-spatial strategies, like fixed response or odour trails.

Post-global ischemic rats do not show persistent sensory motor deficits as damage in the cortex and striatum are generally not that severe. For instance, Capdeville and colleagues (1986) assessed sensorimotor function following global ischemia by testing for several functions (i.e., grasping, several placement reactions, righting reflexes, two equilibrium tests, flexion reflex and spontaneous motility). Three hours following global ischemia, rats' scores were reduced markedly; however, at 24, 48 and 72 hr after ischemia no differences were detected relative to preischemic scores. However, hippocampal CA1, CA4 and hilar regions are severely damaged following global ischemia, which results in long-lasting and profound memory impairments.

1.8 OBJECTIVE OF THE PRESENT THESIS

The purpose of this thesis was to examine the effects of a western diet (WD) on global cerebral ischemia. Nutrition has not only been shown to modify one's risk of an ischemic event,

it has also been shown to influence outcome following different forms of brain injury. For instance, protein energy malnutrition (PEM) has been shown to exacerbate functional impairments following global ischemia in gerbils (Bobyn et al., 2005), while caloric restriction in rats has been shown to attenuate injury and improve functional outcome following focal ischemia (Yu and Mattson, 1999). Saturated fat was shown to exacerbate injury and cognitive deficits in a model of traumatic brain injury in rats (Wu et al., 2003). Accordingly, certain dietary manipulations have the capacity to attenuate injury, while others may aggravate injury. This suggests that outcome following stroke may be improved by following particular dietary regimens and/or avoiding others.

The effects of a WD in normal (i.e., un-injured) rats has been evaluated by several groups (Greenwood and Winocur, 1990, 1996; Molteni et al., 2002; Winocur and Greenwood, 1993), who showed the WD resulted in persistent and widespread spatial learning and memory impairments, which have been associated with decreases in brain-derived neurotrophic (BDNF) levels (Molteni et al., 2004).

The effects of a WD in global cerebral ischemia have yet to be established. Thus, I sought to evaluate the effects of a WD on cell death and spatial learning and memory in the 2-VO model of global ischemia in rats. However, a WD can influence outcome indirectly (i.e., via cardiovascular dysfunction such as increasing blood pressure), as well as directly (i.e., on neural function) (Molteni et al., 2002). Indeed, to establish the influence of a WD independent of any cardiovascular effects, factors which have been shown to influence outcome (i.e., glucose levels, blood pressure, temperature) were kept within normal (i.e., healthy) ranges for the duration of the experiment. The MWM task was selected to assess learning and memory deficits following global ischemia. Briefly, and as mentioned in the preceding section, the MWM task is sensitive

to spatial learning and memory impairments following hippocampal damage (Morris et al., 1982), a region commonly compromised following global ischemia. Additionally, nutritional factors can influence performance in the MWM task (Coscina et al., 1986). The maturation of injury in the hippocampus is well established, and can be seen up to one month following ischemia (Colbourne et al., 1999; Kirino, 1982; Pulsinelli et al., 1982a). For this reason, spatial learning and memory was evaluated acutely (i.e., 2 weeks following cerebral ischemia) and chronically (i.e., 4 weeks following cerebral ischemia), for a more comprehensive assessment of functional outcome.

1.8 REFERENCE LIST

Abbott, R.D., Rodriguez, B.L., Burchfiel, C.M., Curb, J.D. (1994). Physical activity in older middle-aged men and reduced risk of stroke: the Honolulu Heart Program. Am J Epidemiol, 139, 881-893.

Al-Delaimy, W.K., Rexrode, K.M., Hu, B.F., et al. (2004). Folate intake and risk of stroke among women. Stroke, 35, 1259-1263.

American Heart Association. (2004). Heart Disease and Stroke Statistics: 2004 Update. Dallas, Tex: American Heart Association; 2003.

Araki, T., Kato, H., Kogure, K., and Kanai, Y. (1992). Long-term changes in gerbil brain neurotransmitter receptors following transient cerebral ischemia. Br. J. Pharmacol., 107, 437-442.

Ascherio, A., Rimm, E.B., Hernan, M.A., et al. (1998). Intake of potassium, magnesium, calcium, and fiber and risk of stroke among US men. Circulation, 98, 1198-1204.

Axelsson, K., Asplund, K., Norberg, A., Alafuzoff, I. (1988). Nutritional status in patients with acute stroke. Acta Med Scand., 224, 217-224.

Barker, D.J., Lackland, D.T. (2003). Prenatal influences on stroke mortality in England and Wales. Stroke, 34, 1598-1602.

Barnoya, J., Glantz, S.A. (2004). Second-hand smoke: the evidence of danger keeps growing. Am J Med., 116, 201-202.

Basseti, C., Bomio, F., Mathis, J., and Hess, C.W. (1996). Early prognosis in coma after cardiac arrest: a prospective clinical, electrophysiological, and biochemical study of 60 patients. J Neurol Neurosurg Psychiat, 61, 610-615

Bazzano, L.A., He, J., Ogden, L.G., et al. (2002). Dietary intake of folate and risk of stroke in US men and women: NHANES I Epidemiologic Follow-up Study. National Health and Nutrition Examination Survey. Stroke, 33, 1183-1188.

Bazzano, L.A., Serdula, M.K., Liu, S. (2003). Dietary intake of fruits and vegetables and risk of cardiovascular disease. Surr Atheroscler Rep., 5, 492-499.

Bedell, S.E., Delbanco, T.L., Cook, E.G., Epstein, F.H. (1983). Survival after cardiopulmonary resuscitation in the hospital. N Eng J Med, 309, 569-576.

Benjamin, E.J., Wolf, P.A., D'Agostino, R.B., Silbershatz, H., Kannel, W.B., Levy, D. (1998). Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation, 98, 946-952.

Benson, D.W., Williams, G.R., Spencer, F.C., Yates, A.J. (1959). The use of hypothermia after cardiac arrest. Anesth. Analg., 38, 423-428.

Benveniste, H., Drejer, J., Schousboe, A., Diemer, N.H. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem., 43, 1369-1374.

Block, F., Tondar, A., Schmidt, W., and Schwarz, M. (1997). Delayed treatment with rolipram protects against neuronal damage following global ischemia in rats. Neuro Report, 8, 3829-3832.

Block, F. (1998). Global ischemia and behavioural deficits. Progress in Neurobiology, 58, 279-295.

Bobyn, J.P., Corbett, D., Saucier, D.M., Noyan-Ashraf, H.M., Juurlink, B.H.J., Paterson, P.G. (2005). Protein-energy malnutrition impairs functional outcome in global ischemia. Experimental Neurology, 196, 308-315.

Bonita, R., Duncan, J., Truelsen, T., Jackson, R.T., Beaglehole, R. (1999). Passive smoking as well as active smoking increases the risk of acute stroke. Tob Control, 8, 156-60.

Brain Resuscitation Clinical Trial I Study Group. (1986). Randomized clinical study of thiopental loading in comatose survivors of cardiac arrest. N Eng J Med, 314, 397-403.

Brain Resuscitation Clinical Trial II Study Group. (1991). A randomized clinical study of a calcium-entry blocker (lidoflazine) in the treatment of comatose survivors of cardiac arrest. N Eng J Med, 324, 1225-1231.

Broderick, J., Brott, T., Kothari, R., Miller, R., Khoury, J., Pancioli, A., Gebel, J., Mills, D., Minneci, L., Shukla, R. (1998). The Greater Cincinnati/Northern Kentucky Stroke Study: preliminary first-ever and total incidence rates of stroke among blacks. Stroke, 29, 415-21.

Broderick, J.P., Adams, H.P., Jr., Barsan, W., Feinberg, W., Feldmann, E., Grotta, J., Kase, C., Krieger, D., Mayberg, M., Tilley, B., Zabramski, J.M., Zuccarello, M. (1999). Guidelines for the management of spontaneous intracerebral hemorrhage: A statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Stroke, 30, 905-915.

Brooks, D.N., McKinley, W. (1983). Personality and behavioural change after severe blunt head injury – a relative's view. J Neurol Neurosurg Psychiatry, 46, 336-44.

Brown, R.D., Whisnant, J.P., Sicks, J.D., O'Fallon, W.M., Wiebers, D.O. (1996). Stroke incidence, prevalence, and survival: secular trends in Rochester, Minnesota, through 1989. Stroke, 27, 373-380.

Burk, V., Hodgson, J.M., Beilin, L.J., Giangiulioi, N., Rogers, P., Puddey, I.B. (2001). Dietary protein and soluble fiber reduces ambulatory blood pressure in treated hypertensives. Hypertension, 38, 821-826.

Burt, V.L., Whelton, P., Roccella, E.J., Brown, C., Cutler, J.A., Higgins, M., Horan, M.J., Labarathe, D. (1995). Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988-1991. Hypertension, 25, 305-313.

Capdeville, C., Pruneau, O., Allix, M., Plotkine, M., and Baulu, R.G. (1986). Model of global forebrain ischemia in the unanesthetized rat. J. Pharmacol., 17, 553-560.

Chesselet, M.F., Gonzales, C., Lin, C.S., Polsky, K., and Jin, B.K. (1990). Ischemic damage in the striatum of adult gerbils: relative sparing of somatostatinergic and cholinergic interneurons contrasts with loss of efferent neurons. Exp. Neurol., 1110, 209-218.

Clarke, J., Herzberg, G., Peeling, J., Buist, R., Corbett, D. (2005). Dietary supplementation of omega-3 polyunsaturated fatty acids worsens forelimb motor function after intracerebral hemorrhage in rats. Experimental Neurology, 191, 119-127.

Clark, W.M., Lauten, J.D., Lessov, N., Woodward, W., and Coull, B.M. (1995). Time course of ICAM-1 expression and leukocyte subset infiltration in rat forebrain ischemia. Mol. Chem. Neuropathol., 26, 213-230.

Coimbra, C., Drake, M., Boris-Möller, F., and Wieloch, T. (1996). Long-lasting neuroprotective effect of postischemic hypothermia and treatment with an anti-inflammatory/antipyretic drug. Stroke, 27, 1578-1585.

Colbourne, F., Li, H., Buchan A.M. (1999). Continuing postischemic neuronal death in CA1 – influence of ischemia duration, and cytoprotective doses of NBQX and SNX-111, Stroke, 30, 662-668.

Corbett, D., Nurse, S. (1998). The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol., 54, 531-548.

Coscina, D.V., Yehuda, S., Dixon, L.M., Kish, S.J., Leprohan-Greenwood, C.E. (1986). Learning is improved by a soybean oil diet in rats. Life Sciences, 38, 1789-1794.

Cummins, R.O., Eisenberg, M.S., Litwin, P.E., et al. (1987). Automatic external defibrillators used by emergency medical technicians: a controlled clinical trial. JAMA, 257, 1605-1610.

Cummins, R.O., Ornato, J.P., Thies, W.H., Pepe, P.E. (1991). Improving survival from sudden cardiac arrest: the “chain of survival” concept. A statement for health care professionals from the Advanced Cardiac Life Support Subcommittee and the Emergency Cardiac Care Committee, American Heart Association. Circulation, 83, 1832-1847.

Davalos, A., Ricart, W., Gonzalez-Huix, F., et al. (1996). Effect of malnutrition after acute stroke on clinical outcome. Stroke, 27, 1028-1032.

Daviglus, M.L., Orencia, A.J., Dyer, A.R., et al. (1997). Dietary vitamin C, beta-carotene and 30-year risk of stroke: results from the Western Electric Study. Neuroepidemiology, 16, 637-642.

Davis, J.P., Wong, A.A., Schluter, P.J., Henderson, R.D., O'Sullivan, J.D., Read, S.J. (2004). Impact of premorbid undernutrition on outcome in stroke patients. Stroke, 35, 1930-1934.

Dimattia, B.V., Kesner, R.P. (1985). Differential contribution of parietal cortex and hippocampal formation in the retention of a cognitive mapping. Society for Neuroscience Abstracts, 12, 193.2.

Ding, E.L., Mozaffarian, D. (2006). Optimal dietary habits for the prevention of stroke. Seminars in Neurology, 26(1), 11-23.

Earnest, M.P., Yarnell, P.R., Merrill, S.L., Knapp, G.L. (1980). Long-term survival and neurological status after resuscitation from out-of-hospital cardiac arrest. Neurology, 30, 1298-1302.

Eisenberg, M.S., Copass, M.K., Hallstrom, A.P., et al. (1980). Treatment of out-of-hospital cardiac arrests with rapid defibrillation by emergency medical technicians. N Eng J M, 302, 1379-1383.

Ekholm, A., Asplund, B., Siesjö, B.K. (1992). Perturbation of cellular energy state in complete ischemia: relationship to dissipative ion fluxes. Exp Brain Res., 90, 47-53.

Endoh, M., Maiese, K., and Wagner, J. (1994). Expression of inducible form of nitric oxide synthase by reactive astrocytes after transient global ischemia. Brain Res., 651, 92-100.

Erecinska, M., Silver, I.A. (1989). ATP and brain function. J Cereb Blood Flow Metab., 9:2-19.

Farooqui, A.A., Horrocks, L.A. (1994). Involvement of glutamate receptors, lipases, and phospholipases in long-term potentiation and neurodegeneration. J Neurosci Res., 38, 6-11.

Finestone, H.M., Greene-Finestone, L.S., Wilson, E.S., Teasell, R.W. (1995). Malnutrition in stroke patients on the rehabilitation service and at follow-up: prevelance and predictors. Arch Phys Med Rehabil., 76, 310-316.

Finestone, H.M., Greene-Finestone, L.S., Wilson, E.S., Teasell, R.W. (1996). Prolonged length of stay and reduced functional improvement rate in malnourished stroke rehabilitation patients. Arch Phys Med and Rehabil., 77, 340-345.

Fletcher, G.F. (1994). Exercise in the prevention of stroke. Health Rep, 6, 106-110.

FOOD Trial Collaboration. (2003). Poor nutritional status on admission predicts poor outcomes after stroke. Stroke, 34, 1450-1456.

Gariballa, S.E., Parker, S.G., Taub, N., Castleden, C.M. (1998a). Influence of nutritional status on clinical outcome after acute stroke. Am J Clin Nutr., 68, 275-281.

Gariballa, S.E., Parker, S.G., Taub, N., Castleden, C.M. (1998b). A randomized controlled, single-blind trial of nutritional supplementation after acute stroke. JPEN, 22, 315-319.

Giles, W.H., Kittner, S.J., Hebel, J.R., Losonczy, K.G., Sherwin, R.W. (1995). Determinants of black-white differences in the risk of cerebral infarction: the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Arch Intern Med, 155, 1319-1324.

Gillium, R.F. (1999). Risk factors for stroke in blacks: a critical review. Am J Epidemiol, 150, 1266-1274.

Gillman, M.W., Cupples, L.A., Millen, B.E., Ellison, R.C., Wolf, P.A. (1997). Inverse association of dietary fat with development of ischemic stroke in men. JAMA, 278, 2145-2150.

Gillium, R.F., Mussolino, M.E., Ingram, D.D. (1996). Physcial activity and stroke incidence in women and men. The NHANES I Epidemiologic Follow-up Study. Am J Epidemiol, 143, 860-869.

Giugliano, D. (2000). Dietary antioxidants for cardiovascular prevention. Nutr Metab Cardiovasc Dis, 10, 38-44.

