DIETARY CURCUMIN AND CALORIC RESTRICTION AS INTERVENTIONS FOR THE

REVERSAL OF AGE ASSOCIATED FUNCTIONAL DECLINE

DISSERTATION

Presented to the Graduate Council of the Graduate School of Biomedical Sciences

University of North Texas Health Science Center

In partial fulfillment of the requirements

For the degree of

DOCTOR OF PHILOSOPHY

By

Marjana Rahman Sarker

Fort Worth, TX

May 21st, 2015

DIETARY CURCUMIN AND CALORIC RESTRICTION AS INTERVENTIONS FOR THE
REVERSAL OF AGE ASSOCIATED FUNCTIONAL DECLINE

Marjana Rahman Sarker

Approved:

Michael J. Forster, PhD
Major Professor

Susan F. Franks, PhD
Major Professor

Nathalie Sumien, PhD
Committee Member

Eric B. Gonzales, PhD
Committee Member

Derek Schreihofer, PhD
Committee Member

Joseph Warren, PhD
Committee Member

Mehervan Singh, PhD
Dean, Graduate School of Biomedical Sciences

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Mentors:

Michael J. Forster, PhD

Susan F. Franks, PhD

Committee members:

Nathalie Sumien, PhD

Eric B. Gonzales, PhD

Derek Schreihofer, PhD

Joseph Warren, PhD

Colleagues and Peers:

Margaret Rutledge, PhD

Nopporn Thangthaeng, PhD

Ritu Shetty, PhD

Kiran Chaudhari, PhD

Andrew Gdowski

Jessica Wong

All members of AGE and NIDA lab

Family

Ammu and Abbu, thank you for your endless support. Thank you for letting me dream and for giving me everything that I have ever wanted even when times were tough. I love you both. Mariam, you are my rock, thank you for listening to my banter and constant whining throughout this whole PhD process. I love you.

List of tables

List of figures

Chapter III

Chapter IV

List of Abbreviations:

CNS	Central nervous system
ROS	Reactive oxygen species
NOX	NADPH oxidase
Nrf2	Nuclear erythroid-derived 2 related factor
SOD	Superoxide dismutase
CAT	Catalase
GSH-Px	Glutathione peroxidase
ARE	Antioxidant response elements
AD	Alzheimer's disease
PD	Parkinson's disease
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GCL	γ glutamyl cysteine ligase
NK	Natural killer
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
IKKβ	Inhibitor of kappa light polypeptide gene enhancer in B-cells
TNF-α	Tumor necrosis factor alpha
IL-6	Interleukin-6
IL-1β	Interleukin-1 beta
CRP	C-reactive protein
CR	Caloric restriction

FADH	Flavin adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide
WWII	World War II
Keap1	Kelch-like ECH-associated protein 1
VAT	Visceral adipose tissue
SAT	Subcutaneous adipose tissue
AL	Ad libitum
CURAL	Curcumin fed ad libitum
CURCR	Curcumin in the CR paradigm

Glossary

Redox state: term widely used in the research field of free radicals and oxidative stress. It is used to describe the ratio of the inconvertible oxidized and reduced form of a specific redox couple. The redox state of a redox couple is defined by the half-cell reduction potential and the reducing capacity of that couple. Notation for the status of a redox pair – GSSG/2GSH

Nutraceutical: other names include "functional foods", "pharmaconutrients" and "dietary integrators" are terms used to describe nutrients or nutrient-enriched foods that can prevent or treat diseases.

Inflammaging: characterized by the upregulation of the inflammatory response that occurs with advancing age. It is believed to be the consequence of remodeling of the innate and acquired immune system, resulting in chronic pro-inflammatory cytokine production.

Caloric restriction (CR): a dietary regimen that is based on lowering caloric intake without malnutrition**.**

CR mimetics: nutraceuticals and pharmaceuticals that induced similar biochemical modification as caloric restriction.

Keap1-Nrf2-ARE system: the major regulator of cytoprotective responses to oxidative and electrophilic stress.

Rapamycin: a macrolide antibiotic produced by the bacteria *Streptomyces hygroscopicus*

Spermidine: is a polyamine formed from putrescine. It is found in almost all tissues in association with nucleic acids.

Autophagy: describes the ability of the cell to separate parts of their cytoplasm to subject them to lysosomal degradation.

Mitophagy: specific autophagic removal of older and/or dysfunctional mitochondria.

Free radical: molecular species capable of being independent that contains an unpaired electron in its atomic orbital. This makes them highly unstable and reactive; they can either donate or accept an electron from other molecules resulting in oxidant or reductant behavior.

Dietary supplement: means a product intended to supplement the diet that bears or contains, vitamin, a mineral, an herb or other botanical, an amino acid or a dietary substance used to supplement the diet by increasing the total dietary intake.

Michael addition reaction: The 1,4-addition (or conjugate addition) of resonance-stabilized carbanions. The Michael Addition is thermodynamically controlled; the reaction donors are active methylenes such as malonates and nitroalkanes, and the acceptors are activated olefins such as α,β-unsaturated carbonyl compounds.

Polypharmacy: the use of multiple medications and/or the administration of more medications that a clinically indicated, can represent unnecessary drug use. Commonly reported among the elderly.

Sirtuin: are nicotinamide adenine dinucleotide-dependent deacetylases that are highly conserved from bacteria to human and SIR2 was originally shown to extend lifespan in budding yeast. They are highly abundant in the skins of red grapes.

CHAPTER I

Introduction

Aging and neurobehavioral functionality

As life span increases globally, so will the incidence of age related chronic diseases and their associated costs. Changes in tissue structure and deterioration of functional capacity are almost universal in the aged (Oh et al., 2014; Troen, 2003). These changes are notable in both macroscopic and microscopic levels causing a decrease in healthy life span, and ultimately costing the functional independence for older individuals.

The aging process can be divided (Akbaraly et al., 2013) into two categories, "normal" aging and "successful" aging. The latter can be described as a multidimensional complex which has three essential components, (i) lack of disease, (ii) engagement in life, and (iii) maintenance of high cognitive and physical function (Kendig et al., 2014). To understand the factors affecting this process necessitates the utilization of a two-pronged approach: intrinsic (genetic) vs. extrinsic (non-genetic). The gene regulation theory suggests that senescence results from modulation of gene expression (Martin, 1997). Although there is hard evidence that gene expression does change with aging, it is unlikely that only changes in genetic expression directly promotes aging. Emerging evidence suggests that both intrinsic and extrinsic approaches are very important (Troen, 2003; Weinert & Timiras, 2003). Indeed, in most cases the increasing homeostatic imbalance and incidence of pathology with aging is the result of genes that respond to exogenous elements and thereby increase the chances of ending ones lifespan.

Some of the widely supported characteristics of normal aging include (1) increased mortality with age after maturation, (2) changes in biochemical composition in tissues with age, (3) progressive decrease in physiological capacity with age, (4) reduced ability to respond adaptively to environmental stimuli with age, and lastly (5) increased susceptibility and

vulnerability to disease (Troen, 2003). The compound effect of these characteristics results in a dramatic increase in tissue/organ dysfunction and an array of age related degenerative diseases, which encompasses both neurocognitive and cardiovascular systems. Even with an advanced knowledge of the mechanisms related to aging, data from pre-clinical research have are mainly from male cells and animals thereby hindering the progress of translational research and the discovery of new targets (Cubala et al., 2008). Although the National Institutes of Health (NIH) mandated the inclusion of female subjects in clinical trials in 1993, the inclusion of both sexes in preclinical research is almost negligible. One of the primary arguments stated for sex discrepancy in the preclinical phase is the high cost; however, the cost of finding gender differences during clinical trials surpasses any amount of money spent during the preclinical phase (De Vries, 2004). The common assumption is that sex differences are majorly hormone dependent, however the phenomenon of sexual dimorphism is equally important to sex differences reported with disease (Morrow, 2015).

Indeed, a recent study on bone marrow chimeras of XX and XY⁻ gonadal female mice in which the sex chromosome complement of the reconstituted immune system was varied independently of that in the brain reported a significant gender difference. When these mice were tested on several behavioral tests, including tests for motor dysfunction (rotorod test); mice with XY chromosomes in the central nervous system (CNS) had greater neurodegeneration than those with XX chromosomes (Du et al., 2014). Furthermore, there has been extensive research on the role of sex steroid hormones, particularly the role of estrogen and testosterone in neuroprotection (Pike et al., 2009; Spence & Voskuhl, 2012; Wise, 2002). With mounting evidence of adverse consequences for women's health from translating male sex-biased preclinical research for improvements in human health(De Vries, 2004); it is becoming increasingly clear that future preclinical aging studies taking sex into account as a variable can improve the reproducibility of research results (Beery & Zucker, 2011; Clayton & Collins, 2014).

Sexual dimorphism and its influence on oxidative stress is widespread in the animal kingdom (Bokov 2008). The increase in oxidative stress with aging has been implicated as one of the main causes for the decline in neurobehavioral functionality (Dringen, 2000; Forster et al., 1996; Gault & Willems, 2013; Gemma et al., 2007). Additionally, the oxidative stress theory of aging also is one of the most well tested non-genetic theories of aging (Balaban et al., 2005; Finkel & Holbrook, 2000; Popa-Wagner et al., 2013; Salminen & Paul, 2014). Several reports on pathological disorders have suggested age associated increases in oxidative stress to be a causative factor for tissue damage.

Oxidative stress and aging

Reactive oxygen species (ROS) are reactive free radicals that are either the byproducts of oxidative phosphorylation and other incomplete reductive processes or produced specifically by the NADPH oxidase (NOX) family of enzymes. A free radical is an atom or molecule with a single unpaired electron. The presence of an unpaired electron makes it highly reactive and unstable due to its affinity for other free electrons, resulting in a chain reaction (Kalyanaraman, 2013). A chronic oxidative imbalance in the body has been implicated in the oxidative stress theory of aging (Finkel & Holbrook, 2000; Gemma et al., 2007; Harman, 1956). However, ROS in moderate levels are pertinent mediators of several normal physiological processes in a developing body, which include killing invading pathogens and wound healing (Kalyanaraman, 2013; Popa-Wagner et al., 2013; X. Wang & Zhao, 2009). The "free radical theory of aging" has been modified in recent years to propose, "the redox stress theory of aging" in order to accommodate the idea that it is the imbalance of pro-oxidants to antioxidants that deranges cellular mechanisms and causes oxidative damage. Mitochondria are the primary intracellular site for oxygen consumption with around 0.1 to 1% of the consumption funneled to ROS production making it the main site for the pro-oxidant production. Complex I and III on the electron transport chain are the major producers of free radicals. Due to the increase in

production and accumulation of free radicals in the mitochondria with age, the term

"mitochondrial free radical theory of aging" (MFRTA) is now also widely accepted as a plausible

theory of aging.

In normal physiological conditions as described by Carmeli and colleagues, an ideal "golden

triangle" exists which represents the redox state where oxidants, antioxidants and endogenous

biomolecules such as proteins are in place at each apex in a balanced equilibrium (Carmeli et

al., 2002). However an oxidative imbalance perturbing the balanced equilibrium is detrimental to

protein function as it increases susceptibility to protein fragmentation, formation of protein-

protein cross linkages, oxidation of amino acid side chains and generation of carbonyl

derivatives (Berlett & Stadtman, 1997). Aerobic organisms have an intricate antioxidant system,

which includes both enzymatic and non-enzymatic detoxification mechanisms as well as an

adaptive mechanism such as the nuclear factor erythroid-derived 2 related factor (Nrf2) system

that regulates antioxidant gene expression (Motohashi & Yamamoto, 2004; Satoh et al., 2013;

Zimniak, 2011). In normal conditions, these mechanisms are the mediators that balance the

ROS production. Endogenous enzymatic antioxidants include superoxide dismutase (SOD),

catalase (CAT), and the glutathione peroxidases (GSH-Px). Small molecular non-enzymatic

antioxidants include Vitamin E and C, glutathione, ubiquinone, and also beta-carotene

(Kalyanaraman, 2013; Salminen & Paul, 2014). Based on the "golden triangle" physiological

equilibrium, it is not the presence of ROS that causes the severe oxidative stress resulting in

protein damage reported with aging but perhaps the homeostatic imbalance between ROS

production and the endogenous antioxidant defenses (Fusco et al., 2007; Suh et al., 2004).

Most of these enzymatic antioxidant reactions are cytosolic, may require multiple

antioxidants to detoxify certain ROS and are usually present at very low concentrations. The

Keap1-Nrf2-ARE is an endogenous antioxidant system that is activated by mild to moderate

levels of stress induced ROS (Motohashi & Yamamoto, 2004). In a state of mild to moderate

stress, the conformation of Keap1 changes due to modification of its cysteine residues undergoing a Michael addition reaction (Nguyen et al., 2009). The conformational change in Keap1 breaks the bond between Keap1 and Nrf2 releasing a phosphorylated Nrf2, which then undergoes nuclear translocation where it binds to and induces the transcription of antioxidant response elements (ARE)(Itoh et al., 2015; Petri et al., 2012; Satoh et al., 2013). The induction of ARE results in the upregulation of endogenous antioxidant enzyme synthesis as well as an increased production of detoxification enzymes(Itoh et al., 2015; Sykiotis et al., 2011)The induction of glutamate cysteine ligase catalytic (GCLc) is one of the important enzymes expressed via activation of ARE(Bea et al., 2003; Mani et al., 2013). GCLc is a member of a family of antioxidant/detoxification enzymes that is important to maintain cellular redox homeostasis and reduce oxidative damage. GCLc is a rate-limiting enzyme in glutathione (GSH) synthesis. GSH is a major intracellular antioxidant in mammals and is the body's principal non-protein thiol tripeptide and the liver is the major site of its synthesis (DeLeve & Kaplowitz, 1990).

The redox state of a cell depends on the relative amounts of the reduced to oxidized forms of glutathione (GSH/GSSG) (Zhang et al., 2012). This ratio has been commonly used in clinical studies as a sensitive marker for systemic oxidative stress. Depletion of glutathione or a decrease in its redox state has been implicated in several age-associated pathologies such as Alzheimer's disease (AD) and Parkinson's (PD) as well as normal aging itself (Bermejo et al., 2008; Zhu et al., 2006). Glutathione is produced in all major organs as well as organelles including the mitochondria where it can neutralize both superoxide and converts hydrogen peroxide to water and oxygen eliminating the creation of more ROS. Glutathione also protects cell membranes from oxidative damage and helps maintain the sulfhydryl groups of many proteins in the reduced form, which is a requirement for their normal function (Dringen, 2000). In regular physiological conditions, the glutathione redox couple is present in mammalian cells in

concentrations between 1 and 10 mM, with the reduced GSH presiding over oxidized GSSG (Pastore et al., 2003). However, under constant oxidative stress and accumulated oxidative damage as seen with normal aging or age related diseases, a significant decrease in antioxidant defense related to a depletion of intracellular reduced GSH and its ratio has been reported (Rebrin et al., 2011; Robillard et al., 2011; Zhu et al., 2006). Lang et al 1992 observed a significant reduction of GSH in erythrocytes in healthy aged individuals, independent of their gender (Lang et al., 1992). This is because with aging, the enzymes that synthesize GSH which include GCLc and GSH synthetase do not concomitantly increase but actually decline in many tissues. In fact, Suh et al 2004 reported a 50% decrease in both Nrf2 expression and GCL activity in the liver between young and old rats solidifying the notion that one potential mechanism underlying the loss of GSH synthesis in older organisms is a decline in Nrf2 mediated transcription of GCL protein (Suh et al., 2004).

NADPH oxidase, called the NOX family enzymes, has also been implicated in the oxidative stress theory of aging. The phagocytic NOX, most well-known among the NOX family enzymes, uses ROS in the form of oxidative bursts to kill invading pathogens (Cheng et al., 2004; Kalyanaraman, 2013). However, these enzymes have been reported to play a deleterious role in several age related pathologies since their only known function is to generate ROS, primarily superoxide and hydrogen peroxide. And even though a decrease in the ability of macrophages to produce ROS has been observed with aging, there is an overall increase in the oxidative bursts due to an increased incidence of age-associated pathologies. Since hydrogen peroxide is a non-polar molecule, it can easily diffuse through membranes and convert to highly potent hydroxyl radicals or superoxide in other cells and amplify oxidative damage already present in the organism (Krause, 2007).

Based on existing literature and data on the pathology of age associated oxidative stress, there are three main sources for this homeostatic imbalance of the aging body's reductive-

oxidative system: (1) accumulated oxidative damage in the mitochondria and mitochondrial DNA leading to mitochondrial dysfunction and overall increased production of ROS, (2) increased activity of NOX enzymes therefore increased production of superoxide anion and (3) an overall decreased effectiveness of the body's antioxidant system.

If the "free radical theory of aging" were to hold true, and if accumulated oxidative damage and depleted antioxidant capacity were the reason behind aging; then the use of antioxidant supplementation could hypothetically restore diminishing cellular defense and prolong the integrity of neurobehavioral functionality.