Globus, M.Y.-T., Busto, R., Martinez, E., Valdes, I., Dietrich, W.D., and Ginsberg, M.D. (1991). Comparative effect of transient global ischemia on extracellular levels of glutamate, glycine, and γ-aminobutyric acid in vulnerable and nonvulnerable brain regions in the rat. J Neurochem., 57, 470-478.

Gorelick, P.B. (1998). Cerebrovascular disease in African Americans. Stroke, 29, 2656-2664.

Grace, P.A. (1994). Ischaemia-reperfusion injury. Br J Surg., 81, 637-647.

Greenwood, C.E., and Winocur, G. (1990). Learning and memory impairments in rats fed a high saturated fat diet. Behav Neural Biol., 53, 74-87.

Greenwood, C.E., and Winocur, G. (1996). Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty acid intake. Behav Neurosci., 111, 451-459.

Hagan, J.J., Tweedie, F., Morris, G.M., Iversen, S.D. (1986). Lack of task specificy and absence

of posttraining effects of atropine on learning. Behavioral Neuroscience, 100, 483-493.

Hajjar, I.M., Grim, C.E., George, V., Kotchen, T.A. (2001). Impact of diet on blood pressure and age-related changes in blood pressure in the US population: analysis of NHANES III. Arch Intern Med, 161, 589-593.

He, K., Merchant, A., Rimm, E.B., et al. (2003). Dietary fat intake and risk of stroke in male US healthcare professionals: 14 year prospective cohort study. BMJ, 327, 777-782.

He, K., Merchant, A., Rimm, E.B., et al. (2004b). Folate, vitamin B6, and B12 intakes in relation to risk of stroke among men. Stroke, 35, 169-174.

He, K., Song, Y., Daviglus, M.L., et al. (2004a). Fish consumption and incidence of stroke: a meta-analysis of cohort studies. Stroke, 35, 1538-1542.

Heart Protection Study Collaborative Group. (2002). MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet, 360, 23-33.

Heinrich-Noack, P., Prehn, J.H.M., and Krieglstein, J. (1996). TGF-β1 protects hippocampal neurons against degeneration caused by transient global ischemia. Stroke, 27, 1609-1615.

Herlitz, J., Andersson, E., Bang, A., et al. (2000). Experiences from treatment of out-of-hospital cardiac arrest during 17 years in Goteborg. Eur Heart J, 21, 1251-1258.

Hirvonen, T., Virtamo, J., Korhonen, P., Albanes, D., Pietinen, P. (2000). Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers. Stroke, 31, 2301-2306.

Howard, G., Anderson, R., Sorlie, P., Andrews, V., Backlund, E., Burke, G.L. (1994). Ethnic differences in stroke mortality between non-Hispanic whites, Hispanic whites, and blacks: the National Longitudinal Mortality Study. Stroke, 25, 2120-2125.

Howard, G., Wagenknecht, L.E., Burke, G.L., Diez-Roux, A., Evans, G.W., McGovern, P., Nieto, F.J., Tell, G.S. (1998). Cigarette smoking and progression of atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Study. JAMA, 279, 119-124.

Inamura, K., Olsson, Y., Siesjö, B.K. (1987). Substantia nigra damage induced by ischemia in hyperglycemic rats. A light and electron microscopic study. Acta Neuropathol., 75, 131-139.

Iso, H., Stampfer, M.J., Manson, J.E., et al. (2001). Prospective study of fat and protein intake and risk of intraparenchymal hemorrhage in women. Circulation, 103, 856-863.

Jastremski, M., Sutton-Tyrrell, K., Vaagenes, P., et al. (1989). Glucocorticoid treatment does not improve neurological recovery following cardiac arrest. Brain Resuscitation Clinical Trial I Study Group. JAMA, 262, 3427-3430.

Joffres, M.R., Reed, D.M., Yano, K. (1987). Relationship of magnesium intake and other dietary factors to blood pressure: the Honolulu heart study. Am J Clin Nutr, 45, 469-475.

Jorde, R., Bonaa, K.H. (2000). Calcium from dairy products, vitamin D intake, and blood pressure: the Tromso Study. Am J Clin Nutr, 71, 1530-1535.

Kawai, K., Nitecka, L., Joo, F., Saito, N., and Klatzo, I. (1992). Global cerebral ischemia associated with cardiac arrest in the rat. I. Dynamics of early neuronal changes. J Cereb Blood Flow Metab., 12, 238-249.

Keli, S.O., Hertog, M.G., Feskens, E.J., Kromhout, D. (1996). Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Arch Intern Med, 156, 637-642.

Kiely, D.K., Wolf, P.A., Cupples, L.A., Beiser, A.S., Kannel, W.B. (1994). Physical activity and stroke risk: the Framingham Study [published correction appears in Am J Epidemiol. 1995; 141: 178]. Am J Epidemiol, 140, 608-620.

Kirino, T. (1982). Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res., 239, 57-69.

Kittner, S.J., Stern, B.J., Feeser, B.R., Hebel, R., Nagey, D.A., Buchholz, D.W., Earley, C.J., Johnson, C.J., Macko, R.F., Sloan, M.A., Wityk, R.J., Wozniak, M.A. (1996). Pregnancy and the risk of stroke. N Engl J Med, 335, 768-774.

Kleihues, P., Hossmann, K.-A., Pegg, A.E., Koayashi, K., and Zimmermann, V. (1975). Resuscitation of the monkey brain after 1 hour complete ischemia. III. Indications of metabolic recovery. Brain Res., 95, 61-73.

Kohno, K., Ohta, S., Kumon, Y., Mitani, A., Sakaki, S., and Kataoka, K. (1996). Nitric oxide synthase inhibitor reduces delayed neuronal death in gerbil hippocampal CA1 neurons after transient global ischemia without reduction of brain temperature or extracellular glutamate concentration. Brain Res., 738, 275-280.

Kolb, B., Pittman, K., Sutherland, R.J., Whishaw, I.Q. (1982). Dissociation of the contributions of the prefrontal cortex and dorsomedial thalamic nucleus to spatially guided behaviour in the rat. Behavioral Brain Research, 6, 365-378.

Krause, G.S., Kumar, K., White, B.C., Aust, S.D., Wiegenstein, J.G. (1986). Ischemia, resuscitation, and reperfusion: mechanisms of tissue injury and prospects for protection. Am Heart J, 111, 768-780.

Kristian, T., Siesjö, B.K. (1998). Calcium in ischemic cell death. Stroke, 29, 705-718.

Lackland, D.T., Egan, B.M., Ferguson, P.L. (2003). Low birth weight as a risk factor for hypertension. J Clin Hypertens, 5, 133-136.

Larrue, V., von Kummer, R., del Zoppo, G., Bluhmki, E. (1997). Hemorrhagic transformation in acute ischemic stroke. Potential contributing factors in the European Cooperative Acute Stroke Study. Stroke, 28, 957-960.

Leppala, J.M., Virtamo, J., Fogelholm,R., et al. (2000). Controlled trial of alpha-tocopherol and beta-carotene supplements on stroke incidence and mortality in male smokers. Arterioscler Thromb Vasc Biol, 20, 230-235.

Lewington, S., Clarke, R., Qizilbash, N., et al. (2003). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies [published correction appears in Lancet, 361:1060]. Lancet, 360, 1903-1913.

Ljunggren, B., Schutz, H., and Siesjö, B.K. (1974). Changes in energy state and acid-base parameters of the rat brain during complete compression ischemia. Brain Res., 73, 277-289.

Lyden, P.D., Zivin, J.A. (1993). Hemorrhagic transformation after cerebral ischemia: mechanisms and incidence. Cerebrovasc Brain Metab., Rev 5, 1-16.

Manolio, T.A., Kronmal, R.A., Burke, G.L., O'Leary, D.H, Price, T.R. (1996). Short-term predictors of incident stroke in older adults: the Cardiovascular Health Study. Stroke, 27, 1479-1486.

McGee, D., Reed, D., Stemmerman, G., Rhoads, G., Yano, K., Feinleib, M. (1985). The relationship of dietary fat and cholesterol to mortality in 10 years; the Honolulu Heart Program. Int. J. Epidemiol., 14, 97-105.

Mody, I., MacDonald, J.F. (1995). NMDA receptor-dependent excitotoxicity: the role of intracellular Ca2+ release. Trends Pharmac Sci., 16, 356-359.

Molteni, R., Barnard, R.J., Ying, Z., Roberts, C.K., and Gómez-Pinilla, F. (2002). A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience, 112(4), 803-814.

Molteni, R., Wu, A., Vaynman, S., Ying, Z., Barnard, R.J., and Gomez-Pinilla, F. (2004). Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral plasticity associated to the action of brain-derived neurotrophic factor. Neuroscience, 123, 429-440.

Morioka, T., Kalehua, A.N., and Streit, W.J. (1992). Progressive expression of immunomolecules on microglial cells in rat dorsal hippocampus following transient forebrain ischemia. Acta Neuropathol., 83, 149-157.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., O'Keefe, J.O. (1982). Place navigation

impaired in rats with hippocampal lesions. Nature, 297, 681-683.

Mosca, L., Manson, J.E., Sutherland, S.E., Langer, R.D., Manolio, T., Barrett-Connor, E. (1997). Cardiovascular disease in women: a statement for healthcare professionals from the American Heart Association. Circulation, 96, 2468-2482.

Mozaffarian, D., Kumanyika, S.K., Lemaitre, R.N., Olson, J.L., Burke, G.L., Siscovick, D.S. (2003). Cereal, fruit, and vegetable fiber intake and the risk of cerebrovascular disease in elderly individuals. JAMA, 289, 1659-1666.

Myers, R.E. (1979). Lactic acid accumulation as a cause of brain edema and cerebral necrosis resulting from oxygen deprivation. In: Guilleminault RK a G, eds. Advances in Perinatal Neurology New York: Spectrum, 85-114.

Nyswonger, G.D., Helmchen, R.H. (1992). Early enteral nutrition and length of stay in stroke patients. J Neurosci Nurs., 24, 220-223.

Panakhova, E., Buresova, O., Bures, J. (1984). Persistence of spatial memory in the Morris water tank task. International Journal of Psychophysiology, 2, 5-10.

Pappius, H.M. (1989). Cerebral edema and the blood-brain barrier. In: Neuwelt, E.A., ed. Implications of the Blood-Brain Barrier and its Manipulation. Volume 1. New York: Plenum Medical Book Company, 293-309.

Paterson, P.G., Juurlink, B.H.J. (1999). Nutritional regulation of glutathione in stroke. Neurotox Res., 1, 90-112.

Petito, C.K., Morgello, S., Felix, J.C., and Lesser, M.L. (1990). The two patterns of reactive astrocytosis in postischemic rat brain. J Cereb Blood Flow Metab., 10, 850-859.

Pereira, M.A., Pins, J.J. (2000). Dietary fiber and cardiovascular disease: experimental and epidemiologic advances. Curr Artheroscler Rep, 2, 494-502.

Polderman KH. (2008). Induced hypothermia and fever control for prevention and treatment of neurological injuries. Lancet, 371, 1955-1969.

Preventing Obesity and Chronic Diseases Through Good Nutrition and Physical Activity. (2003). National Center for Chronic Disease Prevention and Health Promotion. Available at: http://www.cdc.gov/nccdphp/pe_factsheets/pe_pa.htm.

Pulsinelli, W.A., Brierley, J.B., and Plum, F. (1982a). Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol, 11, 491-498.

Pulsinelli, W.A., Levy, D.E., and Duffy, T.E. (1982b). Regional cerebral blood flow and glucose metabolism following transient forebrain ischemia. Ann Neurol., 11, 499-509.

Pulsinelli, W.A., Waldman, S., Rawlinson, D., Plum, F. (1982). Moderate hyperglycemia augments ischemic brain damage: a neuropathological study in the rat. Neurology, 32, 1239-1246.

Pusswald, G., Fertl, E., Faltl, M., et al. (2000). Neurological rehabilitation of severely disabled cardiac arrest survivors: Part II. Life situation of patients and families after treatment. Resuscitation, 47, 241–248

Qureshi, A.I., Giles, W.H., Croft, J.B., Stern, B.J. (1997). Number of pregnancies and risk for stroke and stroke subtypes. Arch Neurol, 54, 203-206.

Reed, D.M. (1988). A prospective study of cerebral artery atherosclerosis. Stroke, 19(7), 820-825.

Richter, C., Kass, G.E.N. (1991). Oxidative stress in mitochondria: its relationship to cellular Ca2+ homeostasis, cell death, proliferation and differentiation. Chem.-Biol. Interactions, 77, 1-23.

Rodriguez, B.L., D'Agostino, R., Abbott, R.D., Kagan, A., Burchfiel, C.M., Yano, K., Ross, G.W., Silbershatz, H., Higgins, M.W., Popper, J., Wolf, P.A., Curb, J.D. (2002). Risk of hospitalized stroke in men enrolled in the Honolulu Heart Program and the Framingham Study: a comparison of incidence and risk factor effects. Stroke, 33, 230-236.

Roine, R.O., Kajaste, S., Kaste, M. (1993). Neuropsycholgocial sequelae of cardiac arrest. J Am Med Assoc, 269, 237-42.

Rothwell, P.M., Coull, AJ., Giles, M.F., Howard, S.C., Silver, L.E., Bull, L.M., Gutnikov, S.A., Edwards, P., Mant, D., Sackley, C.M., Farmer, A., Sandercock, P.A., Dennis, M.S., Warlow, C.P., Bamford, J.M., Anslow, P. (2004). Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). Lancet, 363,1925-1933.

Sacco, R.L., Boden-Albala, B., Gan, R., Chen, X., Kargman, D.E., Shea, S., Paik, M.C., Hauser, W.A. (1998). Stroke incidence among white, black, and Hispanic residents of an urban community: the Northern Manhattan Stroke Study. Am J Epidemiol, 147, 259-268.

Sairanen, T.R., Lindsberg, P.J., Brenner, M., and Siren, A.-L. (1997). Global forebrain ischemia results in differential cellular expression of interleukin-1β (1L-1β) and its receptor at mRNA and protein level. J Cereb Blood Flow Metab., 17, 107-120.

Saito, K., Suyama, K., Nishida, K., Sei, Y., and Basile, A. (1996). Early increases in TNF-α and IL-1β levels following transient cerebral ischemia in gerbil brain. Neurosci. Lett, 206, 149-152.

Seeburg, P.H. (1993). The molecular biology of the mammalian glutamate receptor channels. Trends Pharmac, 14, 13-20.

Sesso, H.D., Gaziano, J.M., Liu, S., Buring, J.E. (2003). Flavonoid intake and the risk of cardiovascular disease in women. Am J Clin Nutr, 77, 1400-1408.

Shaw, P.J., Bates, D., Cartligde, N.E.F., French, J.M., Heaviside, D., Julian, D.G., and Shaw, D.A. (1987). Neurologic and neuropsychological morbidity following major surgery: comparison of coronary artery bypass and peripheral vasculature surgery. Stroke, 18, 700-707.

Shiga, Y., Onodera, H., Kogure, k., Yamasaki, Y., Yashima, Y., Shouzuhara, H., Sendo, F. (1991). Neurtrophil as a mediator of ischemic edema formation in the brain. Neurosci Lett., 125, 110-112.

Siesjö, B.K. (1981). Cell damage in the brain: a speculative synthesis. J. Cereb. Blood Flow Metab., 1, 155-185.

Siesjö, B.K. (1984). Cerebral circulation and metabolism. J. Neurosurg., 60, 883-908.