Inflammation and aging

The proper functioning of the immune system is an indicator of health and longevity. A positive relationship has been reported between the function of Natural killer (NK) cells, T lymphocytes and longevity. However, this beneficial functioning of the immune system declines with aging. Aging of the immune system has been linked to an increase in the production of pro-inflammatory cytokines like tumor necrosis factor (TNF-α) (De la Fuente et al., 2005; De, la, Fuente, M., 2008). This phenomenon is often referred to as "Inflammaging" and is considered a major contributor to age related chronic disorders such as AD and PD (Franceschi & Bonafe, 2003; Hardeland et al., 2015). With aging, the body suffers from attenuation in autophagy resulting in lower organelle turnover and decreased restoration to healthy tissue, which progressively increases oxidative stress leading to the buildup of chronic inflammation physiologically (Green et al., 2011). The continuous systemic inflammation is a result of the persistence and lack of clear resolution for when tissues are unable to overcome the effects of harmful agents leading to severe tissue deterioration without any relevant symptoms for years. Usually this kind of damage accumulates over time therefore inflammaging or inflammation during old age is a problem because just like oxidative stress, it also occurs due to a homeostatic imbalance. Franchesi et al's 2000 classic paper on "Inflamm-aging" provides an

19

interesting evolutionary perspective to the rise in inflammation reported with aging. Their

argument for this gross augmentation of inflammation with aging is the result of an adaptive

capacity at the organismal level due to a mixture of old; conserved macrophages and newer

evolutionary lymphocyte centered immune response. They further expand on this theory by

stating that proinflammation is a characteristic of both successful and unsuccessful aging and

that this is dependent on a pathological threshold. Those who age successfully take a longer

time to reach this pathological threshold (Franceschi et al., 2000).

Maintaining a precise redox balance status is as important as the physiological acid-

base buffer system of the body for the optimal operation of homeostatic cellular activities. The

nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factor has

been reported to be the master regulator of inflammatory processes and is extremely sensitive

to oxidative stimuli. NF-κB activity is modulated by upstream signaling pathways, which include

IKKβ. The activated IKK complexes phosphorylate the IκB subunits of NF-κB leading to the

degradation of IκB allowing the nuclear translocation of NF-κB and induction of proinflammatory

transcription. In normal conditions, NF-κB activation in response to oxidative stimuli is for a

shorter period and this reaction stops once the area of injury is protected. However, in the

context of aging, this input signal is not well controlled and the NF-κB transcription factor

remains activated. In fact, some of the NF-κB induced proteins like Tumor necrosis factor-α

(TNF-α), Interleukin 1 Beta (IL-1β) and Interleukin 6 (IL-6) themselves are potent NF-κB

activators and form an auto-activating loop (Fisher et al., 1996; Handel et al., 1995). Several

studies on the redox sensitive state of NF-κB have consistently reported increased NF-κB

activity with aging in a variety of tissues, which include heart, liver, kidney as well as the

brain(Csiszar et al., 2008; Donato et al., 2009; Lanzillotta et al., 2015)

In human studies, circulating levels of pro-inflammatory cytokines increase during aging

with increased concentrations of IL-6, TNF-a and IL-1β with aging reported in both the periphery

as well as in the brain(Sankowski et al., 2015; Winklewski et al., 2015). Aging is also associated with increased levels of C-reactive protein (CRP). IL-6 can induce CRP production from hepatocytes by activating the Janus Kinase (JNK) pathways (Black et al., 2004). IL-6 and CRP as well as IL-1β plasma concentrations significantly rise with age associated disease conditions in the elderly. In fact, all three of these cytokines have been reported to share a positive correlation with cognitive and motor dysfunction in aged individuals. Indeed, Sweat et al. 2008 reported a positive correlation between CRP levels and cognitive dysfunction in middle-aged obese women (Sweat et al., 2008). With the proposal of the inflammatory hypothesis of aging by Mc Greer et al 1999, it is now well known that the activation of microglia signifies a primary neuroinflammatory state which is followed by leukocyte invasion as a secondary phenomenon (E. McGeer & McGeer, 2010; P. McGeer & McGeer, 1999). Several studies have suggested this mechanism of action to influence both AD and PD pathogenesis (Anastasio, 2015; Dheen et al., 2007; Polazzi & Monti, 2010; Wilms et al., 2007).

Inflammaging is a pathological phenomenon, which perturbs normal neurobehavioral function and hinders day-to-day living for the elderly. That being said, using anti-inflammatory agents during the course of aging may potentially ameliorate or prevent some of the damaging effects on tissues. Furthermore, the reciprocity in the relationship between oxidative stress and chronic inflammation results in a vicious cycle, which without intervention can exacerbate tissue damage. Potential anti-aging therapies should possess both anti-oxidant and anti-inflammatory actions in order to slow normal and pathological aging and its associated neurobehavioral dysfunction.

Dietary interventions

Interventions that encompass both antioxidant and anti-inflammatory activities will be most beneficial in ameliorating or even preventing age associated pathologies potentially leading to prolonged integrity of neurobehavioral functions. Dietary interventions, modifications and/or

supplements serve as an attractive avenue for the treatment of age-associated diseases, as they are easily accessible to everyone. Indeed, several large-scale comprehensive life style interventional studies such as the one conducted at North Karelia, Finland reported that dietary change was the major factor in the cardiovascular risk reduction compared to pharmacotherapy (Vartiainen et al., 1994). Further, human and animal studies have suggested a bi-directional relationship between energy intake and brain function (Stranahan & Mattson, 2008), wherein, excessive energy intake impairs function, and dietary energy restriction enhances function. Underlying mechanisms for this unique relationship converges on changes in plasticity and neurogenesis. Obesity, which is a common consequence of excessive caloric intake, can accelerate brain aging as well as the risk for neurodegenerative diseases and stroke (Kanoski et al., 2010). Caloric restriction however can protect the brain against aging and delay the onset of neurobehavioral dysfunction.

Caloric Restriction

Lifelong caloric restriction (CR) (usually 20–40% of ad libitum intake) or intermittent short term fasting has long been shown to prolong both the mean and maximum life span of several organisms including rodents (Sohal & Weindruch, 1996; Turturro et al., 1999; Yu, 1996). However, the life span data for primates and humans have so far been inconclusive (Cava & Fontana, 2013; Colman et al., 2014; Mattison et al., 2012). Despite the differences in longevity data, CR has consistently demonstrated improvement in functionality with an overall improvement in health and lower incidences of metabolic diseases, cancer and neurodegenerative disorders across all species (Kuhla et al., 2014; Meydani et al., 2011; Noyan et al., 2015; Trepanowski et al., 2011).

Several theories have been put forward to explain the anti-aging effects of CR, some of which include increased autophagy and attenuation of oxidative stress. Caloric restriction increases mitochondrial biogenesis as well as both whole cell turnoever (autophagy) and

mitochondrial turnover (mitophagy) via inhibition of the mammalian target of rapamycin complex 1 (mTORC1) resulting in a higher organelle turnover as well as quantitatively and qualitatively healthier cells and mitochondria (Haigis & Guarente, 2006; Madeo et al., 2014; Wallace et al., 2010). CR results in an acute depletion of nutrients and is accompanied with a rapid decline in cytosolic acetyl coenzyme A (AcCoA) levels leading to the deacetylation of proteins (Marino, Pietrocola, Madeo et al., 2014; Marino, Pietrocola, Eisenberg et al., 2014; Schroeder et al., 2014). Secondly, the activity of the transacetylases is suppressed and finally deacetylases, such as sirtuins are activated. This cascade of events is crucial for the activation of both AMP kinase and the inhibition of mTORC1 ultimately stimulating autophagy and is primarily hypothesized to be responsible for the extension of lifespan (Eisenberg et al., 2014). The latter hypothesis has been a consistently reproducible action of CR along with the widely ascertained anti-oxidative action that remains in line with the oxidative stress hypothesis of aging. Indeed, several studies have reported CR to stabilize mitochondrial function and improve redox state in several organs including the brain (Dkhar & Sharma, 2014; Radak et al., 2013). It has also been noted than rodents on long term CR had a lower percentage of ROS production attributed to a shift from carbohydrate oxidation to fatty acid oxidation due to nutrient deprivation (Anderson & Weindruch, 2010). CR prevents ROS production by increasing $FADH_2$ relative to NADH thereby bypassing ROS generation at Complex I, which has been reported to be one of the primary sites of ROS production (Murphy, 2009).

Following a CR paradigm not only helps with weight loss but also maintaining a healthy weight (Harvey-Berino, 1999). Varady KA reported a 10-20% decrease in total fat mass for overweight individuals undergoing 15-20% caloric restriction daily for 3 months (Varady, 2011). Obesity is one of the major risk factors for accelerated age related neurodegenerative disorders as well as can cause overall functional decline(Anstey et al., 2011; Spielman et al., 2014; Sturm & Hattori, 2013; Toda et al., 2014; Winklewski et al., 2015). This epidemic of overweight and

obesity developing in the 21st century throughout the world has been predicted to increase

mortality rates (Olshansky, 2005). In humans, CR can provide major and sustained beneficial

effects against obesity, as well as inflammation and oxidative stress. Studies of the CR

paradigm on both vertebrates and invertebrates have yielded successful results of weight loss,

improvement in overall functional capacity to increased life span (Cava & Fontana, 2013;

Timmers et al., 2011; Varady, 2011). Some human CR studies have also reported reduced low-

density lipoptrotein, CRP, blood pressure, and an overall decline in developing cardiovascular

disease.

       The long term study of CR is best exemplified in the study of the World war II Okinanwan

population involuntarily adjusted to a lower calorie diet during their life time due to a shortage of

food (Wilcox 2004, 2007, 2010). This low calorie diet was nutritionally dense with regard to

phytonutrients such as flavonoids and antioxidants. Food intake data from the Okinawans

indicate almost a 20% CR during their lifetime compared to the Japanese average. Medical data

indicate phenotypic effects of CR such as low body mass index and minimal body fat. In

addition, this population shared lower disease specific mortality rates such as cardiovascular

disease and multiple types of cancers. However, this traditional beneficial Okinawan diet

underwent a massive change post WWII with a shift towards higher energy dense foods of

lower quality due to increasing westernization. In recent years, the younger Okinawan

generation actually suffers from high rates of obesity and metabolic syndrome. Long-term CR

studies of humans are extremely challenging and endpoints such as life span are not tenable.

For maximum benefit, individuals would have to reduce their caloric intake regimen by roughly

30 percent, which is equivalent to dropping from 2,500 calories to 1,750 calories a day (Lane et

al., 2002). Furthermore, the applicability of a CR paradigm in humans would be minimally

successful due to compliance issues especially the requirement to adhere to such a harsh

regimen for years on end. Indeed, studies have reported that only 20% of overweight individuals

are capable of losing and maintaining 10% of their weight for a period of at least a year (Allison et al., 2014; Thomas et al., 2014). Most end up gaining even more weight than their body weight from when they started the regimen. The trend of high calorically dense dietary intake is globally spread out. With the increasingly availability and ease of a calorically dense nutrition, implementation of a CR paradigm may be difficult which makes the search for an alternative therapy or a pill that mimics the physiological effects of CR without having to eat less, imperative (Madeo et al., 2014).

The availability of natural (also called nutraceuticals) or synthetic pharmacological agents/compounds could potentially be used to induce the beneficial effects of CR without provoking the discomforts such as weight loss. The term nutraceuticals was first coined or established in Japan; it is also commonly referred to as "functional foods" and is one of the fastest growing segments of the food industry. However, this sector is not FDA regulated. In order to fall under FDA's classification of functional foods or nutraceuticals, these food items have to loosely satisfy 3 categories, (1) they should be from naturally occurring ingredients, (2) they can be consumed as part of the daily diet and (3) when ingested, they should increase or modulate biological processes or mechanisms to prevent or control an ailment (Hardy, 2000). Several prominent CR mimetics includes nutraceuticals and activate CR affected pathways and can mimic the low glucose environment to induce depletion of AcCoA. Resveratrol, a popular nutraceutical has been reported to activate sirtuins. Likewise, other nutraceuticals such as epigallocatechin gallate (EGCG) from green tea has also been reported to induce autophagy via inhibition of acetylation. Table 1 displays the properties of nutraceuticals and other pharmacological agents being currently studied to investigate their CR like effects and mechanisms of actions. Curcumin, the polyphenol of interest for the following studies, is extracted from the rhizome of *Curcuma Longa* and has been reported to have CR like properties of inducing autophagy via its ability of reducing acetylation of histones (Kang et al., 2006;

Sunagawa et al., 2011) as well as its strong anti-proliferative effects on several cancer lines(Rao et al., 1995; Sharma et al., 2001) as well as extend lifespan in invertebrates(Suckow & Suckow, 2006). It has also been reported to exert strong anti-oxidant and anti-inflammatory properties making it an ideal candidate for a CR mimetic.

Curcumin

Turmeric is one of the most widely used spices throughout the world, evident from the variety of names it has been given; has a rich history as a dietary spice and herbal supplement in both ancient China and India (Goel et al., 2008; Gupta et al., 2013; Lee et al., 2013). The active component responsible for the vibrant yellow color of the spice is called curcumin. Curcumin has been indicated as the key component responsible for the major therapeutic properties attributed to turmeric, which include anti-oxidant, anti-inflammatory, anti-mutagenic, anti-microbial activities amongst others (Anand et al., 2011; Shehzad et al., 2011)

Due to its multi-faceted pharmacology, extensive effort has been put towards the possibility of using curcumin to treat or prevent neurodegenerative diseases for which age is the main risk factor(Bigford & Del Rossi, 2014; Mourtas et al., 2014; Siddique et al., 2014). These include AD, PD and cerebrovascular disease, which cause major cognitive and motor dysfunction and for which treatment with curcumin has provided encouraging results on experimental models. The presence of both oxidative stress and inflammation around neurons and glial cells has been previously associated with brain aging and injury (Polazzi & Monti, 2010). Curcumin's molecular structure and its ability to cross the blood brain barrier provide a promising avenue for neuroprotection (Anand et al., 2008; Anand et al., 2011).

Turmeric consists of three important analogues, curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). Curcumin, also known as diferuloylmethane, is a low molecular mass polyphenol compound and is the most abundant amongst the three making up about 77% of the curcuminoids (Lee et al., 2013). It also has been reported to have better anti-

oxidant and anti-inflammatory action compared to the other two curcuminoids. Curcumin consists of two aryl rings containing ortho-methoxy phenolic OH groups that are symmetrically linked to a β-diketone moiety (Fig 1b). The occurrence of intramolecular hydrogen atoms transfer at the β-diketone chain of curcumin leads to the existence of keto and enol tautomeric conformations in equilibrium. It is the o-methoxy groups that make curcumin a strong radical scavenger. The hydrogen bonding interaction between the phenolic OH and the o-methoxy groups in curcumin greatly influences the O-H bond energy as well as the hydrogen atom abstraction by free radicals (Esatbeyoglu et al., 2012). Curcumin also has potent chelating power due to both its o-methoxy groups and diketone moiety. The β-diketone moiety itself is a Michael reaction acceptor which allows curcumin to suppress NF-κB activity is the major transcription factor involved in the production of pro-inflammatory cytokines (Fig 1a) (Anand et al., 2008; Anand et al., 2011; Fujisawa et al., 2004). In addition, the scavenging of free radicals also leads to a decrease in inflammation.

Along with curcumin's molecular structure enabling it to be a strong antioxidant, its binding ability to Keap1 makes it a strong Nrf2 inducer. Curcumin has been reported to change the conformation of Keap1, allowing for nuclear translocation of Nrf2 and interact with antioxidant response elements (ARE). This gives rise to phase II antioxidant genes which includes enzymes that synthesize glutathione (Esatbeyoglu et al., 2012; Trujillo et al., 2014). The GSH based redox regulation is the body's frontline defense system to combat oxidative stress. Taking into account curcumin's strong anti-inflammatory and anti-oxidant actions, an increasing number of studies have been conducted to test its effects on a wide range of diseases including cancer tumorigenesis and neurodegenerative disorders (Bar-Sela et al., 2010; Begum et al., 2008; Hickey et al., 2012).

Curcumin's ability to cross the blood brain barrier makes it a suitable compound to test for age associated brain pathologies that include AD and PD, which impact both cognitive and

motor function (Begum et al., 2008). Indeed, several studies conducted on transgenic rodent models of neurodegenerative disorders have yielded positive results (Begum et al., 2008; Hickey et al., 2012; Siddique et al., 2014; M. S. Wang et al., 2010). However, to date there are only five studies that have investigated the effects of curcumin on oxidative stress and inflammatory markers in normal aging(Calabrese et al., 2007; Jayasena et al., 2013; Kim et al., 2008; Rossi et al., 2008) and only two that have studied its effects on cognitive and motor dysfunction evident with normal aging (Ataie et al., 2010; Dong et al., 2012)

Goals of current research

Aging is characterized by a progressive decline in multiple physiological functions i.e. functional aging/decline. As organisms age, they exhibit a gradual loss in both cognitive and motor activity, but the underlying mechanisms may be influenced by both genetic and environmental factors. Further, genetic and environmental variances exist due to individual differences in compensatory mechanisms to combat aging processes. However, extensive studies have reported on the impact oxidative stress and inflammation have on age associated decline in neurobehavioral functionality. Although major gender differences have been noted with certain age associated neurodegenerative and cardiovascular disorders, application of dietary interventions that have both anti-oxidant and anti-inflammatory properties may to some extent improve functional capacity whilst delaying the aging process.

An extensive review of epidemiological literature on age-associated pathologies strongly suggests accrual of inflammation and oxidative stress during mid-life to be strongly linked to functional decline (Akbaraly et al., 2013; Anstey et al., 2011; Olshansky, 2005; Sturm & Hattori, 2013; Toda et al., 2014; Winklewski et al., 2015). Indeed, even in invertebrate species such as C. elegans, which has been widely used a model for aging, Liu et al 2013 reported that the deterioration of body-wall muscles, which were previously thought to undergo progressive functional aging, does not manifest such a decline until mid-late life (Liu et al., 2013). This information suggests that an anti-aging intervention during late mid-life could carry immense potential for the prevention or alleviation of functional decline in late life.