Siesjö, B.K. (1985). Acid-base homeostasis in the brain: physiology, chemistry, and neurochemical pathology. Progress in Brain Research, 165, 121-154.

Siesjö, B.K. (1988). Acidosis and ischemic brain damage. Neurochem. Pathol., 9, 31-88.

Siesjö, B.K., Ekholm, A., Katsura, K., Memezawa, H., Ohta, S., and Smith, M.-L. (1990). The type of ischemia determines the pathophysiology of brain lesions and the therapeutic response to calcium channel blockade. Pharmacology of Cerebral Ischemia. J. Krieglstein and H. Oberpichler (eds.). Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart, pp. 79-88.

Siesjö, B.K., Ljunggren, B. (1973). Cerebral energy reserves after prolonged hypoxia and ischemia. Arch Neurol., 29, 400-407.

Smith, M.L., Auer, R.N., and Siesjö, B.K. (1984). The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. Acta Neuropathol, 64, 319-332.

Smith, M.L., Kalimo, H., Warner, D.S., Siesjö, B.K. (1988). Morphological lesions in the brain preceding the development of postischemic seizures. Acta Neuropathol., 76, 253-264.

Stamler, J., Rose, G., Elliott, P., et al. (1991). Findings of the International Cooperative INTERSALT Study. Hypertension, 17, 9-15.

Suave, M.J., Dolittle, N., Walker, J., Paul, S., Scheinman, M. (1996). Factors associated with cognitive recovery after cardiopulmonary resuscitation. Am J Crit Care, 5(2), 127-39.

Sunnerhangen, K.S., Johansson, O., Herlitz, J., Grimby, G. (1996). Life after cardiac arrest; a

retrospective study. Resuscitation, 31, 135-140.

Sussman, M.S., Bulkley, G.F. (1990). Oxygen-derived free radicals in reperfusion injury. Meth Enzym., 186, 711-723.

Sutherland, R.J., Dyck, R.H. (1984). Place navigation by rats in a swimming pool. Canadian Journal of Psychology, 38, 322-347.

Sutherland, R.J., Kolb, B., Whishaw, I.Q. (1982). Spatial mapping definitive disruption by hippocampal or medial frontal cortical damage in the rat. Neuroscience Letters, 31, 271-276.

Sutherland, R.J., Whishaw, I.Q., Kolb, B. (1983). A behavioral analysis of spatial localization following electrolytic, kainite- or colchicines-induced damage to the hippocampal formation in the rat. Behavioral Brain Research, 7, 133-153.

Thrift. A.G., Donnan, G.A., McNeil, J.J. (1995). Epidemiology of intracerebral hemorrhage. Epidemiol Rev., 17, 361-381.

Ullegaddi, R., Powers, H.J., Gariballa, S.E. (2004). B-group vitamin supplementation mitigates oxidative damage after acute ischaemic stroke. Clinical Science, 107, 477-484.

Uno, H., Matsuyama, T., Akita, H., Nishimura, H., and Sugita, M. (1997). Induction of tumor necrosis factor-α in the mouse hippocampus following transient forebrain ischemia. J Cereb Blood Flow Metab., 17, 491-499.

Unosson, M., Ek, A.C., Bjurulf, P., von Schenck, H., Larsson, J. (1994). Feeding dependence and nutritional status after acute stroke. Stroke, 25, 366-371.

Van Der Worp, H.B., Sena, E.S., Donnan, G.A., Howells, D.W., Macleod, M.R. (2007). Brain, 130, 3063-3074.

Vasan, R.S., Beiser, A., Seshadri, S., Larson, M.G., Kannel, W.B., D'Agostino, R.B., Levy, D. (2002). Residual lifetime risk for developing hypertension in middle-aged women and men: the Framingham Heart Study. JAMA, 287, 1003-1010.

Wang, Y., Change, C.-F., Chou, J., Chen, H.-L., Deng, X., Harvey, B.K., Dacet, J.L., Bickford, P.C. (2005). Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. Experimental Neurology, 193, 75-84.

Whishaw, I.Q., Kolb, B. (1984). Decortication abolishes place but not cue learning in rats. Behavioral Brain Research, 11, 123-124.

Winocur, G., and Greenwood, C.E. (1993). High-fat diets impair conditional discrimination learning in rats. Psychobiology, 21, 286-292.

Wolf, P.A., Abbott, R.D., Kannel, W.B. (1991b). Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke, 22, 983-988.

Wolf, P.A., D'Agostino, R.B., Belanger, A.J., Kannel, W.B. (1991a). Probability of stroke: a risk profile from the Framingham Study. Stroke, 22, 312-318.

Wolf, P.A., D'Agostino, R.B., O'Neal, M.A., Sytkowski, P., Kase, C.S., Belanger, A.J., Kannel, W.B. (1992). Secular trends in stroke incidence and mortality: the Framingham Study. Stroke, 23, 1551-1555.

Wu, A., Molteni, R., Ying, Z., & Gomez-Pinilla, F. (2003). A saturated-fat diet aggregates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. Neuroscience, 119, 365-375.

Yang, G.-Y., Betz, A.L. (1994). Reperfusion-induced injury to the blood-brain-barrier after middle cerebral artery occlusion in rats. Stroke, 25, 1658-1665.

Yochum, L., Folsom, A.R., Kushi, L.H. (2000). Intake of antioxidant vitamins and risk of death from stroke in postmenopausal women. Am J Clin Nutr, 72, 476-483.

Yochum, L., Kushi, L.H., Meyer, K., Folsom, A.R. (1999). Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. Am J Epidemiol, 149, 943-949.

Yokoyama, T., Date, C., Kokubo, Y., Yoshiike, N., Matsumura, Y., Tanaka, H. (2000). Serum vitamin C concentration was inversely associated with subsequent 20-year incidence of stroke in a Japanese rural community. The Shibata study. Stroke, 360, 23-33.

You, R.X., Thrift, A.G., McNeil, J.J., Davis, S.M., Donnan, G.A. (1999). Ischemic stroke risk and passive exposure to spouses' cigarette smoking. Melbourne Stroke Risk Factor Study (MERFS) Group. Am J Public Health, 89, 572-575.

Yu, Z.F., and Mattson, M.P. (1999). Dietary restriction and 2-Deoxyglucose administration reduced focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. Journal of Neuroscience Research, 57, 830-839.

Zola-Morgan, S., Squire, L.R., Amaral, D.C. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci, 6, 2950-2967.

CHAPTER 2

A 60-DAY WESTERN DIET DOES NOT EXACERBATE INJURY AND SPATIAL LEARNING MEMORY FOLLOWING GLOBAL ISCHEMIA IN RATS

2.1 INTRODUCTION

Global ischemia (ISC), such as occurs with cardiac arrest, causes selective bilateral lesions in the CA1 zone of the hippocampus and profound anterograde amnesia (Zola-Morgan et al., 1986). Clinical trials directed at reducing the extent of neuronal damage by means of pharmacological agents have proven unsuccessful. In lieu of this, attention has been drawn to various modifiable risk factors, such as nutrition. Nutrition influences cerebral ischemia through several mechanisms, such as altering blood pressure (BP), insulin resistance, inflammation, and oxidation (Ding and Mozaffarian, 2006).

The average popular diet of most industrialized western societies, rich in saturated fat and/or refined sugar, is potentially harmful to the brain (Greenwood and Winocur, 1990, 1996; Molteni et al., 2002; Morris et al., 2004; Winocur and Greenwood, 1993, 1999), via an indirect effect of a high-fat diet on cardiovascular dysfunction (e.g., atherosclerosis), and a direct effect of a high-fat diet on neural function (Molteni et al., 2002). Epidemiological stroke studies on diets high in saturated fat have been inconclusive. With increased saturated fat intake, some reported a lower risk of cerebral ischemia (Gillman et al., 1997; Reed, 1990). Similarly, several studies have found a lower mortality (McGee et al., 1985; Reed et al., 1988; Sauvaget et al., 2004), whereas others report no significant association between incidence and mortality from cerebral ischemia and consumption of saturated fat (He et al., 2003).

In addition to affecting stroke risk, dietary manipulations have the capacity to attenuate or aggravate injury. Thus, outcome following stroke may be improved by following particular dietary regimens and/or avoiding others. For instance, Yu and Matson (1999) showed that caloric restriction attenuated injury and improved functional outcome after focal ischemia in rats. Bobyn and colleagues (2005) found that PEM impaired functional outcome in an open field test in

gerbils as long as 10 days following global ISC. Wu and colleagues (2003) showed that a western diet (WD) further exacerbated injury and cognitive impairments following traumatic brain injury. Specifically, they found that relative to controls, rats fed a WD for 4 weeks had longer escape latencies in the Morris water maze (MWM) and significant reductions of brain-derived neurotrophic factor (BDNF) mRNA in the CA3 and dentate gyrus one week following traumatic brain injury (Wu et al., 2003). These findings are in line with additional animal research on WDs, which compromise neuronal function by increasing levels of oxidative stress, reducing levels of BDNF, and consequently impairing spatial learning and memory (Molteni et al., 2002). Indeed, Greenwood and Winocur (1990) and Winocur and Greenwood (1993) showed that providing postweanling rats a WD for 3-months caused severe and widespread learning and memory impairments on numerous tests. Specifically, these authors found that rats fed the largest amount of saturated fatty acids performed the worst relative to rats fed a poly- or monounsaturated fatty acid diet (Greenwood and Winocur, 1996). Molteni and colleagues (2004) also showed that a 2-month WD impaired spatial learning performance in the MWM.

The effects of a WD in global cerebral ISC have yet to be established. Thus, in the present study we sought to delineate the impact of a WD in a well established 2-VO model of global ISC (Smith et al., 1984). This study consisted of 2 experiments. In both experiments rats were placed either on a WD or a low-fat control diet (CD). Since outcome following ISC can be influenced by changes in temperature (Colbourne et al., 1997), glucose (Li and Siesjö, 1997) and activity levels (Gerhardt and Boast, 1988; Weber et al., 1989), it was imperative to establish these profiles in non-ischemic (NO-ISC) animals fed the WD and CD. Thus, experiment 1 evaluated the influence of each diet on temperature, activity, and glucose levels, spatial learning and memory, and hippocampal CA1 cells. Experiment 2 assessed the impact of a WD on the

severity of hippocampal CA1 damage and spatial learning and memory following 8 minutes of forebrain ischemia. We predicted that a WD alone would have no significant effects on temperature, activity, and glucose levels. However, based upon previous literature (Greenwood and Winocur, 1990, 1996; Molteni et al., 2002; Winocur and Greenwood, 1993), we predicted that a WD would impair spatial learning and memory. In experiment 2, based upon findings from Wu and colleagues (2003), we predicted that a WD would affect residual CA1 neurons along with other hippocampal regions (e.g., CA3), and thus impair spatial learning and memory even further.

2.2. Materials and Methods

2.2.1 Animals

Thirty-five male Sprague-Dawley rats (Biosciences Breeding Colony, Edmonton, Alberta, Canada), weighing 45-55 g at the time of arrival, were used in this study. Animals were housed in groups of 4 to 5 until surgery and maintained on a reverse 12-h light/dark cycle (lights on at 10:00 PM) with free access to water. All procedures followed the Canadian Council for Animal Care guidelines and were approved by the Biosciences Animal Care and Use Committee at the University of Alberta.

2.2.2 Diet

Immediately upon arrival, animals in all experiments were placed on a WD (5TJN, Western diet for rodents, TestDiet, Richmond, IN) or CD (5TJS, low fat control for WD, TestDiet, Richmond, IN). The two diets were isocaloric, but differed slightly in energy density. Percent energy (kcal/g) from protein was similar between the WD and CD (16%). The CD

contained 72% energy from carbohydrate and 12% energy from fat. Conversely, the WD contained 44% energy from carbohydrate and 40% energy from fat. Energy density of the WD was 4.49 (kcal/g) and 3.84 (kcal/g) for the CD. However, the amount of vitamins, minerals, fiber and cholesterol per kilocalorie remained equal between the two diets. Animals were fed their respective diets ad libitum throughout the entire experiment, unless otherwise stated. Food intake, however, was not measured throughout the study.

2.2.3 Body weight measurements

Body weight (g) was measured everyday for 7 consecutive days after arrival, and then on a weekly basis until euthanasia. Body weight was also measured at the time of surgery (e.g., core-probe implantation or ISC) and everyday thereafter until animals returned to their pre-surgical weight.

2.2.4 Experiment 1

We examined the effects of a 60-day WD regimen on core temperature, activity, glucose levels and cognition. The NO-ISC animals were fed their respective diets for 30 days, at which time a core telemetry probe was implanted.

i. Core Temperature Telemetry Probe Implantation

Aseptic surgical techniques were used throughout (e.g., autoclaved or hot-bead sterilized instruments, autoclaved surgical drapes). Animals had sterilized core telemetry probes (model TA1OTA-F40; Data Sciences, St. Paul, Minn.) implanted into the peritoneal cavity. Briefly, rats were anesthetized (~20 min) with isoflurane (1.5%–2% maintenance in 30% O_2 and 70% N_2O)

and a 2 cm incision was made in the abdomen into which the sterilized probe was implanted. The muscle was sutured closed and then infiltrated with Marcaine (Sanofi Canada, Markham, ON, Canada). The skin was then closed and treated with a topical antibiotic. These probes remained *in situ* until euthanasia. For 30 consecutive days after core probe implantation, temperature and activity were sampled every 5 minutes by an automated system (DQ3 System, Data Sciences) previously described (Colbourne et al. 1993). The 24-hour periods for temperature and activity were averaged and analyzed.

ii.. Blood Glucose

Following core-probe surgery, animals' blood glucose levels were measured each week for 3 weeks. Animals were briefly anesthetized (~1-3 min) with isoflurane (1.5%–2% maintenance in 30% O_2 and 70% N_2O) and venous blood samples (approximately 100 μL) were collected in heparinized capillary tubes to measure blood glucose levels via a Radiometer ABL810 blood gas analyzer (Radiometer, Copenhagen, Denmark).

iii. Morris Water Maze (MWM)

At 2 and 4-weeks after surgery, rats were tested in a modified version of the MWM task (Morris et al., 1982). Animals were placed snout towards the black circular pool (~140 cm diameter) containing water (~27±1˚C; Brown & Whishaw, 2000). The submerged black platform (~1.5 cm below the surface of the water; ~14 cm in diameter) was maintained in the same location throughout the hidden platform training allowing for the assessment of learning over four days. Following the last trial on the last day, a 60 s probe trial was conducted whereby the platform was removed from the pool and the amount of time spent swimming in each quadrant

was measured allowing for the assessment of memory retention. Animals that learn the task will spend most of the 60 s trial searching for the platform in the target quadrant. After completion of the hidden platform training and probe trial, we administered one day of visible platform training to assess motivation and swim speed. Rats received 4 trials a day and were released from a different quadrant around the perimeter of the pool in a random sequence. The maximum trial length for the hidden and visible platform trials was 90 s, with an inter-trial time of 1 min. If the rat failed to locate the platform within the given time, the rat was removed from the water and placed on the platform for 10 s before returning to the holding cage. Animals that successfully found the platform also remained on the platform for 10 s before being returning to the holding cage. Latency to locate the platform was measured.