Caloric restriction has been widely studied as an anti-aging therapy and has yielded positive results in several species. So far, individuals who practiced lifelong CR for example the Okinawans not only lived a longer life than average; they also had lower levels of inflammation and oxidative stress. These individuals also enjoyed a good amount of functional capacity when compared to age matched individuals from other places. However, the Okinawans did not

practice voluntary caloric restriction and were forced to these dietary conditions due to scarcity of food during WWII. It would be difficult for individuals to follow a strict lifestyle or dietary modification especially with the overabundant accessibility of food. Although the beneficial effects of CR are well known, it is difficult to translate this regimen and presents a huge challenge in clinical practice. With the recent surge in studies being conducted on compounds that can mimic the beneficial effects of caloric restriction with minimal changes in eating habits, curcumin could potentially be a sustainable alternative to CR.

There has, however, been a lack of studies on curcumin and its effects on neurobehavioral dysfunction reported with normal aging. The first project explored midlife obesity as a causative factor for accelerated age associated cognitive impairment. Curcumin was tested as an anti-angiogenic, weight loss therapeutic (Fig 2) and caloric restriction was implemented as negative control. Furthermore, there has also been a lack of reports on the impact of both caloric restriction and curcumin on females and since there are gender differences in age associated pathologies and progression of neurobehavioral functional decline, it is imperative that these questions are answered. While beneficial effects of CR have previously been established, the studies undertaken for this dissertation also addresses the possibility that curcumin and CR implemented concurrently can have additive beneficial effects, or lead to improvements in more domains of age-related impairment than either intervention alone (Fig 3). Longitudinal clinical studies suggest a significant gender-dependent risk for cognitive impairment, inflammation, and oxidative stress. To address possible gender-dependent outcomes in the preclinical setting for combination interventions, the latter study included both male and female mice. Finally, with the analysis of collected serum for inflammatory and oxidative stress markers, the results from the current study can provide information useful in a translational intervention study.

REFERENCES

Akbaraly, T., Sabia, S., Hagger-Johnson, G., Tabak, A.,G., Shipley, M.,J., et al. 2013. Does overall diet in midlife predict future aging phenotypes? A cohort study.126, 411-419.

Allison, D. B., Thomas, D. M., & Heymsfield, S. B. 2014. Energy intake and weight loss. JAMA, 312, 2687-2688.

Anand, P., Sung, B., Kunnumakkara, A.,B., Rajasekharan, K.,N., & Aggarwal, B.,B. 2011. Suppression of pro-inflammatory and proliferative pathways by diferuloylmethane (curcumin) and its analogues dibenzoylmethane, dibenzoylpropane, and dibenzylideneacetone: role of Michael acceptors and Michael donors.. Biochem Pharmacol, 82, 1901-1909.

Anand, P., Thomas, S., Kunnumakkara, A., Sundaram, C., Harikumar, K., et al. 2008. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol, 76, 1590-1611.

Anastasio, T. J. 2015. Computational identification of potential multi-drug combinations for reduction of microglial inflammation in Alzheimer disease. Front.Pharmacol., 6, 116.

Anderson, R. M., & Weindruch, R. 2010. Metabolic reprogramming, caloric restriction and aging. Trends Endocrinol.Metab., 21, 134-141.

Anisimov, V. N., Berstein, L. M., Popovich, I. G., Zabezhinski, M. A., Egormin, P. A., et al. 2011. If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging (Albany NY), 3, 148-157.

Anisimov, V. N., Zabezhinski, M. A., Popovich, I. G., Piskunova, T. S., Semenchenko, A. V., Tyndyk, M. L., Yurova, M. N., Rosenfeld, S. V., & Blagosklonny, M. V. 2011. Rapamycin

increases lifespan and inhibits spontaneous tumorigenesis in inbred female mice. Cell.Cycle, 10, 4230-4236.

Anstey, K., Cherbuin, N., Budge, M., & Young, J. 2011. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obes Rev, 12, 426-437.

Ataie, A., Sabetkasaei, M., Haghparast, A., Moghaddam, A. H., & Kazeminejad, B. 2010. Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress in the rat. Pharmacol.Biochem.Behav., 96, 378-385.

Balaban, R.,S., Nemoto, S., & Finkel, T. 2005. Mitochondria, oxidants, and aging.. Cell, 120, 483-495.

Bar-Sela, G., Epelbaum, R., & Schaffer, M. 2010. Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications.. Curr Med Chem, 17, 190-197.

Batandier, C., Guigas, B., Detaille, D., El-Mir, M. Y., Fontaine, E., Rigoulet, M., & Leverve, X. M. 2006. The ROS production induced by a reverse-electron flux at respiratory-chain complex 1 is hampered by metformin. J.Bioenerg.Biomembr., 38, 33-42.

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., et al. 2006. Resveratrol improves health and survival of mice on a high-calorie diet. Nature, 444, 337-342.

Baur, J. A., & Sinclair, D. A. 2006. Therapeutic potential of resveratrol: the in vivo evidence. Nat.Rev.Drug Discov., 5, 493-506.

Bea, F., Hudson, F. N., Chait, A., Kavanagh, T. J., & Rosenfeld, M. E. 2003. Induction of glutathione synthesis in macrophages by oxidized low-density lipoproteins is mediated by consensus antioxidant response elements. Circ.Res., 92, 386-393.

Beery, A. K., & Zucker, I. 2011. Sex bias in neuroscience and biomedical research. Neurosci.Biobehav.Rev., 35, 565-572.

Begum, A.,N., Jones, M.,R., Lim, G.,P., Morihara, T., Kim, P., et al. 2008. Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease.. J Pharmacol Exp Ther, 326, 196-208.

Berlett, B. S., & Stadtman, E. R. 1997. Protein oxidation in aging, disease, and oxidative stress. J.Biol.Chem., 272, 20313-20316.

Bermejo, P., Martín-Aragón, S., Benedí, J., Susín, C., Felici, E., et al. 2008. Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from Mild Cognitive Impairment. Free Radic Res., 42, 162-170.

Bigford, G. E., & Del Rossi, G. 2014. Supplemental substances derived from foods as adjunctive therapeutic agents for treatment of neurodegenerative diseases and disorders. Adv.Nutr., 5, 394-403.

Black, S., Kushner, I., & Samols, D. 2004. C-reactive Protein.. J Biol Chem, 279, 48487-48490.

Calabrese, V., Guagliano, E., Sapienza, M., Panebianco, M., Calafato, S., et al. 2007. Redox regulation of cellular stress response in aging and neurodegenerative disorders: role of vitagenes. Neurochem.Res, 32, 757-773.

Carmeli, E., Coleman, R., & Reznick, A. 2002. The biochemistry of aging muscle.. Exp Gerontol, 37, 477-489.

Cava, E., & Fontana, L. 2013. Will calorie restriction work in humans? Aging (Albany NY), 5, 507-514.

Chen, C., Liu, Y., Liu, Y., & Zheng, P. 2009. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. Sci.Signal., 2, ra75.

Cheng, G., Ritsick, D., & Lambeth, J. 2004. Nox3 regulation by NOXO1, p47phox, and p67phox.. J Biol Chem, 279, 34250-34255.

Clayton, J. A., & Collins, F. S. 2014. Policy: NIH to balance sex in cell and animal studies. Nature, 509, 282-283.

Colman, R. J., Beasley, T. M., Kemnitz, J. W., Johnson, S. C., Weindruch, R., & Anderson, R. M. 2014. Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat.Commun., 5, 3557.

Csiszar, A., Wang, M., Lakatta, E. G., & Ungvari, Z. 2008. Inflammation and endothelial dysfunction during aging: role of NF-kappaB. J.Appl.Physiol.(1985), 105, 1333-1341.

Cubala, W. J., Landowski, J., & Wichowicz, H. M. 2008. Zolpidem abuse, dependence and withdrawal syndrome: sex as susceptibility factor for adverse effects. Br.J.Clin.Pharmacol., 65, 444-445.

Cusi, K., Consoli, A., & DeFronzo, R. A. 1996. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. J.Clin.Endocrinol.Metab., 81, 4059-4067.

De la Fuente, M., Hernanz, A., & Vallejo, M. 2005. The immune system in the oxidative stress conditions of aging and hypertension: favorable effects of antioxidants and physical exercise. Antioxid Redox Signal, 7, 1356-1366.

De Vries, G. J. 2004. Minireview: Sex differences in adult and developing brains: compensation, compensation, compensation. Endocrinology, 145, 1063-1068.

De, la, Fuente, M. 2008. Role of neuroimmunomodulation in aging. Neuroimmunomodulation, 15, 213-223.

DeLeve, L. D., & Kaplowitz, N. 1990. Importance and regulation of hepatic glutathione. Semin.Liver Dis., 10, 251-266.

Dheen, S. T., Kaur, C., & Ling, E. A. 2007. Microglial activation and its implications in the brain diseases. Curr.Med.Chem., 14, 1189-1197.

Dkhar, P., & Sharma, R. 2014. Late-onset dietary restriction modulates protein carbonylation and catalase in cerebral hemispheres of aged mice. Cell.Mol.Neurobiol., 34, 307-313.

Donato, A. J., Pierce, G. L., Lesniewski, L. A., & Seals, D. R. 2009. Role of NFkappaB in age-related vascular endothelial dysfunction in humans. Aging (Albany NY), 1, 678-680.

Dong, S., Zeng, Q., Mitchell, E. S., Xiu, J., Duan, Y., et al. 2012. Curcumin enhances neurogenesis and cognition in aged rats: implications for transcriptional interactions related to growth and synaptic plasticity. PLoS One, 7, e31211.

Dringen, R. 2000. Metabolism and functions of glutathione in brain. Prog Neurobiol, 62, 649-671.

Du, S., Itoh, N., Askarinam, S., Hill, H., Arnold, A.,P., & Voskuhl, R.,R. 2014. XY sex chromosome complement, compared with XX, in the CNS confers greater neurodegeneration during experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A, 111, 2806-2811.

Eisenberg, T., Knauer, H., Schauer, A., Buttner, S., Ruckenstuhl, C., et al. 2009. Induction of autophagy by spermidine promotes longevity. Nat.Cell Biol., 11, 1305-1314.

Eisenberg, T., Schroeder, S., Andryushkova, A., Pendl, T., Kuttner, V., et al. 2014. Nucleocytosolic depletion of the energy metabolite acetyl-coenzyme a stimulates autophagy and prolongs lifespan. Cell.Metab., 19, 431-444.

Esatbeyoglu, T., Huebbe, P., Ernst, I., Chin, D., Wagner, A., & Rimbach, G. 2012. Curcumin--from molecule to biological function. Angew Chem Int Ed Engl, 51, 5308-5332.

Finkel, T., & Holbrook, N.,J. 2000. Oxidants, oxidative stress and the biology of ageing. Nature, 408, 239-247.

Fisher, G., Datta, S., Talwar, H., Wang, Z., Varani, J., Kang, S., & Voorhees, J. 1996. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature, 379, 335-339.

Forster, M., Dubey, A., Dawson, K., Stutts, W., Lal, H., & Sohal, R. 1996. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc Natl Acad Sci U S A., 93, 4765-4769.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., & De Benedictis, G. 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci., 908, 244-254.

Franceschi, C., & Bonafe, M. 2003. Centenarians as a model for healthy aging. Biochem.Soc.Trans., 31, 457-461.

Fujisawa, S., Atsumi, T., Ishihara, M., & Kadoma, Y. 2004. Cytotoxicity, ROS-generation activity and radical-scavenging activity of curcumin and related compounds. Anticancer Res, 24, 563-569.

Fusco, D., Colloca, G., Lo, M., MR., & Cesari, M. 2007. Effects of antioxidant supplementation to the aging process. Clin Interv Aging, 3, 377-387.

Gault, M.,L., & Willems, M.,E. 2013. Aging, functional capacity and eccentric exercise training.. Aging Dis., 4, 351-363.

Gemma, C., Vila, J., Bachstetter, A., & Bickford, P.,C. 2007. Oxidative Stress and the Aging Brain: From Theory to Prevention, in: D. Riddle R. (Ed.), Brain Aging: Models, Methods, and Mechanisms CRC Press, Boca Raton (FL).

Goel, A., Kunnumakkara, A.,B., & Aggarwal, B.,B. 2008. Curcumin as "Curecumin": from kitchen to clinic.. Biochem Pharmacol, 75, 787-809.

Green, D. R., Galluzzi, L., & Kroemer, G. 2011. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science, 333, 1109-1112.

Gupta, S.,C., Kismali, G., & Aggarwal, B.,B. 2013. Curcumin, a component of turmeric: from farm to pharmacy.. Biofactors, 39, 2-13.

Haigis, M. C., & Guarente, L. P. 2006. Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. Genes Dev., 20, 2913-2921.

Handel, M., McMorrow, L., & Gravallese, E. 1995. Nuclear factor-kappa B in rheumatoid synovium. Localization of p50 and p65. Arthritis Rheum, 38, 1762-1770.

Hardeland, R., Cardinali, D. P., Brown, G. M., & Pandi-Perumal, S. R. 2015. Melatonin and brain inflammaging. Prog.Neurobiol., 127-128C, 46-63.

Hardy, G. 2000. Nutraceuticals and functional foods: introduction and meaning. Nutrition, 16, 688-689.

Harman, D. 1956. Aging: a theory based on free radical and radiation chemistry. J Gerontol., 298-300.

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., et al. 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature, 460, 392-395.

Harvey-Berino, J. 1999. Calorie restriction is more effective for obesity treatment than dietary fat restriction. Ann.Behav.Med., 21, 35-39.

Hickey, M., Zhu, C., Medvedeva, V., Lerner, R., Patassini, S., et al. 2012. Improvement of neuropathology and transcriptional deficits in CAG 140 knock-in mice supports a beneficial effect of dietary curcumin in Huntington's disease. , 7.

Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., et al. 2003. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature, 425, 191-196.

Itoh, K., Ye, P., Matsumiya, T., Tanji, K., & Ozaki, T. 2015. Emerging functional cross-talk between the Keap1-Nrf2 system and mitochondria.. J Clin Biochem Nutr, 56, 91-97.

Jayasena, T., Poljak, A., Smythe, G., Braidy, N., Munch, G., & Sachdev, P. 2013. The role of polyphenols in the modulation of sirtuins and other pathways involved in Alzheimer's disease. Ageing Res.Rev., 12, 867-883.

Kaeberlein, M. 2009. Spermidine surprise for a long life. Nat.Cell Biol., 11, 1277-1278.

Kalyanaraman, B. 2013. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. Redox Biol, 1, 244-257.

Kang, S. K., Cha, S. H., & Jeon, H. G. 2006. Curcumin-induced histone hypoacetylation enhances caspase-3-dependent glioma cell death and neurogenesis of neural progenitor cells. Stem Cells Dev., 15, 165-174.

Kanoski, S. E., Zhang, Y., Zheng, W., & Davidson, T. L. 2010. The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat.. J Alzheimers Dis., 21, 207-219.

Kendig, H., Browning, C.,J., Thomas, S.,A., & Wells, Y. 2014. Health, lifestyle, and gender influences on aging well: an Australian longitudinal analysis to guide health promotion.. Front Public Health, 2.

Khurana, S., Venkataraman, K., Hollingsworth, A., Piche, M., & Tai, T. C. 2013. Polyphenols: benefits to the cardiovascular system in health and in aging. Nutrients, 5, 3779-3827.

Kim, S. J., Son, T. G., Park, H. R., Park, M., Kim, M. S., et al. 2008. Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. J.Biol.Chem. 283, 14497-14505.

Krause, K.,H. 2007.

Aging:a revisited theory based on free radicals generated by NOX family NADPH oxidases. Exp Gerontol, 42, 256-262.

Kuhla, A., Hahn, S., Butschkau, A., Lange, S., Wree, A., & Vollmar, B. 2014. Lifelong caloric restriction reprograms hepatic fat metabolism in mice. J.Gerontol.A Biol.Sci.Med.Sci., 69, 915-922.

Lane, M. A., Ingram, D. K., & Roth, G. S. 2002. The serious search for an anti-aging pill. Sci.Am., 287, 36-41.

Lang, C., Naryshkin, S., Schneider, D., Mills, B., & Lindeman, R. 1992.
Low blood glutathione levels in healthy aging adults. J Lab Clin Med, 120, 720-725.

Lanzillotta, A., Porrini, V., Bellucci, A., Benarese, M., Branca, C., et al. 2015. NF-kappaB in Innate Neuroprotection and Age-Related Neurodegenerative Diseases. Front.Neurol., 6, 98.

LaRocca, T. J., Gioscia-Ryan, R. A., Hearon, C. M.,Jr, & Seals, D. R. 2013. The autophagy enhancer spermidine reverses arterial aging. Mech.Ageing Dev., 134, 314-320.

Lee, W., Loo, C., Bebawy, M., Luk, F., Mason, R., & Rohanizadeh, R. 2013. Curcumin and its derivatives: their application in neuropharmacology and neuroscience in the 21st century.. Curr Neuropharmacol., 11, 338-378.

Li, R., Huang, Y. G., Fang, D., & Le, W. D. 2004. (-)-Epigallocatechin gallate inhibits lipopolysaccharide-induced microglial activation and protects against inflammation-mediated dopaminergic neuronal injury. J.Neurosci.Res., 78, 723-731.

Liu, J., Zhang, B., Lei, H., Feng, Z., Liu, J., et al. 2013. Functional aging in the nervous system contributes to age-dependent motor activity decline in C. elegans. Cell.Metab., 18, 392-402.

Madeo, F., Pietrocola, F., Eisenberg, T., & Kroemer, G. 2014.
Caloric restriction mimetics: towards a molecular definition.. Nat Rev Drug Discov, 13, 727-740.