2.2.5 Experiment 2

We examined the effect of a 60-day WD diet regimen on the severity of hippocampal CA1 injury and functional outcome following ISC in 19 rats. These animals were fed their respective diets for 30 days at which time ISC was induced using a modified 2-Vessel Occlusion (2-VO) model developed by Smith et al. (1984).

i. Forebrain Ischemia (ISC)

Animals were subjected to food deprivation (~ 12 hr) prior to surgery in order to lower glucose levels into a consistent range (4 – 8 mmol / L). Rats were anesthetized (~ 45 min) with isoflurane (1.5%–2% maintenance in 30% O_2 and 70% N_2O) and placed on a heated water blanket (model TP3E, Gaymar, NY) with feedback control from a thermocouple probe (HYPO-33-1-T-G-60-SMG-M, Omega, Stanford, CT) placed subcutaneously on the skull (centre). A

model CSC-32 (Omega) feedback regulator maintained skull temperature (T_s) near 37.5°C via an infrared lamp (175 W) to minimize an unwanted drop in brain temperature during ISC, which would have lessened injury. A 2-cm incision was made along the ventral midline of the neck and the common carotid arteries were isolated. Mean arterial blood pressure (MABP) was measured via a tail artery cannula kept patent by heparinized saline. Ischemia was achieved by transient bilateral carotid artery occlusion and systemic hypotension (35-45 mmHG; BP-1, World Precision Instruments, Sarasota, FL). Systemic hypotension was produced by withdrawing blood from the jugular vein into a heparinized syringe. Following an eight minute 2-VO, clamps were removed and blood was slowly re-infused. Arterial blood samples (approximately 100 μL each) were taken to measure blood pH, pCO_2, pO_2, hemoglobin (ctHb), and glucose levels before and after ISC.

ii. Morris Water Maze

Behavioural testing was identical to that described in experiment 1.

2.2.6 Histopathology

Rats were euthanized 60 days after the initiation of feeding using an overdose of sodium pentobarbital (100 mg/kg i.p.) and transcardially perfused with 0.9% saline followed by 10% neutral-buffered formalin. Extracted brains were embedded in paraffin. Subsequently, 6-μm coronal sections were cut and stained with hematoxylin and eosin. One section at -3.60 mm to Bregma (Paxinos and Watson, 1998) was chosen in which CA1 sector injury was determined. Injury at this level has been shown to correlate highly with injury at more anterior and posterior injury (Colbourne and Corbett, 1995). Viable (healthy-looking, non-eosinophilic) CA1 sector

pyramidal neurons were counted in medial (next to the subiculum), middle, and lateral regions (next to the CA2 zone) of the CA1 zone of both hemispheres. The number of CA1 sector neurons was summated over the left and right hemispheres and expressed as a percent of normal (e.g., non-ischemic animals in experiment 1). The researcher was blinded to the group identity.

2.2.7 Statistics

Temperature, activity, and glucose levels (experiment 1 only) were analyzed using a Student's t-test. Correlations between temperature and activity were achieved by using a Pearson's correlation. Physiology (e.g., glucose levels, MABP), histology, body weight, and MWM data were analyzed using ANOVA (SPSS, v. 15.0. Chicago, Ill.). A p-value of 0.05 was considered statistically significant. All data are reported as mean ± SEM.

2.3 RESULTS

2.3.1 Experiment 1

i. Protocol violations and mortality

No rats were excluded from this experiment. The group sizes were as follows: WD (n=8) and CD (n=8) group.

ii. Body weight measurements

Rat body weight (Figure 2.1) was analyzed using a 2-factor ANOVA (Diet; Time: arrival, days 7, 14, 21, 28, surgery, 35, 42, 56 and euthanasia). There was a significant Time effect ($p<0.001$) as animals gained weight as they aged. There was no significant Diet × Time interaction ($p=0.127$) or a Diet main effect ($p=0.129$).

iii. Glucose Values

Three weekly venous blood samples were collected from rats to measure whether there were any differences in blood glucose levels between animals consuming the WD and CD. There was no significant main effect of diet at any time (data not shown) with the average values being very similar at 9.89 ± 0.24 and 9.14 ± 0.24 in the WD and CD groups, respectively (t-test: p=0.058).

iv. Temperature and Activity

Temperature (Figure 2.2A) and activity (Figure 2.2B) were analyzed using an independent student's t-test, which revealed that the overall 30-day temperature profile was not significantly different (p=0.726) between animals fed the WD (37.4 °C ± 0.1) versus the CD (37.3 °C ± 0.1). However, activity levels were significantly different (p<0.001) between the WD (2.0 ± 0.1) and CD (3.2 ± 0.2). A Pearson's correlation analysis revealed that temperature and activity were significantly correlated in both the WD (r=0.840, p<0.001) as well as the CD (r=0.707, p<0.01). Thus, as expected, it is likely that activity changed core temperature, as the more active animals were, the warmer they became.

v. Morris Water Maze

A repeated measures ANOVA was used to analyze the swim latency data. Collapsing across trials, learning acquisition (Figure 2.3A and 2.3B) was analyzed using a 3-factor ANOVA (Diet; Week: 2 and 4 weeks post surgery; and Day: 4 days of testing). There was no significant main effect of Diet (p=0.368). There was a significant Week × Day interaction (p<0.001), but no

significant interaction effect involving Diet ($p \geq 0.092$). Memory retention (Figure 2.3C), assessed via a probe trial, was analyzed using a 2-factor ANOVA (Diet, Week). There was no significant Week × Diet interaction ($p=0.468$), or a main effect of Week ($p=0.492$) or Diet ($p=0.561$). Animals fed the WD and CD spent an equal amount of time swimming in the target quadrant, which did not vary considerably between weeks. Collapsing across trials, the visible platform (Figure 2.3D) was analyzed using a 2-factor ANOVA (Diet, Week), which revealed no significant Week × Diet interaction ($p=0.647$), and no main effect of Week ($p=0.146$) or Diet ($p=0.103$). Thus, as per the visible platform, the WD and CD did not significantly influence motivation and swim speed.

vi. Histopathology

No hippocampal CA1 pathology was observed in non-ischemic control rats given either CD or a WD (Figure 2.4A), and cell counts were not significantly different between the two diet groups ($p=0.762$).

2.3.2 Experiment 2

i. Protocol violations and mortality

Five rats were excluded owing to surgical problems with isolating and catheterizing the tail artery. The remaining group sizes were as follows: WD (n=11) and CD (n=8).

ii. Body weight measurements

Rat body weight (Figure 2.1B) was analyzed using a 2-factor ANOVA (Diet factor; Time factor: arrival, days 7, 14, 21, 28, surgery, 35, 42, 56 and euthanasia). There was no overall main

effect of Diet (p=0.140); however, there was a significant Time effect (p<0.001) and Diet × Time interaction (p<0.001). Owing to the significant interaction, the data were analyzed for each time point to determine when diet affected body weight. Significant differences occurred at day 7 (p=0.019), day 14 (p=0.019) and euthanasia (p=0.016). At euthanasia, animals fed the WD weighed ~60 g more than animals fed the CD.

iii. Physiology

Physiological variables during surgery are given in Table 2.1. Blood pH, pCO_2, pO_2, concentration of total ctHb, cGlu, T_s and MABP were the same for animals maintained on the WD and CD (p≥0.358).

iv. Morris Water Maze

A repeated measures ANOVA was used to analyze the swim latency data. Collapsing across trials, learning acquisition (Figure 2.3A and 2.3B) was analyzed using a 3-factor ANOVA (Diet, Week and Day). There was a significant Week × Day interaction (p<0.001), but no influence of Diet (p≥0.545) and no main effect of Diet (p=0.177). Memory retention (Figure 2.3C), assessed via a probe trial, was analyzed using a 2-factor ANOVA (Diet, Week). There was no significant Week × Diet interaction (p=0.140), or a main effect of Diet (p=0.864). However, there was a significant main effect of Week (p=0.043). Animals spent more time swimming in the target quadrant on day 16 post-surgery than on day 29 post-surgery. Collapsing across trials, the visible platform (Figure 2.3D) was analyzed using a 2-factor ANOVA (Diet, Week), which revealed no main effect of Week (p=0.274) or Diet (p=0.121), and no significant

Week × Diet interaction (p=0.113). Thus, as per the visible platform, the WD and CD did not significantly influence motivation and swim speed.

We were also interested in examining any differences in latency scores between ISC and NO-ISC animals. Collapsing across trials, learning acquisition was analyzed using a 3 factor ANOVA (Group, Week and Day). There was a significant Week × Day × Group interaction (p<0.001). Accordingly, the data were analyzed separately for each time point to determine when differences between the two groups existed. On day 13 (p<0.001) and 14 (p=0.035) post-surgery, NO-ISC animals were able to locate the platform much faster than ISC animals. By day 15 this effect was no longer present (p=0.845), and did not reappear thereafter. The probe trial, analyzed using a 2 factor ANOVA (Group; Week), revealed a significant Group main effect (p<0.001) with a non-significant Week main effect (p=0.553) and a non-significant interaction (p=0.089). NO-ISC animals spent more time swimming in the target quadrant than ISC animals, indicating that the NO-ISC animals retained more information regarding the location of the hidden platform obtained over the 4 days of learning acquisition training. A 2 factor ANOVA (Group, Week) for the visible platform training data revealed no main effect of Week (p=0.388) or Group (p=0.484), nor a significant Week × Group interaction (p=0.651). Swim speed and motivation did not differ significantly between NO-ISC and ISC animals for the duration of testing.

v. Histopathology

Eight minutes of ISC induced severe CA1 sector necrosis (~92%; p<0.001 for ISC vs. NO-ISC animals), which was not different between rats fed the WD and CD (Figure 2.4A; p=0.363).

2.4 DISCUSSION

This is the first study to assess whether a WD, in the absence of other risk factors associated with cardiovascular dysfunction (e.g., arthrosclerosis, hyperglycemia and altered BP), influences functional recovery and hippocampal CA1 injury following global ISC. We showed that a WD did not potentiate the deleterious effects of an 8 minute insult, which killed approximately 92% of hippocampal CA1 neurons resulting in transient spatial learning deficits (e.g., up to 14 days post-stroke), and long-lasting memory impairments (e.g., up to 30 days post-stroke) in rats. On days 13 and 14 post-stroke, ISC animals took significantly longer to locate the hidden platform, which assesses spatial learning; by day 15 post-stroke, ISC animals' latency scores did not differ from NO-ISC animals. This indicates that ISC animals successfully learned the location of the hidden platform. We assessed memory retention in a probe trial, which involved removing the hidden platform from the pool and measuring the amount of time the animals spent swimming in the target quadrant. In the present study, the probe trial revealed that ISC animals spent significantly less time swimming in the target quadrant up to 30 days post-stroke. Therefore, ISC resulted in long-lasting spatial memory deficits. A WD did not exacerbate spatial learning and memory impairments or cell loss as compared to animals maintained on the CD. These findings suggest that a WD, initiated 30 days prior to ISC, and provided for a total of 60 days, does not aggravate histological and functional outcome following global ISC.

Our findings are different from those of Wu and colleagues (2003), who showed that a high-fat and sucrose diet aggravated injury and spatial learning following traumatic brain injury. Upon closer examination, the disparity between the WD used in the present study and the high-fat and sugar diet used in a study of Wu et al. (2003) may have contributed to the different outcome. In the present study, the approximate energy from fat and sucrose was 40% and 7%,

respectively; in the study of Wu et al. (2003), the approximate energy from fat and sucrose was 39% and 40%, respectively. A diet with high amounts of refined sugar may have induced hyperglycemia, and research over the past two decades has already established that preischemic hyperglycemia aggravates damage following ISC by enhancing intra- and extracellular acidosis (Myers, 1979; Siesjö, 1981, 1984), edema, and postischemic seizures (Siesjö, 1988, 1985). Our goal was to establish the effects of high saturated fat diet in global ISC while controlling for factors such as hyperglycemia, which is already known to exacerbate injury. Indeed, Wu and colleagues (2003) did not measure peri-insult glucose levels, which may have been a factor in their study. In addition, the different models of brain injury, and their varying pathophysiologies, must also be taken into consideration.

We also found that a sustained 60-day WD regimen alone, beginning post-weanling, did not notably influence the number of hippocampal CA1 sector neurons or spatial learning and memory. These findings do not mirror previous work (Greenwood and Winocur, 1990, 1996; Molteni et el., 2002; Winocur and Greenwood, 1993), which showed that a high fat diet alone induced severe learning and memory impairments. However, animals in Greenwood and Winocur's (1990, 1996) and Winocur and Greenwood's (1993) experiments were fed for approximately 4.5 months before any cognitive testing was initiated, whereas in our study animals were only fed for 1.5 months at the time of behavioural testing. The aforementioned authors used post-weanling rats and a diet high in saturated fat (e.g., 40% energy from fat), and found that rats fed a high fat diet performed poorly on the radial arm maze (Greenwood and Winocur, 1990), a standard hippocampus-sensitive test of spatial memory. The present study also used post-weanling rats, along with using a test reliant primarily on hippocampal function (e.g., MWM), and provided a diet similar in fat composition. Thus, it is possible that the duration of

feeding in our study was too short to have an appreciable effect on neuronal plasticity and cognitive function. However, Molteni and colleagues (2002) found significant impairments in spatial learning and memory retention in the MWM task as early as 1 month following the consumption of a diet high in saturated fat and refined sugar, a deficit that was even more pronounced at 2 months. In addition, they found that a high-fat, high sucrose diet significantly reduced levels of hippocampal BDNF mRNA and protein, which correlated significantly with longer escape latencies and greater cognitive impairments. Although we did not measure hippocampal BDNF mRNA or protein levels, the present study did not detect spatial learning and memory impairments in the animals fed a WD as compared to animals maintained on a CD at 1.5 months into the diet regimen. Again, this may be due to nutritional differences in the diets, particularly the large amount of refined sugar in the study of Molteni et al. (2002), which was not the main nutritional constituent of the diet provided in the present study.

It is also possible that the MWM protocol used in the present study was unable to detect small treatment effects. For instance, ISC animals in experiment 2 located the hidden platform as quickly as NO-ISC animals as early as 3 days into testing (day 15 post-surgery). A moving platform paradigm, incorporating more trials per day (e.g., 10 trials per day), may have been a better measure to detect subtle, but very real differences in mean latency scores between the animals on the WD versus the CD. As suggested by Winocur and Greenwood (1999), the Olton's radial arm maze, the Hebb-Williams complex maze series, and a variable-interval delayed alternation test are all sensitive to the deleterious effects of high-fat diets. Thus, future work should consider incorporating one or more of these tests as they have been shown on numerous occasions (Greenwood and Winocur, 1990, 1996; Winocur and Greenwood, 1993) to be

sensitive not only to high-fat diets, but also sensitive to differences between types of fats (e.g., polyunsaturated versus saturated fats).

In summary, a WD provided for 60 days failed to notably influence both histological and cognitive outcome in the 2-VO model of global ISC. While nutrition has been shown to interact with different forms of brain injury to exacerbate injury in animals (global ischemia and protein energy malnutrition by Bobyn et al. 2005; traumatic brain injury and high fat diet by Wu et al., 2003), and humans (e.g., Alzheimer's disease and high fat diets by Kalmijn et al., 1997 and Morris et al., 2003), we showed that a WD did not enhance brain damage and cognitive impairments following global ISC. Even with the majority of CA1 cells killed, one would still expect the WD to have deleterious physiological effects on the remaining CA1 neurons and on other hippocampal regions and thus aggravate learning and memory. Indeed, CA1 sector injury following ISC is less severe at more posterior levels of the hippocampus, and therefore injury should have been assessed there. However, we know that the MWM is a task of spatial learning and memory, which relies primarily on the hippocampus. Since we did not observe any spatial learning and memory impairments between the WD and CD in either experiment, this lends support to the notion that a 60-day WD did not have deleterious effects on neural plasticity and cognition. Nevertheless, before any definitive conclusions can be made regarding the impact of a WD in stroke, several factors must be delineated. First, longer feeding protocols (e.g., 4 months and greater) must be evaluated. Note that potentially-important physiological confounds, including glucose and temperature, must be considered in such studies as the duration of the diet and interactions with ischemia might lead to important differences from that found in our first experiment. Second, the effects of a WD must also be assessed in other types of strokes since the pathophysiology varies substantially between them (e.g., global ISC, focal ISC, hemorrhagic

stroke). Lastly, tasks that have been previously shown to be sensitive to the negative effects of a high-fat diet should be used.

2.5 FIGURES

Figure 2.1

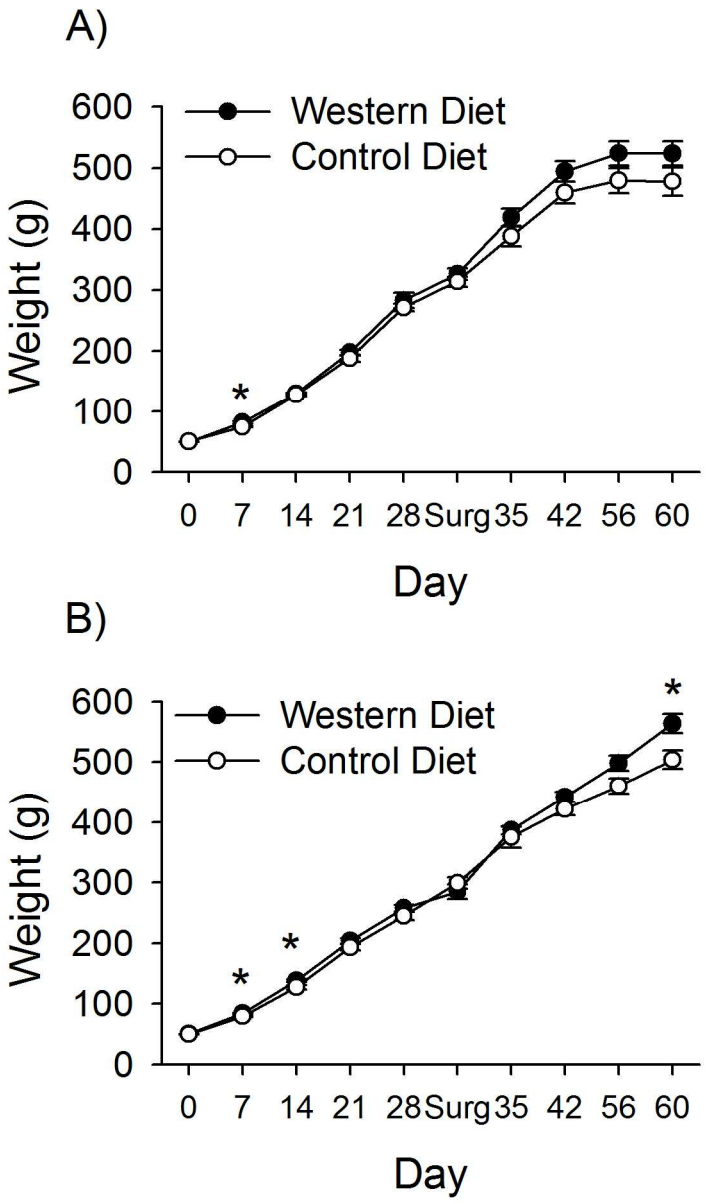

Figure 2.1: Body weight (mean ± SEM) for NO-ISC (A) and ISC (B) animals at several times throughout the study ("Surg" = surgery). Both NO-ISC and ISC animals gained a significant amount of weight over time. In the ISC group only, animals fed the WD were significantly heavier than animals fed the CD at euthanasia (* $p<0.05$).

Figure 2.2

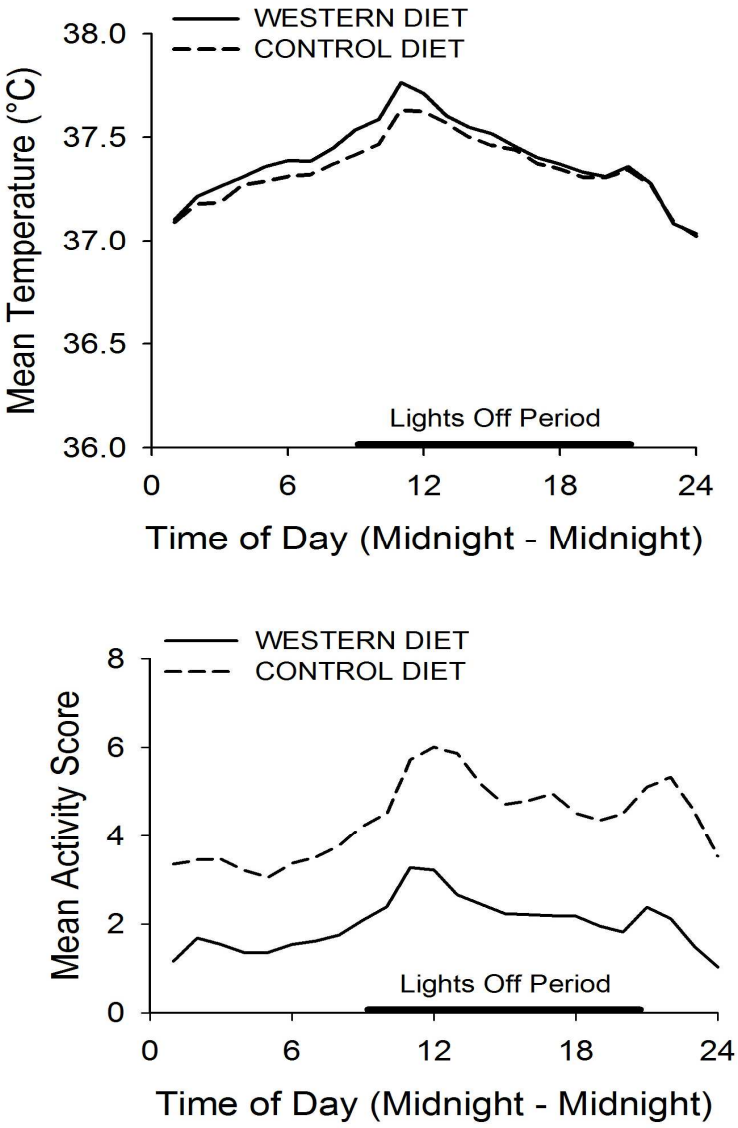

Figure 2.2: Average 24 hour temperature (A) and activity (B) profiles of NO-ISC animals fed the WD and CD from day 30 – 60 measured via a telemetry probe. While basal temperature levels were approximately equal for WD and CD rats, activity levels were significantly decreased in the WD animals. See Results for statistics.

Figure 2.3

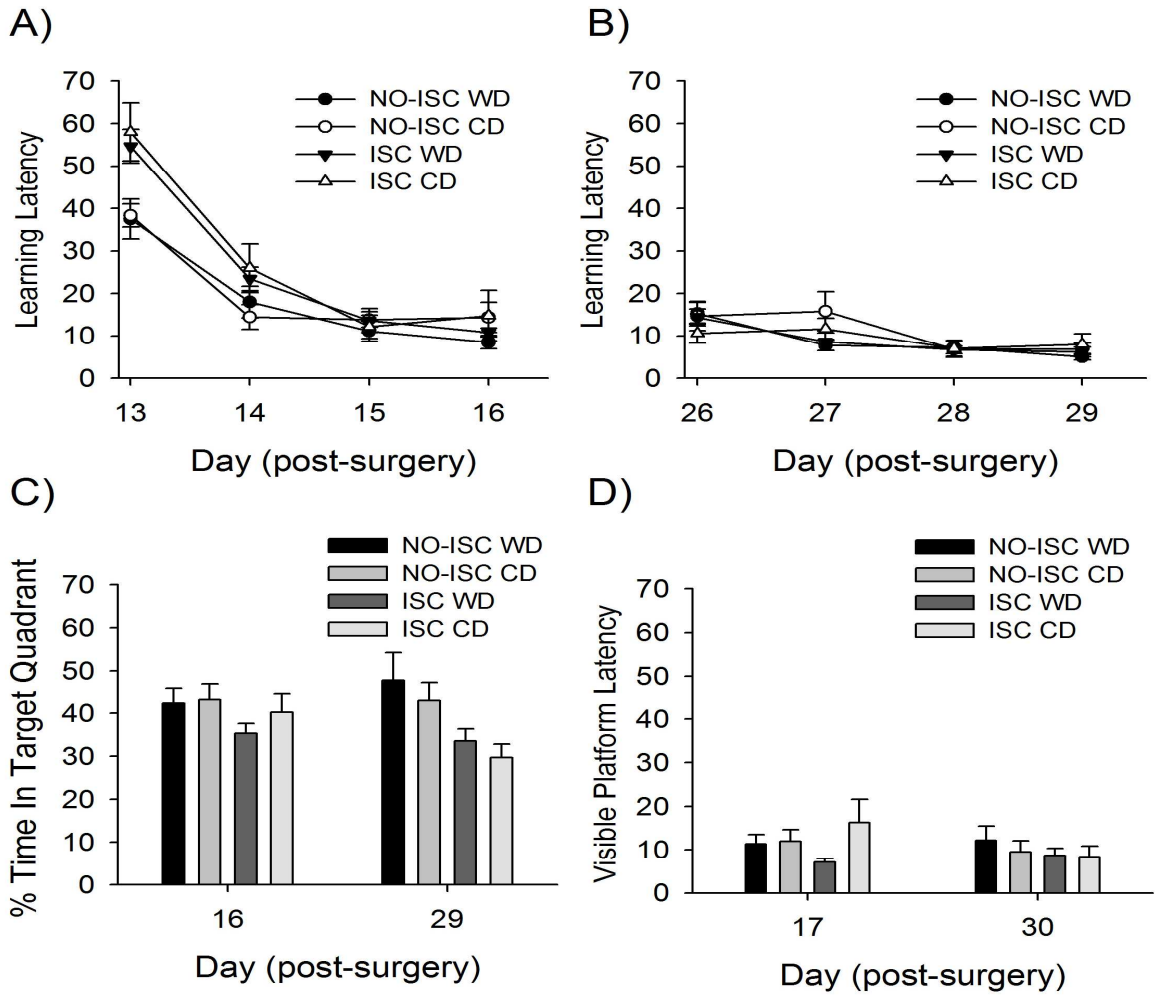

Figure 2.3: Spatial learning and memory for NO-ISC and ISC animals. Learning acquisition (A, B; sec, mean ± SEM), memory retention (C; %, mean ± SEM), and visible platform latencies (D; sec, mean ± SEM) at 2 and 4 weeks post surgery were not influenced by diet in either the NO-ISC or ISC animals. However, 8 min of ISC induced spatial learning deficits at day 13 and 14 post-stroke as compared to NO-ISC animals at the same time point. Forebrain ISC also significantly decreased the amount of time animals spent swimming in the target quadrant as compared to NO-ISC animals. However, visible platform latencies were not different between ISC and NO-ISC animals. See Results for statistics.

Figure 2.4

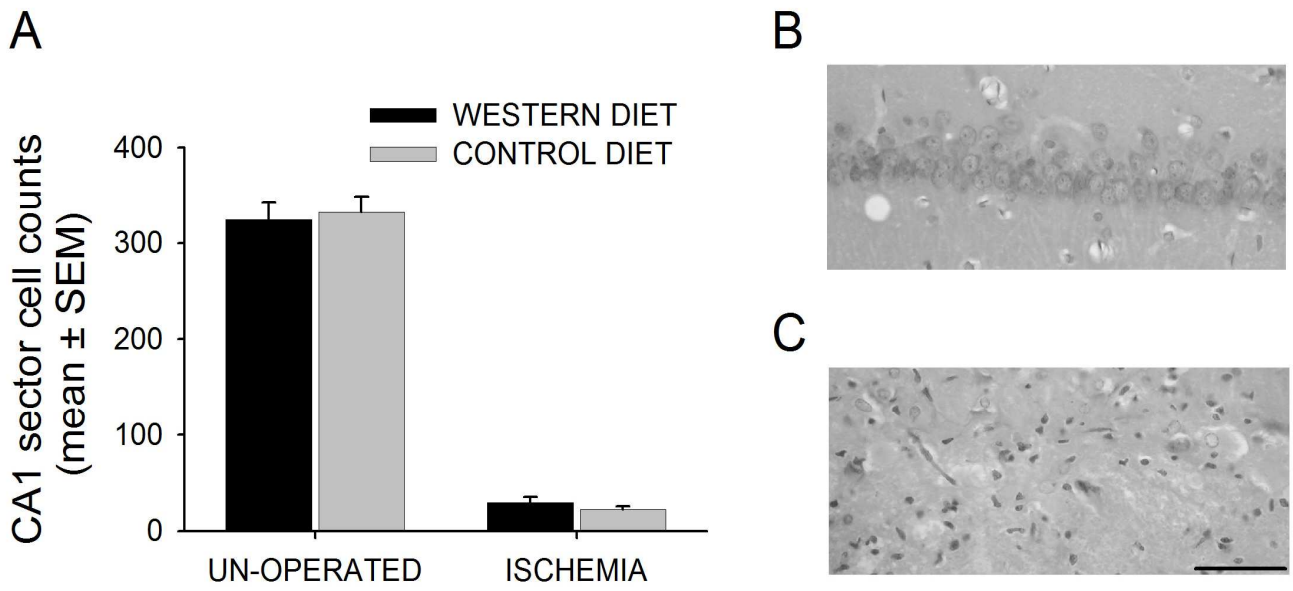

Figure 2.4: Average (± SEM) CA1 sector cell counts (A) in NO-ISC (B) and in rats subjected to 8 min of ISC (C) at a 30 day survival time following the insult. Ischemia caused massive CA1 sector injury that was not significantly affected by diet. Scale bar = 50 µm. The medial CA1 sector is shown at -3.60 mm to Bregma.

2.6 TABLE 2.1 Physiological variables (mean ± SEM) measured during ISC surgery (pH; pCO_2, MABP and pO_2 are in mmHg; glucose is given as mmol / L; ctHb as g / dl; T_s as °C). None of the variables were significantly different between the WD and CD rats

	pH	**pCO_2**	**pO_2**	**cGlu**	**ctHb**	**T_s**	**MABP**
WD	7.36 ± 0.01	39.0 ± 1.2	126.6 ± 1.6	7.8 ± 0.7	12.6 ± 0.2	38.2 ± 0.1	42.0 ± 1.0
CD	7.35 ± 0.01	38.5 ± 1.0	127.1 ± 3.1	7.2 ± 0.7	12.8 ± 0.1	38.1 ± 0.1	42.7 ± 1.0

2.7 REFERENCE LIST

Bobyn, J.P., Corbett, D., Saucier, D.M., Noyan-Ashraf, H.M., Juurlink, B.H.J., Paterson, P.G. (2005). Protein-energy malnutrition impairs functional outcome in global ischemia. Experimental Neurology, 196, 308-315.