Mani, M., Khaghani, S., Gol Mohammadi, T., Zamani, Z., Azadmanesh, K et al. 2013. Activation of Nrf2-Antioxidant Response Element Mediated Glutamate Cysteine Ligase Expression in Hepatoma Cell line by Homocysteine. Hepat Mon., 13, e8394.

Marino, G., Pietrocola, F., Eisenberg, T., Kong, Y., Malik, S. A., et al. 2014. Regulation of autophagy by cytosolic acetyl-coenzyme A. Mol.Cell, 53, 710-725.

Marino, G., Pietrocola, F., Madeo, F., & Kroemer, G. 2014. Caloric restriction mimetics: natural/physiological pharmacological autophagy inducers. Autophagy, 10, 1879-1882.

Martin, G. M. 1997. Genetics and the pathobiology of ageing.
Philos.Trans.R.Soc.Lond.B.Biol.Sci., 352, 1773-1780.

Mattison, J. A., Roth, G. S., Beasley, T. M., Tilmont, E. M., Handy, A. M., et al. 2012. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature, 489, 318-321.

McGeer, E., & McGeer, P. 2010. Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy. J Alzheimers Dis., 19, 355-361.

McGeer, P., & McGeer, E. 1999. Inflammation of the brain in Alzheimer's disease: implications for therapy. J Leukoc Biol, 65, 409-415.

Meydani, M., Das, S., Band, M., Epstein, S., & Roberts, S. 2011. The effect of caloric restriction and glycemic load on measures of oxidative stress and antioxidants in humans: results from the CALERIE Trial of Human Caloric Restriction. J.Nutr.Health Aging, 15, 456-460.

Miller, R. A., Harrison, D. E., Astle, C. M., Baur, J. A., Boyd, A. R., et al. 2011. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. J.Gerontol.A Biol.Sci.Med.Sci., 66, 191-201.

Morrow, E. H. 2015. The evolution of sex differences in disease. Biol.Sex.Differ., 6, 5-015-0023-0. eCollection 2015.

Motohashi, H., & Yamamoto, M. 2004. Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med, 10, 549-557.

Mourtas, S., Lazar, A. N., Markoutsa, E., Duyckaerts, C., & Antimisiaris, S. G. 2014. Multifunctional nanoliposomes with curcumin-lipid derivative and brain targeting functionality with potential applications for Alzheimer disease. Eur.J.Med.Chem., 80, 175-183.

Murphy, M.,P. 2009. How mitochondria produce reactive oxygen species. Biochem J, 417, 1-13.

Nguyen, T., Nioi, P., & Pickett, C. B. 2009. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J.Biol.Chem., 284, 13291-13295.

Noyan, H., El-Mounayri, O., Isserlin, R., Arab, S., Momen, A., et al. 2015. Cardioprotective Signature of Short-Term Caloric Restriction. PLoS One, 10, e0130658.

Oh, J., Lee, Y.,D., & Wagers, A.,J. 2014.
Stem cell aging: mechanisms, regulators and therapeutic opportunities.. Nat Med., 20, 870-880.

Olshansky, S. J. 2005. Projecting the future of U.S. health and longevity. Health.Aff.(Millwood), 24 Suppl 2, W5R86-9.

Owen, M. R., Doran, E., & Halestrap, A. P. 2000. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem.J., 348 Pt 3, 607-614.

Pastore, A., Federici, G., Bertini, E., & Piemonte, F. 2003. Analysis of glutathione: implication in redox and detoxification. Clin Chim Acta, 333, 19-39.

Patrone, C., Eriksson, O., & Lindholm, D. 2014. Diabetes drugs and neurological disorders: new views and therapeutic possibilities. Lancet Diabetes Endocrinol., 2, 256-262.

Petri, S., Korner, S., & Kiaei, M. 2012. Nrf2/ARE Signaling Pathway: Key Mediator in Oxidative Stress and Potential Therapeutic Target in ALS. Neurol.Res.Int., 2012, 878030.

Pike, C., Carroll, J., Rosario, E., & Barron, A. 2009. Protective actions of sex steroid hormones in Alzheimer's disease.. Front Neuroendocrinol, 30, 239-258.

Polazzi, E., & Monti, B. 2010. Microglia and neuroprotection: from in vitro studies to therapeutic applications. Prog.Neurobiol., 92, 293-315.

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., & Buga, A. 2013. ROS and brain diseases: the good, the bad, and the ugly. Oxid Med Cell Longev, 2013.

Radak, Z., Silye, G., Bartha, C., Jakus, J., Stefanovits-Banyai, E., et al. 2013. The effects of cocoa supplementation, caloric restriction, and regular exercise, on oxidative stress markers of brain and memory in the rat model. Food Chem.Toxicol., 61, 36-41.

Ramis, M. R., Esteban, S., Miralles, A., Tan, D. X., & Reiter, R. J. 2015. Caloric restriction, resveratrol and melatonin: Role of SIRT1 and implications for aging and related-diseases. Mech.Ageing Dev., 146-148C, 28-41.

Rao, C. V., Rivenson, A., Simi, B., & Reddy, B. S. 1995. Chemoprevention of colon cancer by dietary curcumin. Ann.N.Y.Acad.Sci., 768, 201-204.

Rebrin, I., Forster, M.,J., & Sohal, R.,S. 2011. Association between life-span extension by caloric restriction and thiol redox state in two different strains of mice.. Free Radic Biol Med., 51, 225-233.

Robillard, J., Gordon, G., Choi, H., Christie, B., & MacVicar, B. 2011. Glutathione restores the mechanism of synaptic plasticity in aged mice to that of the adult. PLoS One, 6, e20676.

Rossi, L., Mazzitelli, S., Arciello, M., Capo, C. R., & Rotilio, G. 2008. Benefits from dietary polyphenols for brain aging and Alzheimer's disease. Neurochem.Res., 33, 2390-2400.

Salminen, L.,E., & Paul, R.,H. 2014. Oxidative stress and genetic markers of suboptimal antioxidant defense in the aging brain: a theoretical review. Rev Neurosci., 25, 805-819.

Sankowski, R., Mader, S., & Valdes-Ferrer, S. I. 2015. Systemic inflammation and the brain: novel roles of genetic, molecular, and environmental cues as drivers of neurodegeneration. Front.Cell.Neurosci., 9, 28.

Satoh, T., McKercher, S., & Lipton, S. 2013. Nrf2/ARE-mediated antioxidant actions of pro-electrophilic drugs.. Free Radic Biol Med, 65, 645-647.

Schneider, C., & Segre, T. 2009. Green tea: potential health benefits. Am.Fam.Physician, 79, 591-594.

Schroeder, S., Pendl, T., Zimmermann, A., Eisenberg, T., Carmona-Gutierrez, D., et al. 2014. Acetyl-coenzyme A: a metabolic master regulator of autophagy and longevity. Autophagy, 10, 1335-1337.

Sharma, R. A., McLelland, H. R., Hill, K. A., Ireson, C. R., Euden, S. A., et al. 2001. Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. Clin.Cancer Res., 7, 1894-1900.

Shehzad, A., Ha, T., Subhan, F., & Lee, Y. 2011. New mechanisms and the anti-inflammatory role of curcumin in obesity and obesity-related metabolic diseases.. Eur J Nutr, 50, 151-161.

Siddique, Y. H., Naz, F., & Jyoti, S. 2014. Effect of curcumin on lifespan, activity pattern, oxidative stress, and apoptosis in the brains of transgenic Drosophila model of Parkinson's disease. Biomed.Res.Int., 2014, 606928.

Soda, K., Kano, Y., Chiba, F., Koizumi, K., & Miyaki, Y. 2013. Increased polyamine intake inhibits age-associated alteration in global DNA methylation and 1,2-dimethylhydrazine-induced tumorigenesis. PLoS One, 8, e64357.

Sohal, R. S., & Weindruch, R. 1996. Oxidative stress, caloric restriction, and aging. Science, 273, 59-63.

Spence, R., & Voskuhl, R. 2012.
Neuroprotective effects of estrogens and androgens in CNS inflammation and neurodegeneration.. Front Neuroendocrinol, 33, 105-115.

Spielman, L. J., Little, J. P., & Klegeris, A. 2014. Inflammation and insulin/IGF-1 resistance as the possible link between obesity and neurodegeneration. J.Neuroimmunol., 273, 8-21.

Stefani, M., Markus, M. A., Lin, R. C., Pinese, M., Dawes, I. W., & Morris, B. J. 2007. The effect of resveratrol on a cell model of human aging. Ann.N.Y.Acad.Sci., 1114, 407-418.

Stranahan, A. M., & Mattson, M. P. 2008.
Impact of energy intake and expenditure on neuronal plasticity. Neuromolecular Med., 10, 209-218.

Sturm, R., & Hattori, A. 2013. Morbid obesity rates continue to rise rapidly in the United States.. Int J Obes (Lond), 37, 889-891.

Suckow, B. K., & Suckow, M. A. 2006. Lifespan extension by the antioxidant curcumin in Drosophila melanogaster. Int.J.Biomed.Sci., 2, 402-405.

Suh, J., Shenvi, S., Dixon, B., Liu, H., Jaiswal, A., Liu, R., & Hagen, T. 2004.
Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid.. Proc Natl Acad Sci U S A, 101, 3381-3386.

Sunagawa, Y., Morimoto, T., Wada, H., Takaya, T., Katanasaka, Y., et al. 2011. A natural p300-specific histone acetyltransferase inhibitor, curcumin, in addition to angiotensin-converting enzyme inhibitor, exerts beneficial effects on left ventricular systolic function after myocardial infarction in rats. Circ.J., 75, 2151-2159.

Sweat, V., Starr, V., Bruehl, H., Arentoft, A., Tirsi, A., Javier, E., & Convit, A. 2008. C-reactive protein is linked to lower cognitive performance in overweight and obese women. Inflammation, 31, 198-207.

Sykiotis, G.,P., Habeos, I.,G., Samuelson, A.,V., & Bohmann, D. 2011. The role of the antioxidant and longevity-promoting Nrf2 pathway in metabolic regulation. Curr Opin Clin Nutr Metab Care, 14, 41-48.

Thomas, D. M., Martin, C. K., Redman, L. M., Heymsfield, S. B., Lettieri, S., et al. 2014. Effect of dietary adherence on the body weight plateau: a mathematical model incorporating intermittent compliance with energy intake prescription. Am.J.Clin.Nutr., 100, 787-795.

Timmers, S., Konings, E., Bilet, L., Houtkooper, R. H., van de Weijer, T., et al. 2011. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell.Metab., 14, 612-622.

Toda, N., Ayajiki, K., & Okamura, T. 2014. Obesity-induced cerebral hypoperfusion derived from endothelial dysfunction: one of the risk factors for Alzheimer's disease. Curr.Alzheimer Res., 11, 733-744.

Trepanowski, J. F., Canale, R. E., Marshall, K. E., Kabir, M. M., & Bloomer, R. J. 2011. Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings. Nutr.J., 10, 107-2891-10-107.

Troen, B.,R. 2003. The biology of aging. Mt Sinai J Med, 70, 3-22.

Trujillo, J., Granados-Castro, L.,F., Zazueta, C., Andérica-Romero, A.,C., Chirino, Y.,I., & Pedraza-Chaverrí, J. 2014. Mitochondria as a target in the therapeutic properties of curcumin.. Arch Pharm (Weinheim), 347, 873-884.

Turturro, A., Witt, W.,W., Lewis, S., Hass, B.,S., Lipman, R.,D., & Hart, R.,W. 1999. Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program.. J Gerontol A Biol Sci Med Sci., 54, B492-B501.

Varady, K. A. 2011. Intermittent versus daily calorie restriction: which diet regimen is more effective for weight loss? Obes.Rev., 12, e593-601.

Vartiainen, E., Puska, P., Pekkanen, J., Tuomilehto, J., & Jousilahti, P. 1994. Changes in risk factors explain changes in mortality from ischaemic heart disease in Finland. BMJ, 309, 23-27.

Wallace, D. C., Fan, W., & Procaccio, V. 2010. Mitochondrial energetics and therapeutics. Annu.Rev.Pathol., 5, 297-348.

Wang, X., & Zhao, X. 2009. Contribution of oxidative damage to antimicrobial lethality.. Antimicrob Agents Chemother, 53, 1395-1402.

Wang, M. S., Boddapati, S., Emadi, S., & Sierks, M. R. 2010. Curcumin reduces alpha-synuclein induced cytotoxicity in Parkinson's disease cell model. BMC Neurosci., 11, 57-2202-11-57.

Wang, Y., Li, M., Xu, X., Song, M., Tao, H., & Bai, Y. 2012. Green tea epigallocatechin-3-gallate (EGCG) promotes neural progenitor cell proliferation and sonic hedgehog pathway activation during adult hippocampal neurogenesis. Mol.Nutr.Food Res., 56, 1292-1303.

Weinert, B.,T., & Timiras, P.,S. 2003. Invited review: Theories of aging. J Appl Physiol (1985), 95, 1706-1716.

Wilms, H., Zecca, L., Rosenstiel, P., Sievers, J., Deuschl, G., & Lucius, R. 2007. Inflammation in Parkinson's diseases and other neurodegenerative diseases: cause and therapeutic implications. Curr.Pharm.Des., 13, 1925-1928.

Winklewski, P. J., Radkowski, M., Wszedybyl-Winklewska, M., & Demkow, U. 2015. Brain inflammation and hypertension: the chicken or the egg? J.Neuroinflammation, 12, 85-015-0306-8.

Wise, P. 2002. Estrogens and neuroprotection. Trends Endocrinol Metab, 13, 229-230.

Yu, B. P. 1996. Aging and oxidative stress: modulation by dietary restriction. Free Radic.Biol.Med., 21, 651-668.

Zhang, H., Limphong, P., Pieper, J., Liu, Q., Rodesch, C., Christians, E., & Benjamin, I. 2012. Glutathione-dependent reductive stress triggers mitochondrial oxidation and cytotoxicity. , 26, 1442-1451.

Zhu, Y., Carvey, P., & Ling, Z. 2006. Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain Res, 1090, 35-44.

Zimniak, P. 2011. Relationship of electrophilic stress to aging. Free Radic Biol Med, 51, 1087-1105.

FIGURE 1: (a) Curcumin binds to IKKβ preventing the phosphorylation of IkB and inhibiting the nuclear translocation of NF-κB. (b) Hydroxyl groups flanking the molecule donate hydrogen to neutralize free radicals. The O-methoxy groups provide steric hindrance and the β diketone bridge provides stability preventing curcumin from becoming a free radical itself.
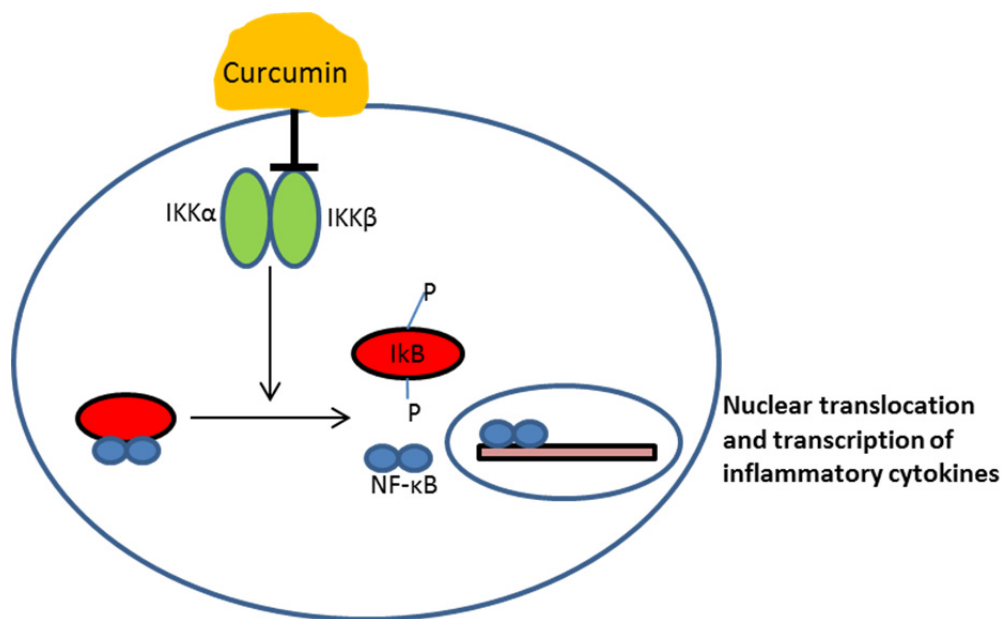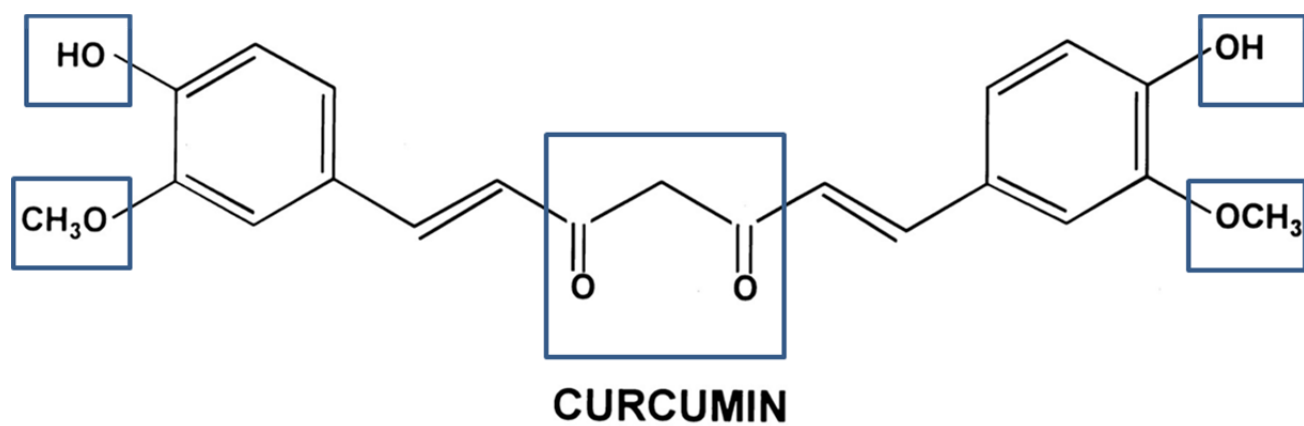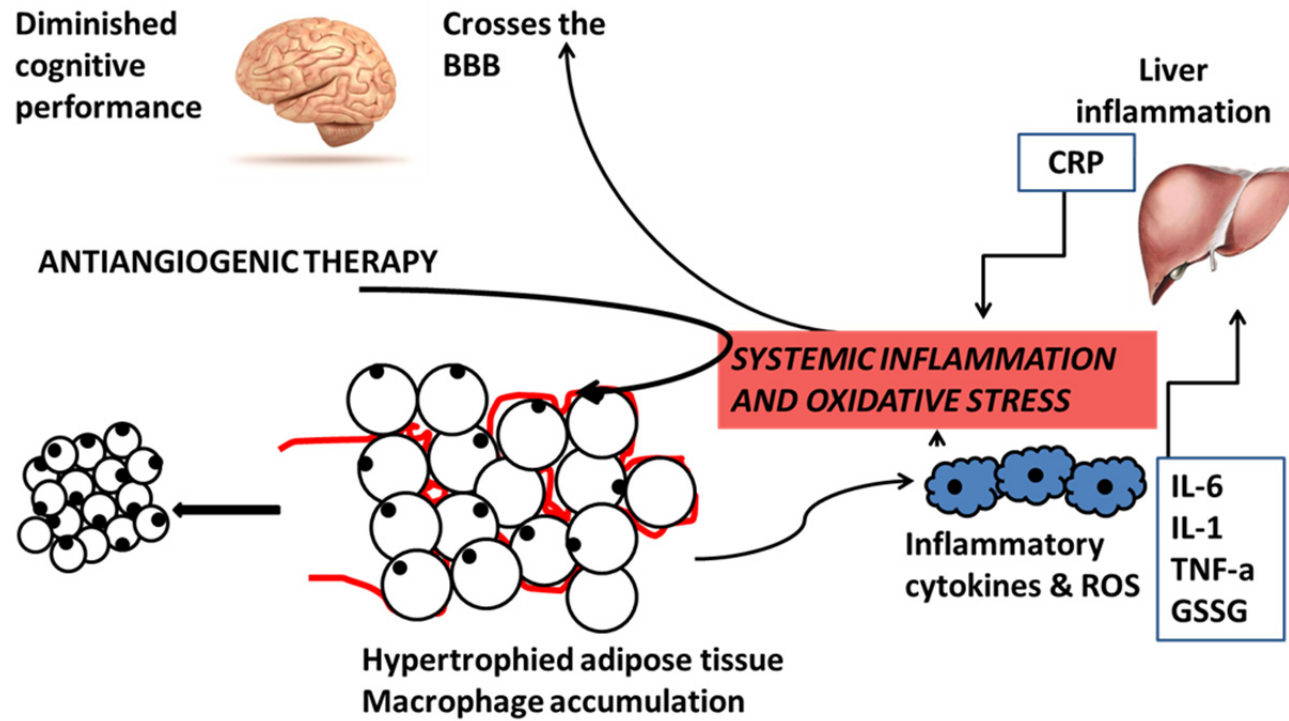
Figure 1a

Figure 1b

Table 1: Biochemical and functional characteristics of caloric restriction mimetics

Table 1: *Biochemical and functional characteristics of caloric restriction mimetics*

Compound	Origin and use	CR-like mechanism	Preclinical effects	References
Rapamycin	Immunosuppressant therapy.	Inhibition of mammalian target of rapamycin complex 1 (MTORC1).	• Extension of maximal lifespan, larger effect sizes in females. • Improves cognition in aging mice. • Decreased number of tumors. • Increases autophagy.	(Anisimov, Zabezhinski et al., 2011; Chen et al., 2009; Harrison et al., 2009; Miller et al., 2011)
Resveratrol	Polyphenol primarily found in red wine (skins of red grapes).	Activation of SIRT1 and inhibition of phosphodiesterase 4 activating AMPK. Upregulates Nrf2. Inhibits cyclooxygenase.	• Extension of lifespan of mice on a high fat diet. • Reduced adipogenesis and enhances insulin stimulated glucose uptake. • Improvement in insulin resistance. • Improves working memory in older Wistar rats. • Increases autophagy	(Baur et al., 2006; Baur & Sinclair, 2006; Howitz et al., 2003; Ramis et al., 2015; Stefani et al., 2007)
Metformin	A biguanide antidiabetic drug, first line of choice for the treatment of type II diabetes.	Indirect activation of AMPK via mild to moderate inhibition of complex 1 in ETC. Inhibition of MTORC1. Upregulation of Nrf2.	• Decreases hepatic glucose production. • Extension of lifespan in C. elegans. • Slight increase in lifespan in certain strains of mice. Moderate improvement in cognition in certain strains of mice	(Anisimov, Berstein et al., 2011; Batandier et al., 2006; Cusi et al., 1996; Owen et al., 2000; Patrone et al., 2014)
Epigallocatechin 3-gallate	Most abundunt catechin in green tea, can cross blood brain barrier.	Inhibition of NF-κB nuclear translocation and acetylation.	• Antioxidant and anti-inflammatory effects. • Reduced weight gain and insulin resistance in mice receiving a high-fat diet. Increases neurogenesis in adult hippocampal progenitor cell cultures. • Reduces microglia activation. Increases autophagy	(Khurana et al., 2013; Li et al., 2004; Schneider & Segre, 2009; Y. Wang et al., 2012)
Spermidine	Naturally occurring polyamine, also found in in food such as fermented soybean and wheat germ.	Nrf2 activation. Inhibition of MTORC1 and acetyltransferases.	• Antioxidant and anti-inflammation, pro-autophagy. • Improvement in age related memory impairment in flies. • Delays age related decline in locomotor activity in mice. • Increases autophagy.	(Eisenberg et al., 2009; Kaeberlein, 2009; LaRocca et al., 2013; Soda et al., 2013)

FIGURE 2: Potential anti-angiogenic mechanism of curcumin and downstream effects. Shrinkage of adiposity results in attenuation of systemic inflammation and oxidative stress and prevents obesity associated cognitive dysfunction

Diminished cognitive performance

Crosses the BBB

Liver inflammation

CRP

ANTIANGIOGENIC THERAPY

SYSTEMIC INFLAMMATION AND OXIDATIVE STRESS

Inflammatory cytokines & ROS

IL-6
IL-1
TNF-a
GSSG

Hypertrophied adipose tissue
Macrophage accumulation

Chapter II

TITLE PAGE:

a. Title: Curcumin mimics the neurocognitive and anti-inflammatory effects of caloric restriction in a mouse model of midlife obesity[1].

b. Authors: Marjana Rahman Sarker[1], Susan Franks[2], Nathalie Sumien[1], Nopporn Thangthaeng[1] Frank Filipetto[2], Michael Forster[1,3]

[1] Department of Pharmacology and Neuroscience, Institute for Aging and Alzheimer's Disease Research (IAADR), University of North Texas Health Science Center, Fort Worth, TX 76107

[2] Family Medicine, Texas College of Osteopathic Medicine, University of North Texas Health Science Center, Fort Worth, TX 76107

[3] Corresponding author, mailing address: CBH 512, 3500 Camp Bowie, Fort Worth TX 76107, Tel no: 8177352092 Fax no: 8177350408, email: Michael.Forster@unthsc.edu

c. Authors last names: Sarker, Franks, Sumien, Thangthaeng, Filipetto, and Forster.

d. Word count: 6927

e. Number of figures: 6

f. No tables

g. Supplemental material will be submitted

h. Dietary curcumin and cognitive status

i. (i) Supplemental Table 1 and 2 available

(ii) Abbreviations used: CR, caloric restriction; IL-6, Interleukin 6; CRP, C-reactive protein; GSH, reduced glutathione; GSSG, oxidized glutathione; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; Nrf2, Nuclear factor (erythroid-derived 2)-like 2.

Abstract

Background. Dietary curcumin was studied for its potential to decrease adiposity and reverse obesity- associated cognitive impairment in a mouse model of midlife sedentary obesity. *Objective*. We hypothesized that curcumin intake, by decreasing adiposity via its antiangiogenic effect, would improve cognitive function in a manner comparable to caloric restriction (CR), a weight loss regimen. *Methods*. 15-month-old male C57BL/6 mice were assigned in groups to receive the following dietary regimens for 12 weeks: (i) a base diet (Ain93M) fed ad libitum (AL), (ii) the base diet restricted to 70% of ad libitum (CR) or (iii) the base diet containing curcumin fed AL (1000 mg/kg diet, CURAL). Blood markers of inflammation, interleukin 6 (IL-6) and C-reactive protein (CRP), as well as an indicator of redox stress (GSH: GSSG ratio), were determined at different time points during the treatments, and visceral and subcutaneous adipose tissue were measured upon completion of the experiment. After 8 weeks of dietary treatment, the mice were tested for spatial cognition (Morris water maze) and cognitive flexibility (discriminated active avoidance). *Results.* The CR group showed significant weight loss and reduced adiposity, whereas CURAL mice had stable weight throughout the experiment, consumed more food than the AL group, with no reduction of adiposity. However, both CR and CURAL groups took fewer trials than AL to reach criterion during the reversal sessions of the active avoidance task, suggesting an improvement in cognitive flexibility. The AL mice had higher levels of CRP compared to CURAL and CR, and GSH as well as the GSH: GSSG ratio were increased during curcumin intake, suggesting a reducing shift in the redox state. *Conclusions.* The results suggest that, independent of their effects on adiposity; dietary curcumin and caloric restriction have positive effects on frontal cortical functions that could be linked to anti-inflammatory or antioxidant actions.

Keywords: curcumin, obesity, caloric restriction, inflammation, cognition, oxidative stress

Introduction

Complementary and alternative therapies have significant potential to expand current treatment options for obese patients, especially those who can be classified as overweight and class 1 obese yet do not meet clinical criteria for appetite suppressant drugs or bariatric surgery (1). Very often these classifications can be linked to a sedentary lifestyle, wherein energy intake significantly exceeds that expended, resulting in a chronic positive energy imbalance, weight gain and adiposity. Indeed, a recent report by Sturm and Hattori (2) revealed that only 6.6% of the obese population in the US are morbidly obese, making a strong case for more studies being conducted on alternate therapies for the bulk of the obese population. The current study focused on the therapeutic potential of the polyphenol curcumin, which has gained an increased interest in recent years as a potential treatment for obesity-related comorbidities as well as neurodegenerative disorders (3-5). Because it has been shown to have anti-angiogenic effects (6), administration of curcumin could potentially decrease the vascularization around expanding adipose tissue, resulting in attenuation of adiposity and its associated inflammation and oxidative stress (7, 8).

In testing curcumin as an alternative approach for decreasing adiposity in class 1 obese individuals, the accumulated weight and adiposity of middle-aged, ad libitum (AL)-fed laboratory mice was targeted as a preclinical model. Traditional experimental obesity models (also previously used to study curcumin) have targeted excessive weight gain associated with high fat diets or leptin deficiency (6). However, the accumulation of weight in these experimental models could be considered a translational analogue of morbid obesity, as these mice often weigh more than two times that of their age-matched controls (6, 9). Moreover, control groups in these studies are fed ad libitum; a condition that itself leads to significant weight gain and adiposity under typical laboratory housing conditions. Recent reviews have highlighted the observation that control rodents kept on ad libitum (AL) feeding often double their weight during adulthood and show an array of obesity-associated conditions by midlife (10). A recent analysis of data on

7 strains of commonly used laboratory rodents suggested that beneficial effects of chronic caloric restriction on life-span were directly proportional to the degree of weight gained by midlife under the AL feeding regimen (31). These results challenge the use of AL feeding as a control condition and, perhaps more importantly, suggest that under standard laboratory conditions, middle-aged AL fed mice are translational analogues of overweight and mildly obese humans (10). The Centers for Disease Control and Prevention reports that almost 40% of the US obese population fall within the range of middle age, suggesting that this age group is a significant target for obesity intervention (11).

In addition to addressing therapeutic potential as an anti-obesity medication, it was of interest to determine if curcumin would also influence co-morbid conditions linked to obesity via inflammation and oxidative stress. Mild cognitive impairment has been detected in association with obesity in human studies (12-14), and animal studies of CR imply that improved cognitive function is present in subjects that maintain lower weight (15, 16). Moreover, based on the results from a study conducted on more than 8000 Swedish twins, it was concluded that being overweight or obese during midlife, independent of diabetes and vascular diseases, significantly increased the risk of late life dementia, Alzheimer's disease and vascular dementia (17). The same trend has been also reported in several other longitudinal studies with a more diverse population (12-14, 18).

Increased levels of inflammatory and oxidative stress markers related to obesity have been linked to impaired cognitive performance in middle-aged individuals in previous studies. For example, inflammatory markers such as interleukin 6 (IL-6) and C-reactive protein (CRP) are reported to be inversely related to cognitive capacity in middle aged obese women (19-21). Other pro-inflammatory markers such as IL-1β and TNF-α have also been associated with poor memory (22, 23). The association of inflammation with poor cognition may also be influenced by the reciprocal relationship inflammation shares with oxidative stress in several pathologies, which also includes chronic obesity (24-26). Both IL-6 and TNF-α, whose levels are increased in

59

several pathologies, have been reported to promote the production of reactive oxygen species, a process which reinforces activation of pro-inflammatory transcription factors. A very sensitive indicator of oxidative stress in tissues and blood is the redox state of the endogenous antioxidant glutathione or reduced glutathione (GSH) (27). Clinically, a decrease in the ratio of reduced to oxidized glutathione in red blood cells has been linked to both mild cognitive impairment and Alzheimer's disease (27, 28). Further, a decrease in this ratio has also been associated with poor cognitive performance and overall functional decline in several *in vivo* age-associated disease studies (29, 30).

The rationale for the current study was to evaluate curcumin as a safe, inexpensive and potentially effective intervention for weight loss, using a preclinical model of midlife sedentary obesity, inflammation, and comorbid cognitive impairment. The effect of curcumin was compared with outcomes for the middle-aged mice subjected to caloric restriction implemented gradually to maintain a healthy weight loss, improve cognition and reduce inflammation. The hypothesis for this study was that dietary curcumin would promote weight loss and reduce adiposity, thereby improving cognition via a concurrent decrease in inflammation and oxidative stress.

Materials and Methods:

Mice and diets.

Fifteen-month-old C57BL/6JNia male mice, maintained at the National Institute on Aging (NIA) colony under ad libitum (AL) feeding of NIH-31(31), were shipped to UNT Health Science Center where they were assigned in groups of 19 to receive the following dietary regimens for 12 weeks: (i) a base diet fed ad libitum (AL), (ii) the base diet restricted to 70% of AL (CR) or (iii) the base diet containing curcumin fed AL (1000 mg/kg diet, CURAL). Caloric restriction was implemented incrementally (relative to that consumed by AL groups), by 10% of AL during the first week, 20% in the second, and by 30% in the third week. Feed of the CR group remained fixed at this level for the remainder of the experiment. The obese control group (AL) was fed a

60

purified maintenance diet (AIN 93M) (58M1 Test Diet, Richmond, IN) (**Supplemental Table 1**)

ad libitum. The CURAL group was fed the same maintenance diet supplemented with 1000 mg

of curcumin (Sigma-Aldrich, St. Louis, MO)/kg of diet (Catalog no: 1815457-201)

(**Supplemental Table 1**). The CR group received a diet fortified with vitamins and minerals

formulated by Test Diet (Catalog no: 1815458-209) (**Supplemental Table 1**). All animals were

fed the AL diet for 2 weeks to acclimate them on the purified diet (because they were fed a

grain-based diet at NIA), following which they were randomly assigned to one of the three

treatment groups. Body weight was measured once every week, and food intake for CURAL

and AL was estimated at 4, 8 and 12 weeks of treatment. Behavior tests were initiated after 8

weeks of dietary treatment and continued until the last week of the treatment period. Mice

underwent tail bleeds at 8 and 12 weeks following treatment, and visceral (VAT) and

subcutaneous (SAT) adipose tissue (which include epididymal and gluteal adipose tissue

respectively) was collected after mice had been euthanized at the conclusion of the study. The

study protocol was reviewed and approved by the Institutional Animal Care and Use Committee

of UNTHSC.

Assessment of systemic inflammation.

All protein concentrations were determined using the BCA Protein Assay Kit from Pierce

Biotechnology (Rockford, IL) with bovine serum albumin as a standard. Serum was separated

from blood and the inflammatory markers were measured using ELISA. CRP was measured

using a mouse CRP kit from R&D Systems (Minneapolis, MN) and IL-6 was measured using a

kit from Life Technologies (Carlsbad, CA).

Assessment of redox state.