Brown, R.W., Whishaw, I.Q. (2000). Similarities in the development of place and cue navigation by rats in a swimming pool. Dev Psychobiol., 37, 238-245.

Colbourne, F., Nurse, S. M., Corbett, D. (1993). Temperature changes associated with forebrain ischemia in the gerbil. Brain Research, 602: 264-7.

Colbourne, F., Corbett, D. (1995). Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. Journal of Neuroscience, 15, 7250-7260.

Colbourne, F., Sutherland, G., Corbett, D. (1997). Postischemic hypothermia. A critical appraisal with implications for clinical treatment. Molecular Neurobiology, 14(3), 171-201.

Ding, E.L., Mozaffarian, D. (2006). Optimal dietary habits for the prevention of stroke. Seminars in Neurology, 26(1), 11-23.

Gerhardt, S.C., Boast, C.A. (1988). Motor activity changes following cerebral ischemia in gerbils are correlated with the degree of neuronal degeneration in hippocampus. Behav. Neurosci., 102, 301-303.

Gillman, M.W., Cupples, L.A., Millen, B.E., Ellison, R.C., Wolf, P.A. (1997). Inverse association of dietary fat with development of ischemic stroke in men. JAMA, 278, 2145-2150.

Greenwood, C.E., Winocur, G. (1990). Learning and memory impairments in rats fed a high saturated fat diet. Behav. Neural. Biol., 53, 74-87.

Greenwood, C.E., Winocur, G. (1996). Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty acid intake. Behav. Neurosci., 111, 451-459.

He, K., Merchant, A., Rimm, E.B., et al. (2003). Dietary fat intake and risk of stroke in male US healthcare professionals: 14 year prospective cohort study. BMJ, 327, 777-782.

Kalmijn, S., Launer, L.J., Ott, A., Witteman, J.C., Hofman, A., Breteler, M.M. (1997). Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann. Neurol., 42, 776-782.

Li, P.-A., Siesjö, B.K. (1997). Role of hyperglycaemia-related acidosis in ischaemic brain damage. Acta. Physiol. Scand., 161, 567–580.

McGee, D., Reed, D., Stemmerman, G., Rhoads, G., Yano, K., Feinleib, M. (1985). The relationship of dietary fat and cholesterol to mortality in 10 years; the Honolulu Heart Program. Int. J. Epidemiol., 14, 97-105.

Molteni, R., Barnard, R.J., Ying, Z., Roberts, C.K., Gómez-Pinilla, F. (2002). A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience, 112(4), 803-814.

Molteni, R., Wu, A., Vaynman, S., Ying, Z., Barnard, R.J., Gómez-Pinilla, F. (2004). Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioural plasticity associated to the action of brain-derived neurotrophic factor. Neuroscience, 123, 429-440.

Morris, M.C., Evans, D.A., Bienias, J.L., Tangney, C.C., Tangney, C.C. (2003). Dietary fats and the risk of incident Alzheimer disease. Arch. Neurol., 60, 194-200.

Morris, M.C., Evans, D.A., Bienias, J.L., Tangney, C.C., Wislon, R.S. (2004). Dietary fat intake and 6-year cognitive change in an older biracial community population. Neurology, 62, 1573-1579.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., O'Keefe, J.O. (1982). Place navigation impaired in rats with hippocampal lesions. Nature, 297, 681-683.

Myers, R.E. (1979). Lactic acid accumulation as a cause of brain edema and cerebral necrosis resulting from oxygen deprivation. In: Guilleminault RK a G, eds. Advances in Perinatal Neurology New York: Spectrum, 85-114.

Paxinos G, Watson C. (1998). The rat brain in stereotaxic coordinates. 4th ed. San Diego CA, USA: Academic Press.

Reed, D.M. (1988). A prospective study of cerebral artery atherosclerosis. Stroke, 19(7), 820-825.

Reed, D.M. (1990). The paradox of high risk of stroke in populations with low risk of coronary Heart disease. Am. J. Epidemiol., 131, 579-588.

Sauvaget, C., Nagano, J., Allen, N., Grant, E.J., Beral, V. (2004). Animal protein, animal fat, and cholesterol intakes and risk of cerebral infarction mortality in the adult health study. Stroke, 35, 1531-1537.

Siesjö, B.K. (1981). Cell damage in the brain: a speculative synthesis. J. Cereb. Blood Flow Metab., 1, 155-185.

Siesjö, B.K. (1984). Cerebral circulation and metabolism. J. Neurosurg., 60, 883-908.

Siesjö, B.K. (1985). Acid-base homeostasis in the brain: physiology, chemistry, and neurochemical pathology. Progress in Brain Research, 165, 121-154.

Siesjö, B.K. (1988). Acidosis and ischemic brain damage. Neurochem. Pathol., 9, 31-88.

Smith, M.L., Auer, R.N., Siesjö, B.K. (1984). The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. Acta. Neuropathol., 64, 319-332.

Weber, K.O., Tranmer, B., Schurer, L., Baethmann, A., Kempski, O. (1989). Physical activity determines survival of mongolian gerbils from forebrain ischemia. In: Hartmann A, Kuschinsky W, eds. Cerebral Ischemia and Calcium. Berlin-Heidelberg-New York: Springer 79-82.

Winocur, G., Greenwood, C.E. (1993). High-fat diets impair conditional discrimination learning in rats. Psychobiology, 21, 286-292.

Winocur, G., Greenwood, C.E. (1999). The effects of high fat diets and environmental influences on cognitive performance in rats. Behavioural Brain Research, 101, 153-161.

Wu, A., Molteni, R., Ying, Z., Gómez -Pinilla, F. (2003). A saturated-fat diet aggregates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. Neuroscience, 119, 365-375.

Yu, Z.F., and Mattson, M.P. (1999). Dietary restriction and 2-Deoxyglucose administration reduced focal ischemic brain damage and improve behavioural outcome: evidence for a preconditioning mechanism. Journal of Neuroscience Research, 57, 830-839.

Zola-Morgan, S., Squire, L.R., Amaral, D.C. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J. Neurosci., 6, 2950-2967.

CHAPTER 3

PROLONGED WESTERN DIET REGIMEN ON STROKE SEVERITY AND FUNCTIONAL OUTCOME FOLLOWING GLOBAL ISCHEMIA IN RATS

3.1 INTRODUCTION

In the previous chapter, we examined the effects of a diet high in saturated fat on stroke severity and functional outcome. Results suggested that a WD did not exacerbate hippocampal CA1 damage nor spatial learning and memory deficits in the MWM task following global ischemia. A thorough review of the existing literature on WDs and its associated negative effects on the brain highlighted several imitations with our study. Briefly, feeding duration proved to be consistently and markedly longer in other experiments (Greenwood and Winocur's, 1990, 1996l; Winocur and Greenwood's, 1993) as compared to our experiment. Specifically, these studies used a diet similar in composition to the one used in our study (i.e., ~40% energy from fat), but fed animals 3 months longer than we did before any cognitive testing was instituted. Others (e.g., Molteni et al., 2002; Wu et al., 2003) who have found significant deleterious effects of WDs as early as 1.5 months after initiating feeding were using diets different in nutritional composition to the diet we used (i.e., high in saturated fat and refined sugar versus simply high in saturated fat). Importantly, a 4.5 month feeding protocol in rats will be more clinically relevant, and thus serve to more accurately reflect the human condition. For instance, a two-week diet comprised of fast-food like meals is not as likely to influence recovery following stroke as a two month diet would. Indeed, we are interested in delineating the effects of a more long-term chronic WD regimen, and how this affects brain damage and functional outcome following stroke. Thus, for a WD to have an appreciable effect on neuronal plasticity and cognition, a longer feeding protocol is necessary.

Two additional limitations in chapter two may have clouded the results. One, the MWM protocol that was employed in chapter two might not have been sensitive enough. For instance, ISC animals' latencies were comparable to that of our NO-ISC animals (i.e., unoperated

controls) by as early as day 3 into testing. If a behavioural measure fails to reveal acute impairments between animals with and without ischemic injury, the likelihood that this same measure will effectively detect any dietary influences is very slim. For this reason, a more demanding MWM protocol is essential. Lastly, in chapter two, 92% of CA1 cells were abolished. If diet did have an effect on the degree of CA1 damage sustained, it would have been difficult to determine this owing to a possible floor effect. For this reason, CA1 sector injury following ischemia should be assessed at more posterior levels of the hippocampus, where injury is less severe and treatment effects can be detected (Corbett and Nurse, 1998).

With these modifications in place, we endeavoured to investigate the effects of a 120-day HF diet regimen on stroke severity and functional outcome following global ischemia. Animals were fed their respective diets for 90 days, at which point a forebrain ischemia was induced, and twenty days later cognitive deficits evaluated. We predicted that a WD would aggravate CA1 cell death and spatial learning and memory impairments.

3.2 Materials and Methods

3.2.1 Animals

Thirty-six male Sprague-Dawley rats (Biosciences Breeding Colony, Edmonton, Alberta, Canada), weighing 60-80 g at the time of arrival, were used in this study. Animals were housed in groups of 4 to 5 until surgery and maintained on a reverse 12-h light/dark cycle (light on at 10:00 PM) with free access to food and water. All procedures followed the Canadian Council for Animal Care guidelines and were approved by the Biosciences Animal Care and Use Committee at the University of Alberta.

3.2.2 Diet

Immediately upon arrival, animals in all experiments were placed on a WD (5TJN, Western diet for rodents, TestDiet, Richmond, IN) or CD (5TJS, low fat control for WD, TestDiet, Richmond, IN). The two diets were isocaloric, but differed slightly in energy density. Percent energy (kcal/g) from protein was similar between the WD and CD (16%). The CD contained 72% energy from carbohydrate and 12% energy from fat. Conversely, the WD contained 44% energy from carbohydrate and 40% energy from fat. Energy density of the WD was 4.49 (kcal/g) and 3.84 (kcal/g) for the CD. However, the amount of vitamins, minerals, fiber and cholesterol per kilocalorie remained equal between the two diets. Animals were fed their respective diets ad libitum throughout the entire experiment, unless otherwise stated. Food intake, however, was not measured throughout the study.

3.2.3 Body weight measurements

Body weight (g) was measured everyday for 7 consecutive days after arrival, and then on a weekly basis until euthanasia. Body weight was also measured at the time of surgery and everyday thereafter until animals returned to their pre-surgical weight

3.2.4 Forebrain Ischemia

After 90 days of feeding, animals were subjected to 8 minutes of global ischemia. Animals were subjected to food deprivation (~ 12 hr) prior to surgery in order to lower glucose levels into a consistent range (4 – 8 mmol / L). Rats were anesthetized (~ 45 min) with isoflurane (1.5%–2% maintenance in 30% O_2 and 70% N_2O) and placed on a heated water blanket (model TP3E, Gaymar, NY) with feedback control from a thermocouple probe (HYPO-33-1-T-G-60-

SMG-M, Omega, Stanford, CT) placed subcutaneously on the skull (centre). A model CSC-32 (Omega) feedback regulator maintained skull temperature (T_s) near 37.5°C via an infrared lamp (175 W) to minimize an unwanted drop in brain temperature during ISC, which would have lessened injury. A 2-cm incision was made along the ventral midline of the neck and the common carotid arteries were isolated. Mean arterial blood pressure (MABP) was measured via a tail artery cannula kept patent by heparinized saline. Ischemia was achieved by transient bilateral carotid artery occlusion and systemic hypotension (35-45 mmHG; BP-1, World Precision Instruments, Sarasota, FL). Systemic hypotension was produced by withdrawing blood from the jugular vein into a heparinized syringe. Following an eight minute 2-VO, clamps were removed and blood was slowly re-infused. Arterial blood samples (approximately 100 μL each) were taken to measure blood pH, pCO_2, pO_2, hemoglobin (ctHb), and glucose levels before and after ISC.

3.2.5 Morris Water Maze

Twenty days following surgery, animals were trained in a modified version of the MWM task described by Driscoll and colleagues (2006). The platform was moved to a new location every second day according to a random sequence of locations. The platform remained in the same location for two consecutive days. This allowed for the assessment of learning on the first day (new platform location), and of retention on the second day (same platform location). The platform moved to a new location every two days until 4 different location-day pairs were completed (for a total of 8 consecutive days). After completion of the hidden platform training, we administered one day of visible platform training. Rats received 8 trials on each day and were released twice from each quadrant around the perimeter of the pool in a pseudorandom sequence.

Animals had a maximum of 60 s to locate the platform. If animals failed to locate the platform within 60 s, they were removed from the water and placed on the platform for 10 s before returning to the holding cage. Animals who successfully found the platform also remained on the platform for 10 s before being returning to the holding cage. Latency to locate the platform was measured. All other details (e.g., pool size, water temperature) were identical to those described in experiment 2 under 'Morris Water Maze' (section 2.2.4*ii*).

3.2.6 Histopathology

Rats were euthanized (i.e., 30 days after ischemia) using an overdose of sodium pentobarbital (100 mg/kg, intraperitoneal (i.p.)) and transcardially perfused with 0.9% saline followed by 10% neutral-buffered formalin. Extracted brains were embedded in paraffin. Subsequently, 6-micrometre coronal section were cut and stained with hematoxylin-eosin. Two sections, one at -3.60 mm to Bregma, and the other at -4.60 mm to Bregma (Paxinos and Watson, 1998), were chosen in which CA1 sector injury was determined (Colbourne and Corbett, 1995). Viable (healthy-looking, non-eosinophilic) CA1 sector pyramidal neurons were counted in medial (next to the subiculum), middle, and lateral regions (next to the CA2 zone) of the CA1 zone of both hemispheres. The number of CA1 sector neurons was summated over the left and right hemispheres and expressed as a percent of normal. The researcher was blinded to the group identity.

3.2.7 Statistics

Physiology (e.g., glucose levels, MABP), histology, body weight, and MWM data were analyzed using ANOVA (SPSS, v. 15.0. Chicago, Ill.). A p-value of ≤ 0.05 was considered statistically significant. All data are reported as mean ± SEM.

3.3 RESULTS

3.3.1 Protocol violations and mortality

Five rats were excluded from the study. One animal had to be euthanized during surgery because he was too heavy (e.g., 1060 g) and the desirable MABP (~45 mmHg) could not be achieved even after withdrawing approximately 16 ml of blood. One animal died during surgery of unknown causes, likely due to anaesthetic complications. Three animals developed post-ischemic seizures and thus had to be euthanized. The remaining group sizes were as follows: HF (n=15) and LF (n=16) group.

3.3.2 Body weight measurements

Rat body weight (Figure 3.1) was analyzed using a 2-factor ANOVA (Diet factor; Time factor: arrival, 1 month, 2 months, surgery and euthanasia). There was a significant Diet x Time interaction ($p<0.001$), an overall main effect of Diet ($p=0.004$) and Time ($p<0.001$). Owing to the significant interaction, the data were analyzed for each time point to determine when diet affected body weight. Significant differences occurred at 2 months ($p<0.001$), 3 months ($p=0.001$) and approached significance at euthanasia ($p=0.056$).