Prior to the processing of erythrocytes, the collected blood was centrifuged at 2,500g for 10

min at 4ºC; the plasma was removed to be analyzed for inflammatory cytokines. The white

blood cells were removed and the remaining red blood cells were lysed with 4 times its volume

of ice cold double distilled water and incubated for 10 min on ice. The samples were then

centrifuged at 10,000g for 15 min at 4ºC. Some of the supernatant was collected to measure protein concentration and the rest received an equal volume of metaphosphoric acid to stop the oxidation process and stored at -80ºC until assayed. Total glutathione (tGSH) and oxidized glutathione (GSSG) were measured from erythrocytes collected during the blood draws according to manufacturer's instructions, and the concentration of reduced glutathione (GSH) was then calculated using the formula: tGSH-(2*GSSG) /GSSG and GSH: GSSG was calculated. The GSH: GSSG kit was purchased from Cayman Chemical (Ann Harbor, MI).

Cognitive skills assessment.

At the end of week 8 of dietary treatment, mice underwent behavior tests to analyze domains of cognitive performance linked to hippocampal (spatial learning) versus frontal cortical (cognitive flexibility) functions.

Spatial learning and memory

Spatial learning and memory were measured using a Morris water maze (MWM) test similar to that described previously (32, 33). This test provided a measure of the efficiency of mice in swimming to a hidden, safe platform from different locations within a cylindrical tank filled with opacified water. A pre-training phase was conducted in two sessions during which mice did not have access to spatial information (cues) in the testing room, to ensure that the mice had learned the motor components of swimming and climbing onto the platform prior to testing for spatial performance. During subsequent testing, a computerized tracking system recorded the length of the path taken by the mouse to reach the platform, as well as the swimming speed (Any-maze; Stoelting Co., Wood Dale, IL, USA), with spatial cues available in the open tank and the hidden platform in a fixed location. Testing was conducted over nine sessions (1-4, Tuesday-Friday and 5-9, Monday-Friday the following week). Each session consisted of five trials separated by an ITI of 90s during which the mouse had to swim to the platform from one of four different starting points. A learning index was calculated as the average path length on sessions 2, 3 and 4, the initial learning phase. Probe trials were conducted immediately

following sessions 2, 4, 5, 7, and 9, during which the platform was submerged to a depth that prevented the mice from climbing onto it. The platform was raised after 30 s, and the trial was ended when the mouse successfully located it. The percentage of time spent in a 40 cm diameter annulus around the platform location was calculated as a measure of spatial bias for the platform location. Memory retention for the platform location was tested during one additional session conducted one week after session 9.

Cognitive flexibility

An acrylic T-maze (black on the sides with a clear top) was used for the discriminated avoidance task (34, 35). The maze was divided into three compartments with a stem and goal arms that rested on a grid floor wired to deliver 0.67-mA scrambled shock to the feet of the mice. The test consisted of three sessions separated by 1 h. Each mouse was trained to leave the start box and run to a designated correct goal within 5 s after lifting of the start door. On the first trial of the first session (acquisition), the correct goal arm was designated as the one opposite from the mouse's first arm choice (determined on a separate preference trial). On subsequent trials, shock was initiated 5 s after the opening of the start door if the mouse had not entered the correct goal arm, or immediately upon entry into the incorrect arm. In both cases, shock was continued until the correct goal arm was entered or a maximum of 60 s had elapsed. A correct avoidance trial was recorded when the mouse entered the correct goal arm within 5s of the opening of the start door. The mouse was then allowed to stay in the correct goal arm for 10 s after which the mouse was removed and placed in a holding cage for 1 min. A session ended when the mouse had made a correct avoidance on the last two trials and on at least 4 of the last 5 trials. The second and third sessions of avoidance training were reversals such that the mice were required to run to the goal arm opposite that to which they had previously been trained. Ability to learn the avoidance problem was considered inversely proportional to the number of trials required to reach criterion in each of the sessions. Learning efficiency during the second and third sessions was considered indicative of cognitive flexibility (36, 37).

Measures of performance on the MWM test were initially considered in a 3 x 2 x 4 analysis of variance (ANOVA) with Diet as a between-groups factor and Test phase (session 1-4 vs 5-8) and Session as within-subject factors. The number of trials required to reach the correct avoidance criterion was considered in a two-way ANOVA with Diet as a between groups factor and Sessions as a within subject factor. In the context of a significant main effect of Diet or a Diet x Session interaction, planned comparisons were conducted between diet groups using single degree of freedom F tests within the Diet x Session interaction (i.e., using the cell means and overall error term). A similar approach was applied to analysis of data for body weight and food intake, with weeks as the within subject factor. One-way between groups ANOVA was applied to data for CRP, IL6, GSH, GSH: GSSG and GSSG, followed by planned comparisons among diet groups. The alpha level was set to 0.05 for all analyses.

Results

Body weight, food intake and adiposity. The CR group had significant loss of body weight compared to mice on curcumin or AL beginning in week 3, and they continued to lose weight until week 6, after which they maintained stable weight for the rest of the treatment period (Fig.1). Both CURAL and AL groups maintained stable body weight throughout the 12-week dietary treatment, and there was no difference in body weight between the CURAL and AL group at any time point. However, in spite of the lack of difference in body weight compared to AL mice, CURAL mice had a higher food intake (almost 25%) compared to AL at week 12 of the dietary treatment period (Fig. 1, right ordinate). Analyses of variance for body weight and food intake supported these observations, revealing a significant effect of diet, weeks, and the interaction of those factors (All $P<0.03$).

At the end of the 12-week treatment, the CR group had markedly lower amounts of both VAT and SAT when compared with the AL group (Fig. 2), but there was no decrease in adipose

tissue associated with intake of curcumin. The fat loss in the CR group was responsible for a main effect of diet in analyses of variance on VAT and SAT (all $P<0.001$).

Inflammation and redox state. There was no significant effect of diet on concentration of IL-6 at 8 or 12 weeks (all $P<0.27$, **Supplemental Table 2**). However, the obese control (AL) on average had a higher concentration of serum CRP than the CURAL or CR group at both 8 and 12 weeks of treatment. Data analysis indicated a significant main effect of Diet ($P=0.006$) in the absence of an interaction with weeks ($P=0.770$) (Fig. 3). Curcumin-treated mice had a higher concentration of reduced glutathione (Fig. 4a) and a more reducing redox state (GSH: GSSG) compared to mice on CR and those fed AL (all $P < 0.05$) (Fig. 4b). There was, however, no difference in GSSG among the different diet groups ($P >0.446$) (Fig. 4c).

Spatial learning and cognitive flexibility. The distance to reach the hidden platform (path length) was determined during sessions 1-9 to assess the efficiency with which the mice located the platform regardless of their swimming speed. Over sessions 1-4, all groups showed improvement in their efficiency to reach the platform (Fig. 5A.), and there was no apparent difference among the treatment groups in the spatial learning index (**Supplemental Table 2**) or during probe trials conducted after sessions 2 and 4 (data not shown). Performance during the second week remained relatively stable for the treatment groups. Data analysis failed to indicate a significant effect of diet or interaction of diet with test phase or session (all $P>0.62$). Probe trial data calculated from percent time spent in the 40-cm annulus around the target site (not shown) confirmed that mice in the different treatment groups had acquired a spatial bias for the platform location that was maintained through session 9. However, analysis of the probe data failed to indicate a significant main effect or interaction involving diet (all $P>0.15$).

Cognitive flexibility was tested by first establishing a discriminated avoidance response, followed by two additional sessions separated by 1 hour in which the required correct response was reversed. Figure 6 shows the initial learning versus the average number of trials taken during sessions 2 and 3 to reach criterion (reversal). Mice kept on CR took fewer trials to reach

criterion compared to AL and CURAL in the initial learning phase. During the reversal sessions, both the CR and CURAL groups took fewer trials to reach criterion when compared with AL. A two-way ANOVA on the acquisition phase and reversal phase with diet and phase as the independent variables confirmed the mentioned observations, all $P<0.03$ (Fig. 6).

Discussion:

The effects of curcumin have not previously been considered in a model of midlife sedentary obesity. The main findings of this study were as follows: 1. Curcumin was ineffective in decreasing adiposity, but increased apparent energy intake in the absence of weight gain; 2. Calorically restricted mice lost both weight and had significantly reduced visceral and subcutaneous adipose tissue; 3. Curcumin intake promoted a more reducing redox state and a lower level of inflammation and 4. Curcumin and caloric restriction improved executive function but did not affect spatial learning. Overall, these results suggest that dietary curcumin produces an improvement of cognitive function similar to CR, in the absence of a significant effect on body weight or adiposity. This effect may involve an anti-inflammatory effect similar to that of CR, or an antioxidant mechanism.

Caloric restriction has been extensively studied in mice and other non-human primates. These studies have suggested that CR can extend lifespan and delay onset of age-related diseases for which oxidative stress and inflammation are also established risk factors (31, 38-40). While it has been argued extensively that CR attenuates aging and disease processes independently of the effect on adiposity, it remains likely that the anti-obesity effect is a key factor (38, 41). In this study, a 12-week regimen of CR, implemented gradually, not only reduced adiposity, but also improved frontal cortical functions and decreased plasma C-reactive protein (CRP), a biomarker of systemic inflammation. The beneficial effect of long-term CR on discriminated avoidance learning has been reported previously (15, 16), although the current findings suggest additionally that this outcome is evident after only 3 months of CR, when its implementation is delayed until middle age. This outcome favors the view that apparent long-

term benefits of CR regimens on cognition are at least partly attributable to reversible physiological actions (42-44). However, in apparent contrast to previous investigations, there was no effect of short-term CR on oxidative stress, when measured as the ratio of GSH: GSSG in erythrocytes (39). The different outcome in the current and previous studies of CR could be attributed to a difference in severity of the CR regimens (30 vs. 40%), the approach to measurement of GSH and GSSG, or the duration of the regimens.

Although the beneficial effects of CR are well known, it has been difficult to translate this regimen to a clinical setting because of the reluctance of most individuals to change eating behavior and modify food choices (45). A sustainable alternative or adjunct to the practice of caloric restriction may involve compounds that mimic the beneficial effects of caloric restriction and do not require a change in eating habits (46). Curcumin, a widely studied polyphenol, has gained significant interest as a caloric restriction mimetic, and our current findings of improved cognitive function and attenuated inflammation and oxidative stress would seem to confirm this suggestion. Moreover, despite the absence of weight loss in the CURAL group, the current studies do not rule out the possibility that curcumin intake is associated with a metabolic effect. The mice under the curcumin regimen showed a noticeable increase in food intake relative to their age-matched obese control, yet no net change in energy balance was detectable as weight gain. This finding would imply that curcumin increases energy expenditure, although no measurements of respiration, body temperature or physical activity were available from these studies for confirmation of this hypothesis. Several papers have reported curcumin's ability to activate AMP kinase (AMPK) a cellular energy sensor and stimulant of ATP production implicated as a physiological mechanism of the CR effect (6, 47, 48). Kim et al (49) found that both curcumin and its active metabolite tetrahydrocurcumin were effective in activating AMPK and inactivating Acetyl CoA carboxylase. Such an increase in energy expenditure would be offset by an increase in energy intake under conditions of AL feeding.

The curcumin-fed mice also had low levels of systemic inflammation, which was measured via serum CRP, widely considered a translational index of low grade inflammation (50, 51). Mice under caloric restriction also had lower levels of CRP compared to the obese control (AL). These findings are consistent with previous reports that both curcumin and caloric restriction have strong anti-inflammatory effects (41, 52-56). For mice under CR, it seems likely that the attenuation of inflammation can be directly linked to reduction of adiposity. However, the lack of effect of curcumin on adiposity suggests a different anti-inflammatory mechanism of action. Previous studies have reported on curcumin's ability to decrease inflammation via the inhibition of the nuclear translocation of nuclear factor kappa-light-chain-enhancer (NF-κB) of activated B cells. The nuclear translocation of NF-κB results in the transcription of pro-inflammatory cytokines such as IL-6, IL-1β and TGF-β. These cytokines can then induce CRP synthesis by hepatocytes (57). In vitro studies have reported curcumin binding to IKKβ and thereby preventing the activation of this kinase leading to downstream inhibition of NF-κB activity (58)

Despite the effects of curcumin and CR on CRP, no effect of treatments on serum IL-6 was evident in the current study. However, TNF-α is a stronger inducer of CRP than IL-6 and IL-1β alone (59), and is expressed in and secreted by adipocytes. The concentration of TNF-α correlates with the degree of visceral adiposity and, based on the current study, CR significantly reduced adipose tissue whereas curcumin did not. For this reason, curcumin and CR may have acted via different pathways to reduce inflammation. Additionally, both CR and curcumin were hypothesized to improve redox state; however, only dietary curcumin improved the redox state. There was a significant increase in both reduced glutathione and total glutathione but no significant differences in oxidized glutathione levels amongst the groups, suggesting that curcumin may increase glutathione synthesis. Curcumin has previously been reported to up-regulate the expression of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) via conformational change of Kelch ECH associating protein 1 by either alkylation or oxidation, thereby allowing the nuclear translocation of Nrf2 and increasing glutathione synthesis (60, 61). Although CR has

68

been reported to do the same (62), the lack of an increase in reduced glutathione could be associated with the shorter intervention period implemented in this study. In addition, several studies have reported on curcumin's strong anti-oxidant activity, which can be attributed to its molecular structure enabling it to be a strong free radical quencher (54, 63, 64).

Previous studies from our laboratory as well as others have determined impaired cognition to be strongly associated to increased oxidative stress. Indeed, lower reduced to oxidized glutathione ratios are correlated with poorer cognitive performance (27, 28, 30). Our findings further strengthen this suggestion, with mice under the curcumin regimen displaying better executive function than the obese controls. However, the same cannot be concluded with regard to mice under the caloric restriction regimen. Previous studies have also reported on a strong association of inflammation with cognition, which may be the contributing factor for better executive function and acquisition that occurred in mice under the CR regimen in the current study. Although both of these treatments had positive effects for fronto-cortical functioning, they did not have any effect on hippocampal dependent spatial learning. Neither the learning nor overall performance on the spatial learning task was affected by the interventions. This finding is congruent with the progression of cognitive dysfunction as evident from neurological assessments in the non-demented aging population. More often than not, older dementia free individuals suffer from difficulties on tasks that stress attention, cognitive flexibility and other executive functions (65-67) than hippocampal-dependent spatial learning and long-term memory. Results from the current study and that from human cognitive evaluations may indicate curcumin to be a potential therapeutic for fronto-cortical dysfunction reported with normal aging.

Finally, the dosage we used for this study was decided based on a previous obesity related study (6) and translates roughly to a human dose of only 500mg. Future studies should test a higher translatable dose corresponding to suggested doses for individuals suffering from severe inflammation-related diseases such as rheumatoid arthritis. Based on the calculation that uses the body surface area and metabolic activity of the species index by Reagan-Shaw et al. the

human equivalent dose in this study falls below the 1500mg that has been recommended for daily use (53). Follow-up studies with higher doses may further elucidate on a dose-response related to cognitive function.

In conclusion, the present findings from this study did not support the hypothesis that reduction of adiposity is responsible for improved cognition following curcumin intake. However, our findings suggest that both caloric restriction and dietary curcumin can improve obesity-associated comorbidities.

Literature Cited

1. Cooke D, Bloom S. The obesity pipeline: current strategies in the development of anti-obesity drugs. Nat Rev Drug Discov. 2006;5:919-31.

2. Sturm R, Hattori A. Morbid obesity rates continue to rise rapidly in the United States. Int J Obes (Lond). 2013;37:889-91.

3. Bar-Sela G, Epelbaum R, Schaffer M. Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications. Curr Med Chem. 2010;17:190-7.

4. Begum AN, Jones MR, Lim GP, Morihara T, Kim P, Heath DD, Rock CL, Pruitt MA, Yang F, Hudspeth B, Hu S, Faull KF, Teter B, Cole GM, Frautschy SA. Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. J Pharmacol Exp Ther. 2008;326:196-208. doi: 10.1124/jpet.108.137455.

5. Gupta SC, Kismali G, Aggarwal BB. Curcumin, a component of turmeric: from farm to pharmacy. Biofactors. 2013;39:2-13. doi: 10.1002/biof.1079.

6. Ejaz A, Wu D, Kwan P, Meydani M. Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. J Nutr. 2009;139:919-25. doi: 10.3945/jn.108.100966.

7. Cao Y. Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nat Rev Drug Discov. 2010;9:107-15. doi: 10.1038/nrd3055.

8. Beavers KM, Beavers DP, Newman JJ, Anderson AM, Loeser RFJr, Nicklas BJ, Lyles MF, Miller GD, Mihalko SL, Messier SP. Effects of total and regional fat loss on plasma CRP and IL-6 in overweight and obese, older adults with knee osteoarthritis. Osteoarthritis Cartilage. 2015;23:249-56. doi: 10.1016/j.joca.2014.11.005.

9. Latham JR, Pathirathna S, Jagodic MM, Choe WJ, Levin ME, Nelson MT, Lee WY, Krishnan K, Covey DF, Todorovic SM, Jevtovic-Todorovic V. Selective T-type calcium channel blockade alleviates hyperalgesia in ob/ob mice. Diabetes. 2009;58:2656-65.

10. Martin B, Ji S, Maudsley S, Mattson MP. "Control" laboratory rodents are metabolically morbid: why it matters. Proc Natl Acad Sci. 2010;107:6127-33. doi: 10.1073/pnas.0912955107.

11. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA. 2014;311:806-14. doi: 10.1001/jama.2014.732.

12. Anstey K, Cherbuin N, Budge M, Young J. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obes Rev. 2011;12:426-37. doi: 10.1111/j.

13. Deckers K, van Boxtel MP, Schiepers OJ, de VM, Muñoz Sánchez JL, Anstey KJ, Brayne C, Dartigues JF, Engedal K, Kivipelto M, Ritchie K, Starr JM, Yaffe K, Irving K, Verhey FR, Köhler S. Target risk factors for dementia prevention: a systematic review and Delphi consensus study on the evidence from observational studies. Int J Geriatr Psychiatry. 2015;30:234-46. doi: 10.1002/gps.4245.

14. García-Ptacek S, Faxén-Irving G, Cermáková P, Eriksdotter M, Religa D. Body mass index in dementia. Eur J Clin Nutr. 2014;68:1204-9. doi: 10.1038/ejcn.2014.199.

15. Dubey A, Forster M, Lal H, Sohal R. Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. Arch Biochem Biophys. 1996;333:189-97.

16. Spangler E, Long J, Bennett K, Marshall M, Minor R, de Cabo R. Will calorie restriction stave off age-related brain dysfunction, specifically to learning and memory? A review and

critique of the rodent literature. In: Everitt A, Rattan S, Couteur D, de Cabo R, editors. Calorie restriction, Aging and Longevity. London: Springer; 2010. p. 177-90.

17. Xu WL, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. Neurology. 2011;76:1568-74. doi: 10.1212/WNL.0b013e3182190d09.

18. Gupta A, Mayer EA, Sanmiguel CP, Van Horn JD, Woodworth D, Ellingson BM, Fling C, Love A, Tillisch K, Labus JS. Patterns of brain structural connectivity differentiate normal weight from overweight subjects. Neuroimage Clin. 2015;7:506-17. doi: 10.1016/j.nicl.2015.01.005.

19. Sweat V, Starr V, Bruehl H, Arentoft A, Tirsi A, Javier E, Convit A. C-reactive protein is linked to lower cognitive performance in overweight and obese women. Inflammation. 2008;31:198-207. doi: 10.1007/s10753-008-9065-3.

20. Weuve J, Ridker PM, Cook NR, Buring JE, Grodstein F. High-sensitivity C-reactive protein and cognitive function in older women. Epidemiology. 2006;17:183-9.

21. Lekander M, von EJ, Schultzberg M, Andreasson AN, Garlind A, Hansson LO, Nilsson LG. Cytokines and memory across the mature life span of women. Scand J Psychol. 2011;52:229-35. doi: 10.1111/j.1467-9450.2010.00865.

22. Arfanakis K, Fleischman DA, Grisot G, Barth CM, Varentsova A, Morris MC, Barnes LL, Bennett DA. Systemic inflammation in non-demented elderly human subjects: brain microstructure and cognition. PLoS One. 2013;8doi: 10.1371/journal.pone.0073107.

23. Guerreiro RJ, Santana I, Brás JM, Santiago B, Paiva A, Oliveira C. Peripheral inflammatory cytokines as biomarkers in Alzheimer's disease and mild cognitive impairment. Neurodegener Dis. 2007;4:406-12.

24. Kim YW, West XZ, Byzova TV. Inflammation and oxidative stress in angiogenesis and vascular disease. J Mol Med (Berl). 2013;91:323-8. doi: 10.1007/s00109-013-1007-3.

25. West XZ, Malinin NL, Merkulova AA, Tischenko M, Kerr BA, Borden EC, Podrez EA, Salomon RG, Byzova TV.
Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands. Nature. 2010;467:972-6. doi: 10.1038/nature09421.

26. Chaudhari N, Talwar P, Parimisetty A, Lefebvre d C, Ravanan P. A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. Front Cell Neurosci. 2014;8:213. doi: 10.3389/fncel.2014.00213.

27. Bermejo P, Martín-Aragón S, Benedí J, Susín C, Felici E, Gil P, Ribera JM, Villar AM. Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from Mild Cognitive Impairment. Free Radic Res. 2008;42:162-70. doi: 10.1080/10715760701861373.

28. Torres LL, Quaglio NB, de Souza GT, Garcia RT, Dati LM, Moreira WL, Loureiro AP, de Souza-Talarico JN, Smid J, Porto CS, Bottino CM, Nitrini R, Barros SB, Camarini R, Marcourakis T. Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis. 2011;26:59-68. doi: 10.3233/JAD-2011-110284.

29. Dixit S, Bernardo A, Walker JM, Kennard JA, Kim GY, Kessler ES, Harrison FE. Vitamin C deficiency in the brain impairs cognition, increases amyloid accumulation and deposition, and oxidative stress in APP/PSEN1 and normally aging mice. ACS Chem Neurosci. 2015doi: 10.1021/cn500308h.

30. Mazzetti AP, Fiorile MC, Primavera A, Lo BM. Glutathione transferases and neurodegenerative diseases. Neurochem Int. 2015;82C:10-8. doi: 10.1016/j.neuint.2015.01.008.

31. Turturro A, Witt WW, Lewis S, Hass BS, Lipman RD, Hart RW. Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. J Gerontol A Biol Sci Med Sci. 1999;54:B492-501.

32. deFiebre NC, Sumien N, Forster MJ, deFiebre CM. Spatial learning and psychomotor performance of C57BL/6 mice: age sensitivity and reliability of individual differences.. Age. 2006;28:235. doi: 10.1007/s11357-006-9027-3.

33. Sumien N, Sims MN, Taylor HJ, Forster MJ. Profiling psychomotor and cognitive aging in four-way cross mice. Age (Dodr). 2006;28:265-82. doi: 10.1007/s11357-006-9015-7.

34. Forster MJ, Lal H. Within-subject behavioral analysis of recent memory in aging mice.. Behav Pharmacol. 1992;3:337-49.

35. McDonald S,R., Sohal R,S., Forster M,J. Concurrent administration of coenzyme Q10 and alpha-tocopherol improves learning in aged mice. Free Radic Biol Med. 2005;38:729-36.

36. Thangthaeng N, Sumien N, Forster MJ. Dissociation of functional status from accrual of CML and RAGE in the aged mouse brain. Exp Gerontol. 2008;43:1077-85. doi: 10.1016/j.exger.2008.08.045.

37. Chaudhari K, Wong M J, Vann HP, Sumien N. Exercise training and antioxidant supplementation independently improve cognitive function in adult male and female GFAP-APOE mice. J Sport Health Sci. 2014;3:196-205. doi: dx.doi.org/10.1016/j.jshs.2014.04.004.

38. Sohal RS, Forster MJ. Caloric restriction and the aging process: a critique. Free Radic Biol Med. 2014;73:366-82. doi: 10.1016/j.freeradbiomed.2014.05.015.

39. Rebrin I, Forster MJ, Sohal RS. Association between life-span extension by caloric restriction and thiol redox state in two different strains of mice. Free Radic Biol Med. 2011;51:225-33. doi: 10.1016/j.freeradbiomed.2011.04.006.

40. Pires RC, Souza EE, Vanzela EC, Ribeiro RA, Silva-Santos JC, Carneiro EM, Boschero AC, Amaral ME. Short-term calorie restriction improves glucose homeostasis in old rats: involvement of AMPK. Appl Physiol Nutr Metab. 2014;39:895-901. doi: 10.1139/apnm-2013-0520.

41. Harvie M, Pegington M, Mattson M, Frystyk J, Dillon B, Evans G, Cuzick J, Jebb S, Martin B, Cutler R, Son T, Maudsley S, Carlson O, Egan J, Flyvbjerg A, Howell A. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease riskmarkers: a randomized trial in young overweight women. Int J Obes (Lond). 2011;35:714-27. doi: 10.1038/ijo.2010.171.

42. Forster MJ, Sohal BH, Sohal RS. Reversible effects of long-term caloric restriction on protein oxidative damage. J Gerontol A Biol Sci Med Sci. 2000;55:B522-9.

43. Forster M, Lal H. Estimating age-related changes in psychomotor function: influence of practice and of level of caloric intake in different genotypes. Neurobiol Aging. 1999;20:167-76.

44. Spindler SR. Rapid and reversible induction of the longevity, anticancer and genomic effects of caloric restriction. Mech Ageing Dev. 2005;126:960-6.

45. Burke LE, Steenkiste A, Music E, Styn MA. A descriptive study of past experiences with weight-loss treatment. J Am Diet Assoc. 2008;180:640-7. doi: 10.1016/j.jada.2008.01.012.

46. Madeo F, Pietrocola F, Eisenberg T, Kroemer G.

Caloric restriction mimetics: towards a molecular definition. Nat Rev Drug Discov. 2014;13:727-40. doi: 10.1038/nrd4391.

47. Pu Y, Zhang H, Wang P, Zhao Y, Li Q, Wei X, Cui Y, Sun J, Shang Q, Liu D, Zhu Z.

Dietary curcumin ameliorates aging-related cerebrovascular dysfunction through the AMPK/uncoupling protein 2 pathway. Cell Physiol Biochem. 2013;32:1167-77. doi: 10.1159/000354516.

48. Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P, Kwun I, Shen C.

Novel insights of dietary polyphenols and obesity. J Nutr Biochem. 2014;25:1-18. doi: 10.1016/j.jnutbio.2013.09.001.

49. Kim T, Davis J, Zhang AJ, He X, Mathews ST.

Curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells. Biochem Biophys Res Commun. 2009;388:377-82. doi: 10.1016/j.bbrc.2009.08.018.

50. Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem. 2004;279:48487-90.

51. Lee BC, Lee J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim Biophys Acta. 2014;1842:446-62. doi: 10.1016/j.bbadis.2013.05.017.

52. Banerjee M, Tripathi L, Srivastava V, Puri A, Shukla R.

Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. Immunopharmacol Immunotoxicol. 2003;25:213-24.

53. Wang J, Vanegas S, Du X, Noble T, Zingg J, Meydani M, Meydani S, Wu D.

Caloric restriction favorably impacts metabolic and immune/inflammatory profiles in obese mice

but curcumin/piperine consumption adds no further benefit. Nutr Metab (Lond). 2013;10doi: 10.1186/1743-7075-10-29.

54. Shen LR, Xiao F, Yuan P, Chen Y, Gao QK, Parnell LD, Meydani M, Ordovas JM, Li D, Lai CQ. Curcumin-supplemented diets increase superoxide dismutase activity and mean lifespan in Drosophila. Age (Dordr). 2013;35:1133-42. doi: 10.1007/s11357-012-9438-2.

55. Gupta S, Kumar B, Nag T, Agrawal S, Agrawal R, Agrawal P, Saxena R, Srivastava S. Curcumin prevents experimental diabetic retinopathy in rats through its hypoglycemic, antioxidant, and anti-inflammatory mechanisms. J Ocul Pharmacol Ther. 2011;27:123-30. doi: 10.1089/jop.2010.0123.

56. Bradford P. Curcumin and Obesity. Biofactors. 2013;39:78-87. doi: 10.1002/biof.1074.

57. Taylor AW, Ku NO, Mortensen RF. Regulation of cytokine-induced human C-reactive protein production by transforming growth factor-beta. J Immunol. 1990;145:2507-13.

58. Cohen AN, Veena MS, Srivatsan ES, Wang MB. Suppression of interleukin 6 and 8 production in head and neck cancer cells with curcumin via inhibition of Ikappa beta kinase. Arch Otolaryngol Head Neck Surg. 2009;135:190-7. doi: 10.1001/archotol.135.2.190.

59. Calabró P, Willerson J, Yeh E. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation. 2003;108:1930-2.

60. Trujillo J, Granados-Castro LF, Zazueta C, Andérica-Romero AC, Chirino YI, Pedraza-Chaverrí J. Mitochondria as a target in the therapeutic properties of curcumin. Arch Pharm (Weinheim). 2014;347:873-84. doi: 10.1002/ardp.201400266.

61. Tang Y, Chen A. Curcumin eliminates the effect of advanced glycation end-products (AGEs) on the divergent regulation of gene expression of receptors of AGEs by interrupting leptin signaling. Lab Invest. 2014;94:503-16. doi: 10.1038/labinvest.2014.

62. Sykiotis GP, Habeos IG, Samuelson AV, Bohmann D. The role of the antioxidant and longevity-promoting Nrf2 pathway in metabolic regulation. Curr Opin Clin Nutr Metab Care. 2011;14:41-8. doi: 10.1097/MCO.0b013e32834136f2.

63. Priyadarsini KI. Chemical and structural features influencing the biological activity of curcumin. Curr Pharm Des. 2013;19:2093-100.

64. Priyadarsini KI, Maity DK, Naik GH, Kumar MS, Unnikrishnan MK, Satav JG, Mohan H. Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Radic Biol Med. 2003;35:475-84.

65. Sperling RA, Dickerson BC, Pihlajamaki M, Vannini P, LaViolette PS, Vitolo OV, Hedden T, Becker JA, Rentz DM, Selkoe DJ, Johnson KA. Functional alterations in memory networks in early Alzheimer's disease. Neuromolecular Med. 2010;12:27-43. doi: 10.1007/s12017-009-8109-7.

66. Zheng D, Dong X, Sun H, Xu Y, Ma Y, Wang X. The overall impairment of core executive function components in patients with amnestic mild cognitive impairment: a cross-sectional study. BMC Neurol. 2012;12:138. doi: 10.1186/1471-2377-12-138.