3.3.3 Physiology

Physiological variables during surgery are given in Table 3.1. Animals in the WD has significantly elevated fasting glucose levels (p=0.014). Blood pH, PCO_2, PO_2, concentration of total hemoglobin (ctHb), skull temperature (T_s) and mean arterial blood pressures (MABP) were the same for animals maintained on the WD and CD ($p \geq 0.112$). These measurements did not significantly correlate with CA1 injury at either -3.60 or -4.60 mm to Bregma (r=-0.329 to 0.329, $p \geq 0.071$)

3.3.4 Histopathology

CA1 sector necrosis was not significantly different between the WD and CD at either -3.60 mm to Bregma (p=0.121) or -4.80 mm to Bregma (p=0.063). However, there was tremendous variability in hippocampal CA1 cell damage across animals at both levels of the hippocampus (i.e., -3.60 and -4.60 mm to Bregma; Figure 3.2). For instance, at -3.60 mm to Bregma, injury ranged from severe (~85% – 99% necrosis; n=7: WD=2, CD=5), moderate (~55% – 85% necrosis; n=10: WD=4, CD=6), mild (~25% – 55% necrosis; n=3: WD=1, CD=2), to none (~0% – 25%; n=10: WD=8, CD=2). While the Levene's test of equality of error variance revealed homogeneity of variance between the WD and CD (p=0.680 at -3.60 mm to Bregma; p=0.683 at -4.60 mm to Bregma), we do have a problem with external validity, in that our stroke model did not produce severe and consistent CA1 injury in both the WD and CD. For this reason, the effects of a WD on CA1 sector injury should be interpreted with caution.

3.3.5 Morris Water Maze

Since ischemic damage was inconsistent across animals, it is difficult to delineate whether the observable behavioral impairments exhibited by animals were due to the dietary

manipulation (i.e., WD versus CD) or to the varying degrees of ischemic injury. For this reason, the behavioral data collected were best analyzed using correlational analyses. A Pearson's correlation revealed that the extent of brain damage predicted the extent of behavioral deficits. For instance, there was a significant negative correlation between hippocampal CA1 cell death and MWM learning acquisition latencies in animals fed the WD (at -3.60 mm to Bregma r=-0.774, $p<0.01$; at -4.80 mm to Bregma r=-0.824, $p<0.01$) and CD (at -3.60 mm to Bregma r=-0.762, $p<0.01$; at -4.80 mm to Bregma r=-0.785, $p<0.01$). The same patterns of result were seen with the memory retention component of the MWM task. For instance, hippocampal CA1 cell death and memory retention latencies were negatively and significantly correlated in animals fed the WD (at -3.60 mm to Bregma r=-0.774, $p<0.01$; at -4.80 mm to Bregma r=-0.839, $p<0.01$) and CD (at -3.60 mm to Bregma r=-0.713, $p<0.01$; at -4.80 mm to Bregma r=-0.731, $p<0.01$). These data suggest that as the number of remaining hippocampal CA1 cells increases (e.g., less hippocampal CA1 sector damage), latency to locate the hidden platform decreases (e.g., the animal is able to learn the location of the platform quicker and/or remember the platform location better). Conversely, these data also suggest that as the number of remaining hippocampal CA1 cells decreases (e.g., greater hippocampal CA1 sector damage), latency to locate the hidden platform increases (e., the animals takes longer to learn the location of the hidden platform and/or does not remember the platform location as well.

3.4 DISCUSSION

This is the first study to assess the effects of a prolonged (i.e., 90 days) WD feeding protocol on stroke severity and functional outcome following global ischemic injury. However, ischemic injury, as assessed by hippocampal CA1 cell counts, was largely variable. While

identical procedures were employed in this study as in chapter two (i.e., animals fasted 12 hours prior to surgery, skull temperature and physiological variables monitored during surgery, etc), the findings were anything but the same; in the previous study we saw consistent damage across all ischemic animals. Note however, that animals in the present study were more than double in weight and age as compared to animals in our previous study. The 2-VO model developed by Smith and colleagues (1984) used rats weighing between 325-400 g, similar in weight to the animals in our previous study. Even research in the area of aging and global ischemia uses rats around 600 g (26 – 28 months of age) at the time of ischemia (Sutherland et al., 1996). In the present study however, animal body weight ranged from 558 to 850 g. It may be the case that the 2-VO model that was developed by Smith et al. (1984) and used in this study does not suffice when it comes to heavier animals. However, this explanation does not account for the animals weighing in at 560 g at the time of surgery who did not sustain any observable CA1 damage.

The irregularity with which damage was observed cannot be fully explained at the present time, but one or several plausible explanations may be held accountable. For one, it may simply be the case that heavier rats are more resistant to global ischemic injury. For instance, obese rats may experience steeper drops in brain temperature, not reflected by skull temperature; however, only skull temperature was regulated in the present study. Indeed, research has found an existing dissociation between rectal, skull and brain temperature (Busto et al., 1987; Colbourne et al., 1993; Minamisawa et al., 1990). For instance, Colbourne and colleagues (1993a) measured skull and brain temperature at the start of, and for up to 180 minutes following global ischemia. They found that at the start of surgery and occlusion, brain temperature was approximately 1°C lower than skull temperature. However, at the end of occlusion and the beginning of reperfusion, brain temperature was at 32.5°C (mild hypothermia), while skull

temperature was at 37°C (normothermia); however, as time elapsed, this difference decreased, but did not become identical until 30 minutes following global ischemia. A separate study by Colbourne and colleagues (1993b) found that extended anaesthesia (e.g., 85 min following ischemia) caused prolonged brain hypothermia, but normothermic skull and rectal readings, as compared to control animals (e.g., without extended anaesthesia). The animals in the present study were very large; at times surgery did take longer than usual, and as a result animals were maintained under anaesthesia for a longer period of time. Hypothermia during ischemia and for the first few minutes of reperfusion significantly decreases glutamate release and thus the severity of CA1 necrosis (Colbourne et al., 1993a). Clearly, brain temperature, as opposed to skull temperature, needs to be regulated in order for more consistent CA1 necrosis to be achieved (Nurse et al., 1992). Since we only regulated skull temperature, animals' brain temperature may have dropped to hypothermic levels, and hence unbeknownst to us, animals were sufficiently cooled. We know from previous research that cooling is neuroprotective following global ischemia in rats and cardiac arrest in humans (for a review see Colbourne et al., 1997).

By the same token, obese rats may be able to better compensate for reductions in blood pressure. Since rats have a complete Circle of Willis, hypotension to levels below 50 mmHg is essential to achieving consistent and severe CA1 cell death. In lieu of this, it seems appropriate to incorporate cerebral blood flow measurements using Laser Doppler Flowmetry, which assesses microvasculature blood perfusion. Hippocampal cerebral blood flow should be at ~1% of control values during occlusion and hypotension below 50 mmHg (Smith et al., 1984). Perhaps a reduction in blood pressure to 50 mmHg in obese rats does not reduce cerebral blood flow values to similar values (i.e., ~1% of controls), leading to variable and moderate damage at best.

Moreover, while occlusion times of 8 minutes in smaller animals (i.e., not as heavy) consistently and sufficiently gives rise to permanent brain damage, perhaps longer occlusion times are required with heavier animals, such as those seen in the present study. Indeed, Sutherland and colleagues (1996), investigating the effects of age in the 2-VO model of global ischemia, used occlusion times of 12 minutes, which produced consistent damage in their aged animals weighing approximately 600 g. Overall, future studies must deductively address the aforementioned issues, along with others that may arise, using obese animals. In doing so, an effective model of global ischemia for obese rats will be established so that longer feeding protocols (e.g., 4 months and greater) can be evaluated.

3.5 FIGURES

Figure 3.1

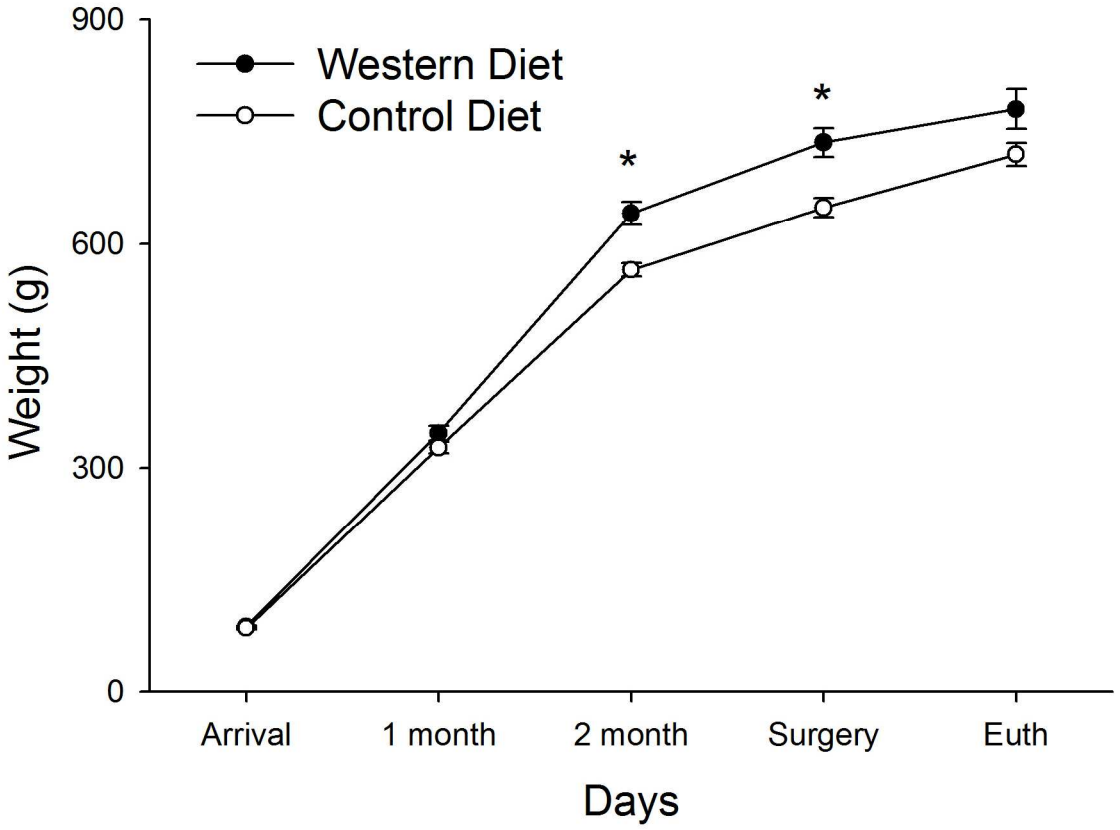

Figure 3.1: Body weight (mean ± SEM) for ischemic animals fed the WD and CD at several times throughout the study. Animals on both diets gained a significant amount of weight over time. However, a significant weight differences was only observed at 2 months and at the time of ischemic surgery between the WD and CD (* $p<0.001$).

Figure 3.2

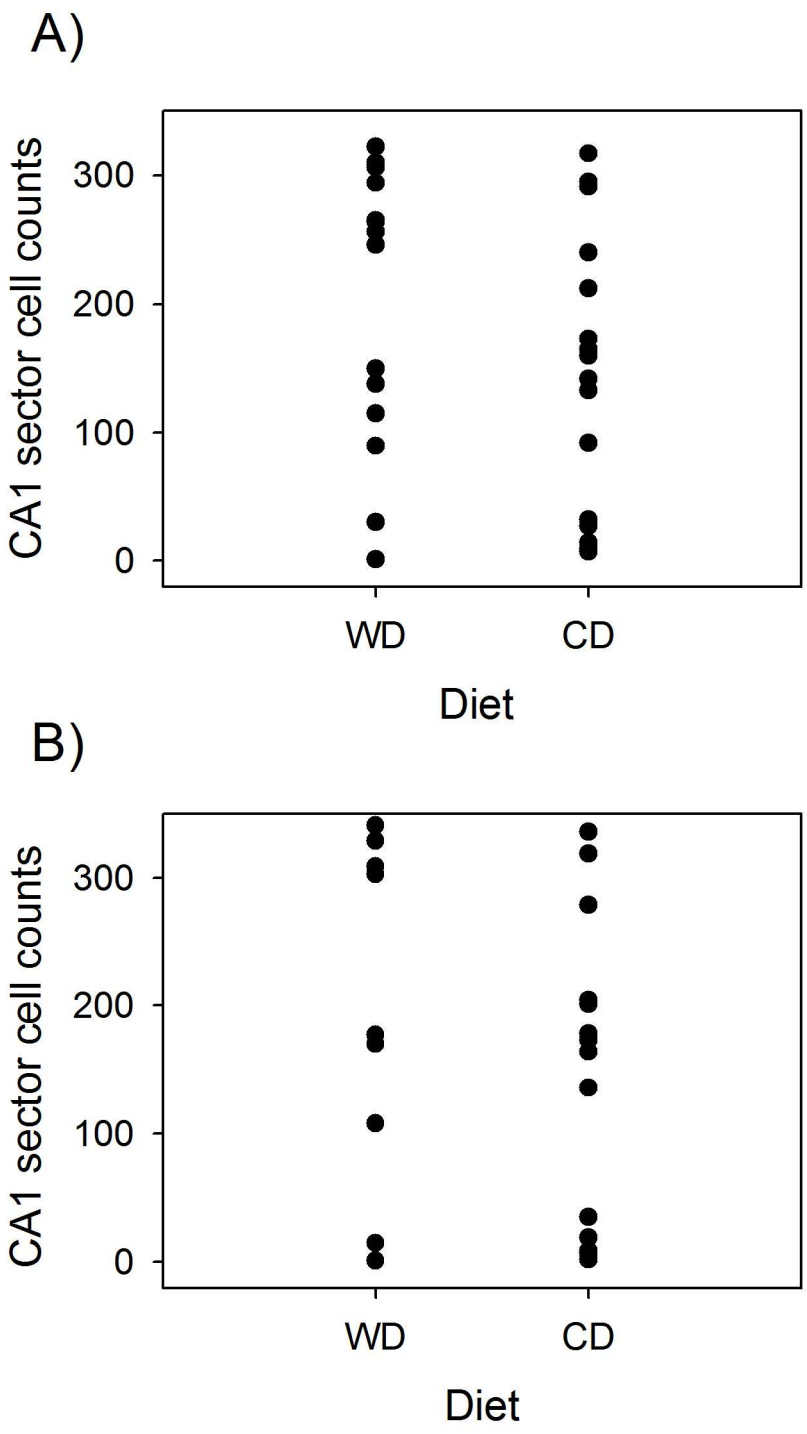

Figure 3.2: Hippocampal CA1 sector cell counts (A) at -3.60 mm to Bregma (B) and at -4.60 mm to Bregma. In both the WD and CD, eight minutes of ischemia caused variable CA1 sector injury, with severe CA1 damage in some and no observable CA1 damage in others.

3.6 TABLE 3.1 Physiological variables measured during global ISC surgery (pH; pCO_2, BP and pO_2 are in mmHg; glucose is given as mmol / L; ctHb as g / dl; T_s as °C). Fasting glucose levels were significantly higher in animals fed the WD versus the CD. None of the other variables were significantly different among the WD and CD rats (see Results).

	pH	**pCO_2**	**pO_2**	**cGlu**	**ctHb**	**T_s**	**MABP**
WD	7.34 ± 0.01	45.18 ± 1.81	129.53 ± 1.35	*15.91 ± 0.97	14.52 ± 0.12	37.78 ± 0.11	41.33 ± 0.44
CD	7.35 ± 0.01	45.18 ± 0.95	129.94 ± 1.76	12.23 ± 1.02	14.40 ± 0.12	37.58 ± 0.05	40.45 ± 0.43

3.6 REFERENCE LIST

Busto, R., Dietrich, W.D., Globus, M.Y.-T., Valdes, I., Scheinberg, P., Ginsberg, M.D. (1987). Small differences in intra-ischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cereb Blood Flow Metab., 7, 729-738.

Colbourne, F., Corbett, D. (1995). Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. Journal of Neuroscience, 15, 7250-7260.

Colbourne, F., Nurse, S.M., Corbett, D. (1993a). Temperature changes associated with forebrain ischemia in the gerbil. Brain Research, 602, 264-267.

Colbourne, F., Nurse, S.M., Corbett, D. (1993b). Spontaneous postischemic hyperthermia is not required for severe CA1 ischemic damage in gerbils. Brain Research, 623, 1-5.

Colbourne, F., Sutherland, G., Corbett, D., (1997). Postischemic hypothermia. A critical appraisal with implications for clinical treatment. Molecular Neurobiology 14(3), 171-201.

Corbett, D., Nurse, S. (1998). The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol., 54, 531-548.

Driscoll, I., Howard, S.R., Stone, J.C., Monfils, M.H., Tomanek, B., Brooks, W.M., Sutherland, R.J. (2006). The aging hippocampus: a multi-level analysis in the rat. Neuroscience, 139, 1173-1185.

Greenwood, C.E., Winocur, G., 1990. Learning and memory impairments in rats fed a high saturated fat diet. Behav. Neural. Biol. 53, 74-87.

Greenwood, C.E., Winocur, G., 1996. Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty acid intake. Behav. Neurosci. 111, 451-459.

Minamisawa, H., Mellergard, P., Smith, M-L., Bengtsson, F., Theander, S., Boris-Moiler, F., Siesjö, B.K. (1990). Preservation of brain temperature during ischemia in rats. Stroke, 21, 758-764.

Molteni, R., Barnard, R.J., Ying, Z., Roberts, C.K., Gómez-Pinilla, F., 2002. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112(4), 803-814.

Nurse, S.M., Neuman, R.S., Corbett, D. (1992). Hypothermic protection against ischemic insult: assessment using histological, behavioral and electrophysiological measures. Soc. Neurosci Abstr., 18, 1586.

Paxinos, G., Watson, C. (1998). The rat brain in stereotaxic coordinates. 4th ed. San Diego CA, USA: Academic Press.

Rajdev, S., Hara, K., Kikubo, Y., Mestril, R., Dillmann, W., Weinstein, P.R., et al. (2000). Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. Ann Neurol, 47(6), 782-791.

Smith, M.L., Auer, R.N., Siesjö, B.K., 1984. The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. Acta. Neuropathol. 64, 319-332.

Sutherland, G.R., Dix, G.A., Auer, R.N. (1996). Effect of age in rodent models of focal and forebrain ischemia. Stroke, 27, 1663-1668.

Winocur, G., Greenwood, C.E., 1993. High-fat diets impair conditional discrimination learning in rats. Psychobiology 21, 286-292.

Yenari, M.A., Giffard, R.G., Sapolsky, R.M., Steinberg, G.K. (1999). The neuroprotective potential of heat shock protein 70 (HSP70). Mol Med Today, 5(12), 525-531.

CHAPTER 4

DISCUSSION

4.1 Major Findings

Brain damage from cerebral ischemia is a very prominent and debilitating injury. In humans, drugs therapies to attenuate brain damage and its associated neurological deficits have yet to be identified. Note that many pharmacological treatments (with the exception of hypothermia) in the past have offered neuroprotection in animals, but failed once they reached clinical trials. Briefly, inappropriate animals stroke models, over reliance on histological outcome without functional assessments, and short-term end points have all contributed to the disconnect between research outcomes in experimental versus clinical settings (Gladstone et al., 2002). As a result, effective stroke therapies remain elusive, which in part contributed to the recent shift in attention from drug treatments to modifiable factors such as nutrition. In contrast to nutrition, drug treatments are generally more expensive, especially in parts of the world where health care and drug coverage are not widely available (i.e., United States of America), not to mention are more invasive, such as unwanted drug side effects and drug interactions. Even more promising is recent literature suggesting that dietary constituents and/or regimens have neuroprotective properties independent of risk factors (i.e., influencing blood pressure, cardiovascular health, etc.). For instance, a recent clinical study by Ullegaddi and colleagues (2004) found that B-group vitamin supplementation for 14 days following stroke mitigated oxidative damage after acute ischemic stroke as per C-reactive protein concentrations (a marker of tissue inflammation).

With increasing incidences of obesity, predominantly in the Western part of the world, researchers have started to seriously investigate the impact of a so called ‘Western diet’ (i.e., high in refined sugar and/or saturated fat) on outcome following brain injury. In an animal model of traumatic brain injury, Wu and colleagues (2003) showed that a WD provided for 30 days

aggravated brain damage and cognitive deficits, and depleted BDNF mRNA in the CA3 and dentate gyrus of the hippocampus, one week following injury. Even in normal animals (i.e., with no brain damage) WDs have been shown to markedly and negatively effect cognitive faculties (Greenwood and Winocur, 1990, 1996; Winocur and Greenwood, 1993, 1999). However, no studies to date have examined the impact of a WD on stroke severity and functional outcome. Since we already know that various factors (i.e., increased blood pressure, increased glucose levels, increased brain temperature, decreased oxygen levels), worsen stroke outcome, we rigorously controlled for them to help isolate the effects of a WD on stroke severity and functional outcome. We therefore chose to address this question in a well-established and well controlled experimental model of global ischemia in rats.

4.1.1 Cell death and cognition not worsened by a 60-day western diet

The experiments detailed in chapter 2 assessed the impact of a WD in NO-ISC and ISC animals. The first experiment looked at whether a WD impacted glucose, temperature, activity, cognition and/or histology in NO-ISC animals; with the exception of activity, the findings were unremarkable. Temperature profiles, CA1 cell counts, and spatial learning and memory were not significantly different in animals fed the WD versus the CD. Overall, animals fed the CD were more active than animals fed the WD; however, because histology was similar between the two dietary groups, the need to further investigate this issue was unnecessary for our purposes. With no apparent confounding variables (i.e., similar glucose levels, temperature profiles in the WD and CD) from experiment 1, we felt the next appropriate step was to investigate the histological and cognitive consequences of a WD following a global ischemic insult. Global ischemia is known to abolish hippocampal CA1 sector cells and induce grave learning and memory

impairments, with no sensory motor impairments. For this reason, we chose the MWM as our functional outcome assessment tool, a standard hippocampus-sensitive test of spatial learning and memory. We quantified hippocampal CA1 sector cell death because one, that region is especially vulnerable to global ischemic injury so one can expect to see consistent and significant injury, and two, the luminar distribution and large size of CA1 cells makes them rather simple to quantify (Corbett and Nurse, 1998). Here we found that while global ischemia induced severe CA1 cell necrosis and memory deficits at 30 days post ischemia, a WD did not enhance histological and functional injury. However, we were interested to see if a longer feeding regimen would have a more profound affect on the brain. Thus, we turned our attention to assessing the impact of a WD on global ischemia following a 4-month feeding regimen.

4.1.2 Why a longer feeding protocol made it difficult to assess stroke outcome

The experiment detailed in chapter 3 sought to address several limitations of experiment 2 (chapter 2), one of which was a feeding protocol that may not have been long enough to have a pronounced effect on the brain. For this reason, we assessed the consequences of a 4-month WD on stroke severity and cognition. However, routine CA1 cell counts under the microscope revealed that there was remarkable variability in the amount of observable CA1 necrosis across all animals. Numerous plausible explanations have been put forth to account for this outcome. One, it is entirely feasible that the model of global ischemia developed my Smith et al. (1984) and used in the present study, while efficiently induces brain damage in smaller animals, is not entirely effective in more obese animals. In short, occlusion times may need to be longer (i.e., 12 minutes versus 8 minutes as in Sutherland et al.'s (1996) experiment using older animals), and hypotension may need to maintained below 40 mmHg and not 50 mmHg in order to reduce

cerebral blood flow values to ~1% of controls in the hippocampus. Accordingly, cerebral blood flow measurements are the best way to determine blood perfusion in desired brain regions (i.e., hippocampus). Indeed, if one endeavoured to develop a model of global ischemia for obese rats, it would be imperative to use Laser Doppler Flowmetry to measure cerebral blood flow in selected and commonly vulnerable brain structures. In which case, one should absolutely measure brain and skull temperature throughout surgery to determine whether there is a more significant drop in brain temperature (i.e., greater than 1.5°C) than skull temperature in heavier rats than average sized rats. It is also feasible that the aspirated blood during ischemia cooled significantly whilst sitting in the syringe, and thus cooled the brain when it was reinfused; therefore, future studies must be sure to keep that blood warmed. One or all of these factors may be responsible for the variability in injury seen in chapter 3.

4.2 Future Direction

The brain is the organ with the second greatest concentration of lipids that are directly involved in the functioning of membranes; and because the brain can neither synthesize nor store energy reserves, it is the daily diet that provides the immediate source of energy to the brain and thereby influences brain function. In fact, research has shown that the levels of lipids, cholesterol, oxidized cholesterol, and oxidative stress that follow meals containing trans fats, saturated fat, or glucose are what affect the endothelium for approximately 18 hours of the day (Spence, 2006). Thus, it comes as no surprise that several lines of animal research (e.g., Greenwood and Winocur, 1990, 1996; Winocur and Greenwood, 1993) have illustrated the nocuous effects of a diet high in saturated fat on cognition. While the neurobiological mechanisms underlying this impairment remain elusive, authors suggest that saturated fat intake

contributes to increased insulin resistance and decreased glucose uptake, the latter being an important factor in the cognitive decline associated with normal aging.

With all of these factors in mind, one must question the influence of saturated fatty acids in the diseased brain. In a rat model of traumatic brain injury, a diet high in saturated fat and refined sugar proved pernicious (Wu and colleagues, 2003); however, these authors should re-examine this issue with control of glucose. While we attempted to evaluate the effects of a WD in a rat model of global ischemia, several issues precluded us from reaching any definitive conclusions. Nevertheless, it remains essential to delineate the effects of a WD on stroke severity and functional outcome following global ischemia in rats; to effectively assess this using a longer feeding protocol, a reliable and adequate model of global ischemia must be established in obese rats. Moreover, several authors (e.g., Molteni et al., 2002; Wu et al., 2003) have shown that a WD exerts its deleterious effects through depleting BDNF levels and increasing oxidative stress levels in the hippocampus; however, their diet contained excessive amounts of saturated fat and refined sugar, whereas the diet used in the present study was comprised mainly of copious amounts of saturated fat. Thus, addressing mechanistic questions, such as BDNF and oxidative stress, is essential in understanding how specifically a diet high in saturated fat alters learning, memory, and recovery.

It is also critical to evaluate a WD in other stroke models (e.g., hemorrhagic stroke, focal ischemia) as the differences in pathophysiology between them are remarkable. For instance, in focal ischemia there is a significant gradation of ischemia from the core of the lesion (e.g., where regional cerebral blood flow drops below 10% of control values) to its outermost boundary, also known as the penumbral region (e.g., where regional cerebral blood flow levels are kept at up to 40% of control values owing to retrograde perfusion by anastomosis from adjacent arteries)

(Choi et al., 1996). First introduced by Astrup and colleagues (1981), the penumbra is a region of viable tissue that is hypoperfused, electrically silent, and functionally impaired. Neuroprotective strategies have thus focused on treatments able to salvage the penumbral tissue and minimize neurological impairments. In stark contrast, global ischemia does not have a penumbral region; instead, global ischemia is modelling a pattern of injury (such as that seen in cardiac arrest patients), where distinct neuronal populations have very different vulnerabilities to ischemia. In other words, global ischemia leads to neuronal cell death in isolated regions (e.g., hippocampus, striatum) whereas focal ischemia produces an adjoining mass of damaged brain tissue (e.g., the infarct). While some therapies have proved beneficial in both global and focal ischemia (e.g., hypothermia), other treatments have worked in focal ischemia but are not possible in global ischemia (e.g., tissue plasminogen activator), due mainly to differences in pathophysiology. Accordingly, although an adverse effect of a WD was not found in global ischemia, it is imperative to evaluate a WD in focal ischemia and hemorrhagic stroke for a more comprehensive understanding that saturated fats play in stroke.

4.3 CONCLUSIONS AND CLINICAL SIGNFICANCE

In summary, my thesis looked at the long-term effects a WD on stroke severity and functional outcome in a rat model of global ISC. I found that a WD provided for 30-days prior to brain injury did not exacerbate hippocampal CA1 cell death, or spatial learning and memory impairments in the WD group. I carried out a second experiment that evaluated the effects of a WD provided to animals for 90-days prior to brain injury. However, histology revealed that hippocampal CA1 damage across animals was inconsistent, possibly owing to how obese the rats became. A number of plausible explanations (e.g., cerebral blood flow does not drop as low in certain brain region in obese animals) and corrective strategies (e.g., measure brain temperature

along with skull temperature, use Laser Doppler Flowmetry to measure cerebral blood flow in the CA1 region of the hippocampus) have been put forth; these will help establish an effective model of global ischemia in more obese rats to then effectively evaluate the effects of a WD in global ischemia. Results from these studies may have some significance for clinical cardiac arrest in patients. The WD provided in the present thesis reflects upper levels of human fat consumption. What's more, older adults are at a higher stroke risk and particularly vulnerable to the adverse effects of dietary fat. Clearly, continued efforts to characterize the effect of saturated fat on stroke outcome are warranted, which may help improve dietary habits of older adults and lead to new approaches to the treatment of stroke.

4.4 REFERENCE LIST

Astrup,J. Siesjo,BK. Symon,L. (1981). Thresholds in cerebral ischemia—the ischemic penumbra. *Stroke*, 12, 723–725.

Choi DW. Ischemia induced neuronal apoptosis. (1996). Curr Opin Neurobiol, 6, 667–72.

Corbett, D., Nurse, S. (1998). The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol., 54, 531-548.

Gladstone, D.J., Black, S.E., Hakim, A.M. (2002). Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions. Stroke, 33, 2123-2136.

Greenwood, C.E., Winocur, G. (1990). Learning and memory impairments in rats fed a high saturated fat diet. Behav. Neural. Biol., 53, 74-87.

Greenwood, C.E., Winocur, G. (1996). Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty acid intake. Behav. Neurosci., 111, 451-459.

Molteni, R., Barnard, R.J., Ying, Z., Roberts, C.K., Gómez-Pinilla, F. (2002). A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience, 112(4), 803-814.

Smith, M.L., Auer, R.N., Siesjö, B.K. (1984). The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. Acta. Neuropathol., 64, 319-332.

Sutherland, G.R., Dix, G.A., Auer, R.N. (1996). Effect of age in rodent models of focal and forebrain ischemia. Stroke, 27, 1663-1668.

Ullegaddi, R., Powers, H.J., Gariballa, S.E. (2004). B-group vitamin supplementation mitigates oxidative damage after acute ischaemic stroke. Clinical Science, 107, 477-484.

Winocur, G., Greenwood, C.E. (1993). High-fat diets impair conditional discrimination learning in rats. Psychobiology, 21, 286-292.

Winocur, G., Greenwood, C.E. (1999). The effects of high fat diets and environmental influences on cognitive performance in rats. Behavioural Brain Research, 101, 153-161.

Wu, A., Molteni, R., Ying, Z., Gómez -Pinilla, F. (2003). A saturated-fat diet aggregates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. Neuroscience, 119, 365-375.