67. Buckner RL. Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron. 2004; 44:195-208.

FIGURE 1 Body weight (left ordinate) and estimated food intake (right ordinate) over a span of 12 weeks of dietary treatment. Data are means ±SEM, n=18-19, for: AL, base diet fed ad libitum; CR, base diet restricted to 70% AL, CURAL, curcumin in base diet fed AL. Letters indicate differences, *P* < 0.05: (a) different from AL, (b) different from CURAL.
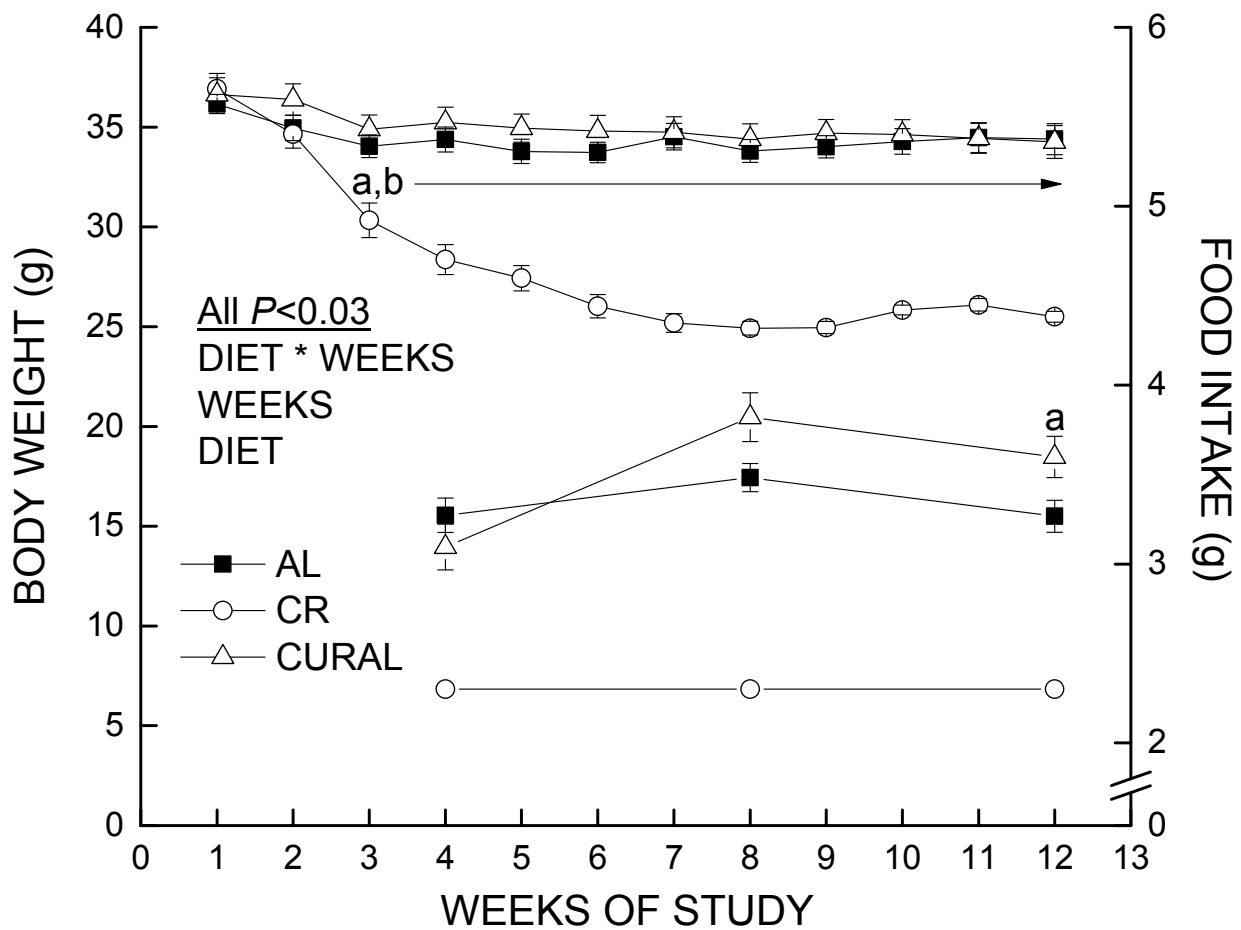
FIGURE 2 Adipose tissue weights after 12 weeks of dietary treatment. Data are means ± SE, n=18-19. Visceral adipose tissue (VAT) was collected from epididymis and, subcutaneous adipose tissue SAT was collected from the gluteal region. Letters indicate differences, $P < 0.05$: (a) different from AL, (b) different from CURAL.
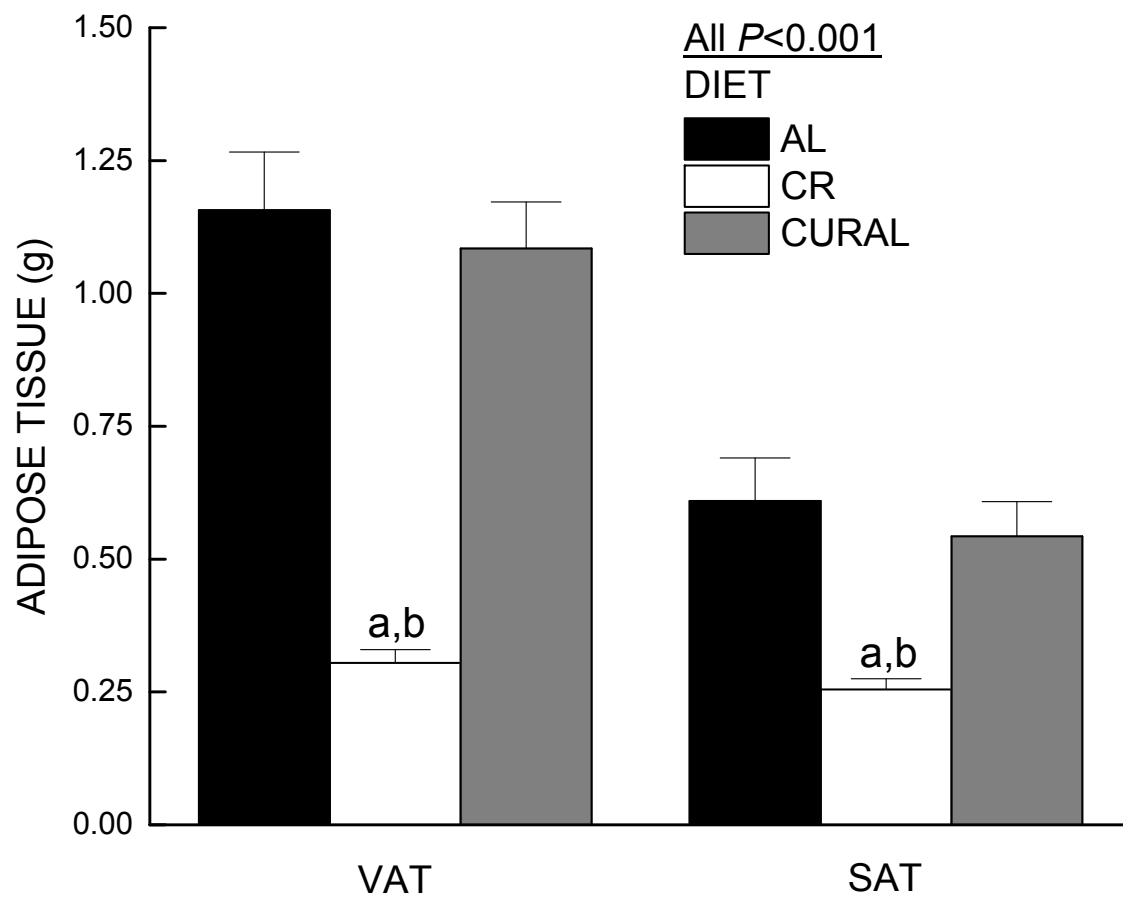
FIGURE 3 Serum concentration of C-reactive protein as a function of diet group. Data represent the average of measurements at 8 and 12 weeks of treatment. Bars represent means +SE, n=8-10. (a) *P*<0.05 when compared with AL
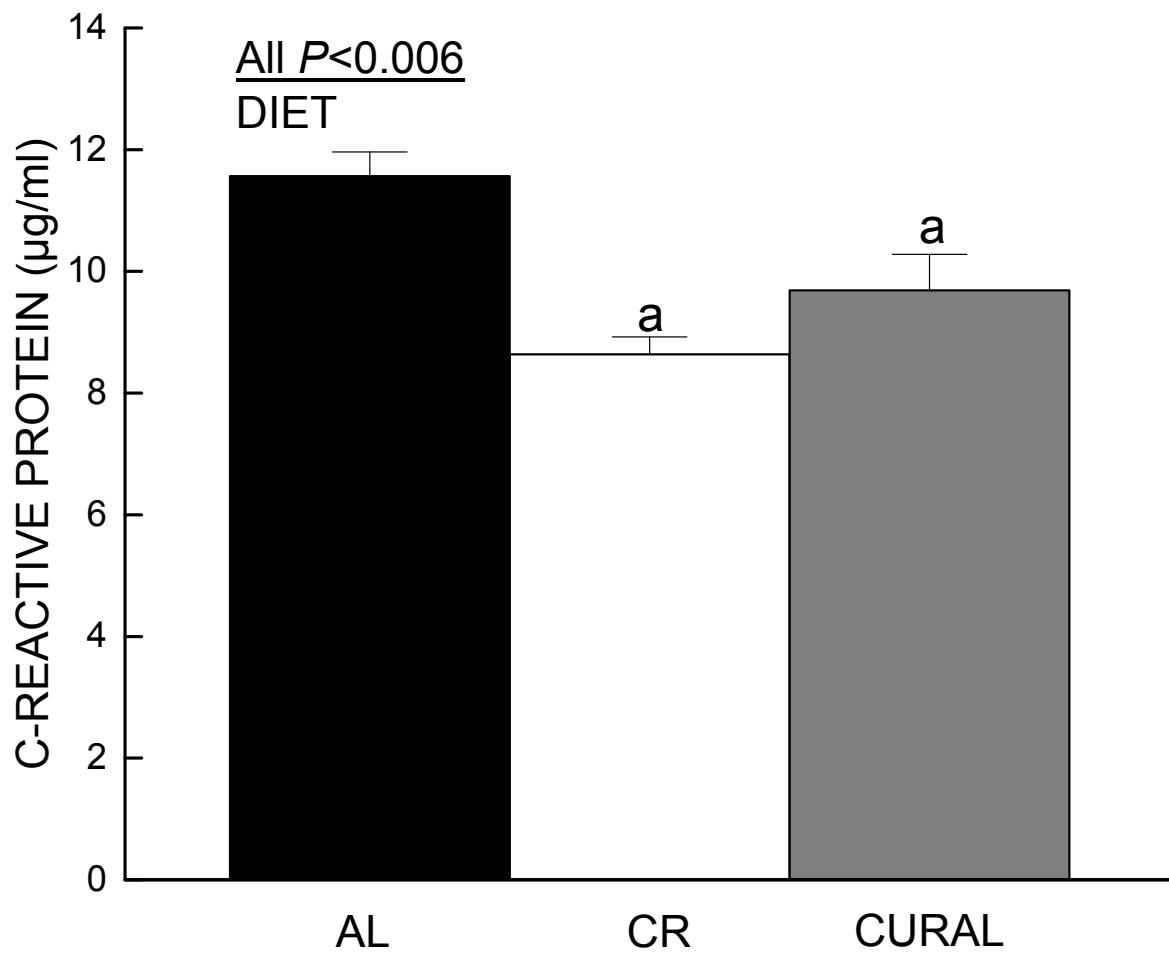
FIGURE 4 Concentration of (A) reduced glutathione, (B) GSH/GSSG and (C) oxidized glutathione in erythrocytes after 8 weeks of dietary treatment. Bars represent means ± SE, n=8-10. Letters indicate differences, $P < 0.05$: (a) different from CURAL, (c) different from CR.

A

All *P*<0.05
DIET

GSH (μmol/mg protein)

a,c

AL   CR   CURAL

B

All *P*<0.05
DIET

GSH/GSSG

a,c

AL   CR   CURAL

C

GSSG (μmol/mg protein)

AL   CR   CURAL
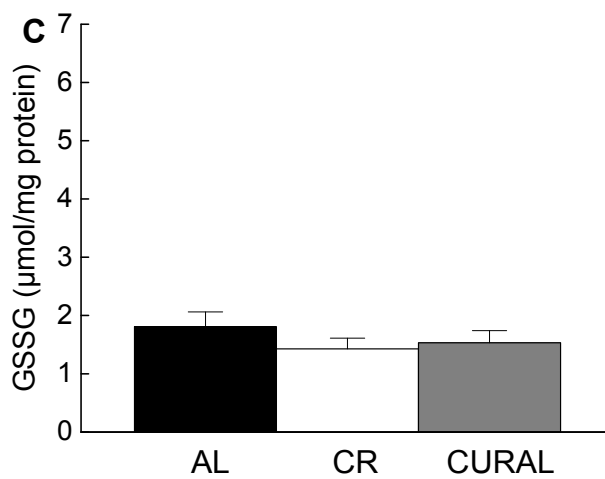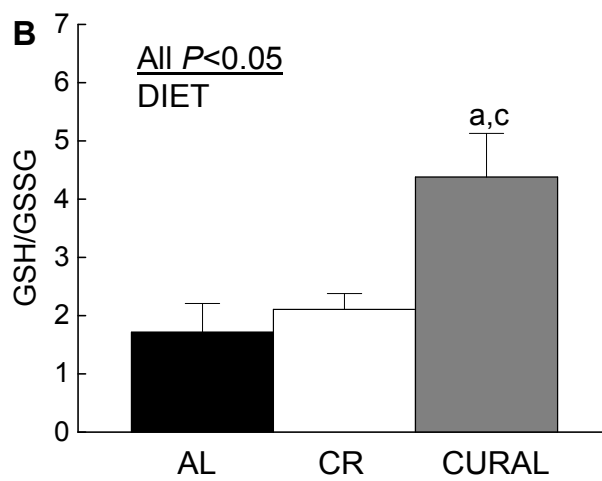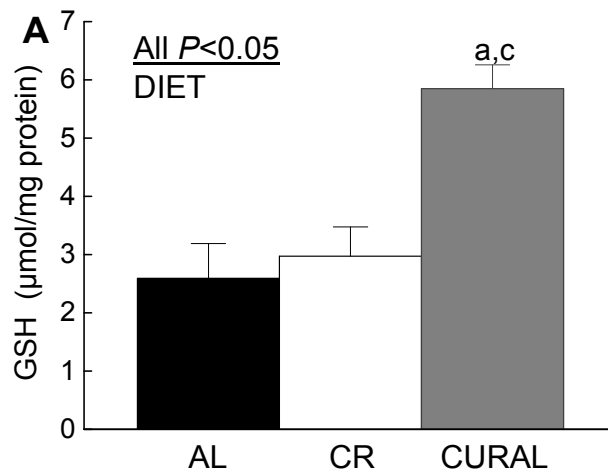
FIGURE 5 (A) Effect of dietary treatment on the efficiency to locate the hidden platform (path length) as a function of training sessions. (B) Effect of treatments on learning index defined as the average of the path length recorded from each mouse during sessions 2, 3 and 4. All data represent means ± SE, n=18-19.

A

All *P*<0.001
SESSION
- ■ AL
- ○ CR
- △ CURAL

y-axis: PATHLENGTH (cm), 200 – 1200

x-axis: SESSIONS, 1 2 3 4 -- -- 5 6 7 8 9
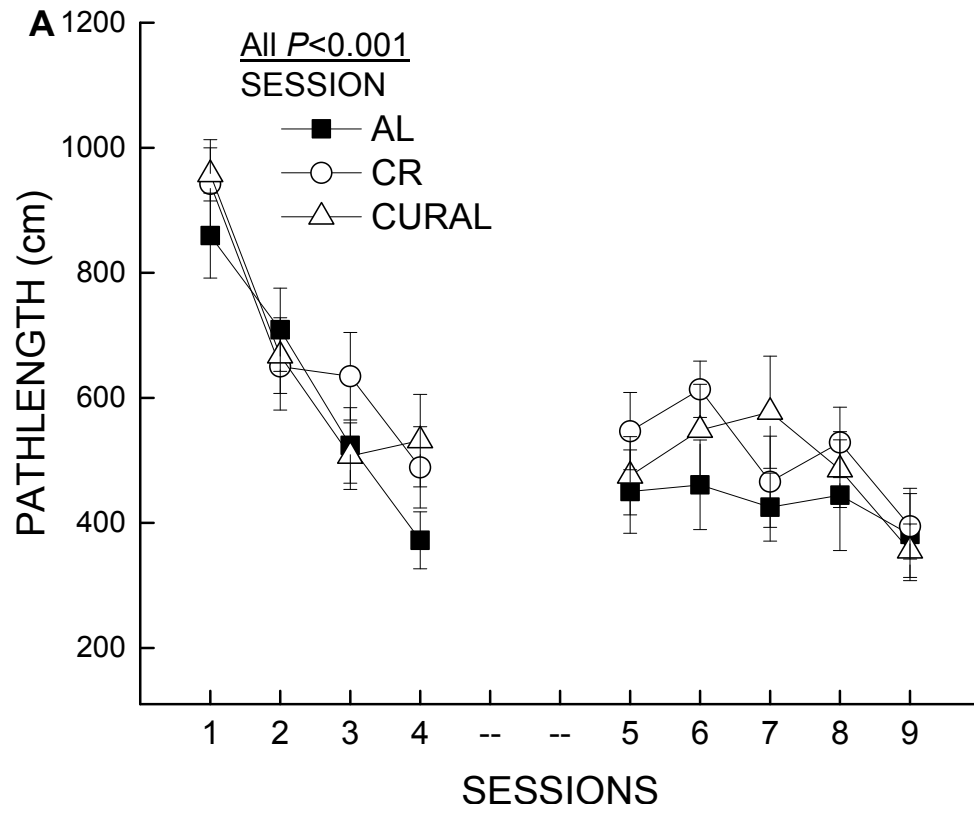
FIGURE 6 Effect of dietary treatment on learning and reversal of an active avoidance response to one of two locations in a T-maze. During the first session mice learned the avoidance response, and reversals of the correct location occurred during two subsequent sessions which were combined (AVG) to assess cognitive flexibility. All data are expressed as the mean number of trials (± SE) needed to meet the correct avoidance criterion, n=18-19. Letters indicate differences, *P* < 0.03: (a) different from AL, (b) different from CURAL.
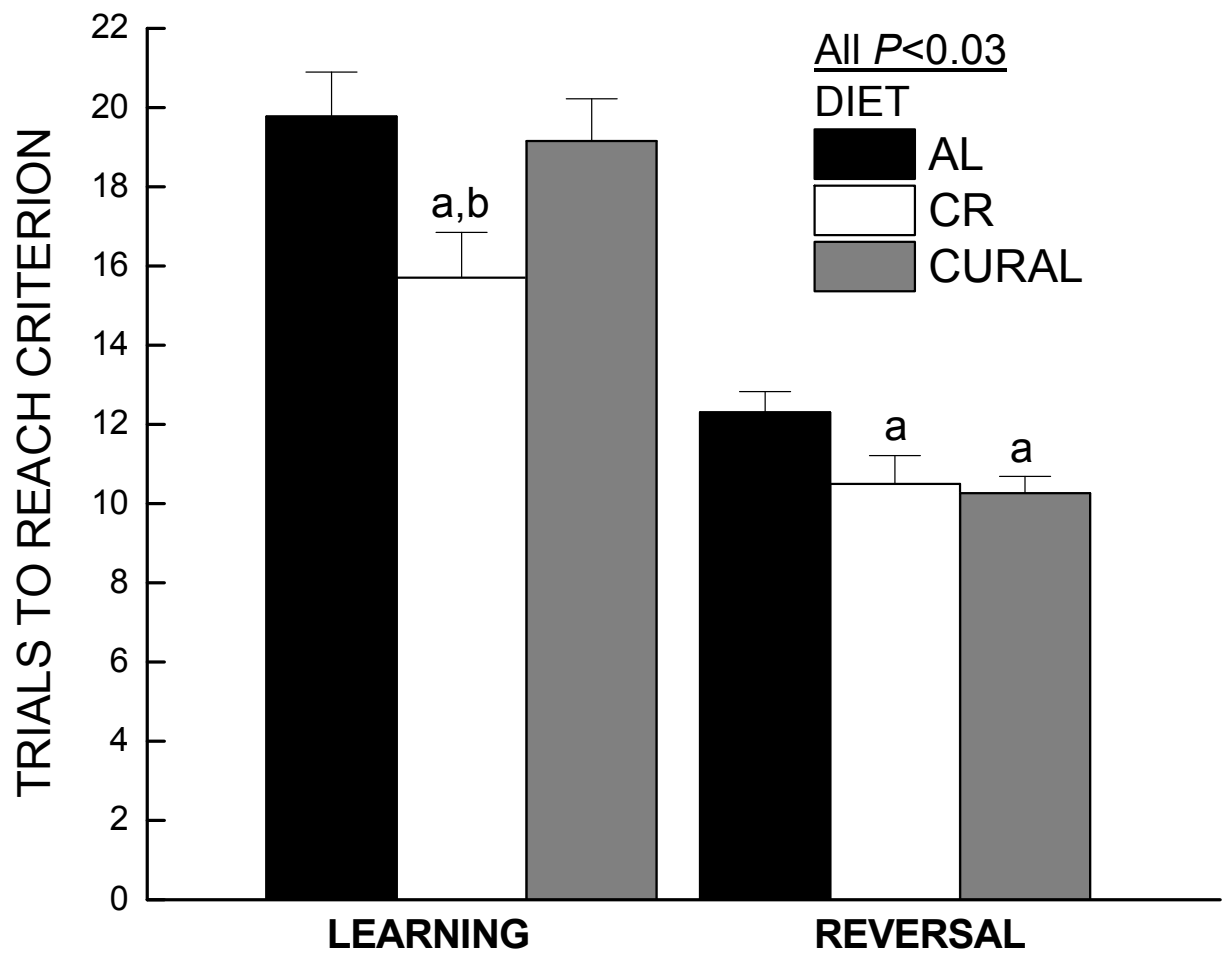
Ingredient (g/kg diet)	AL[1]	CR[1]	CURAL[1,2]
Casein	140.0	140.0	140.0
Curcumin	0	0	1.0
L-Cystine	1.8	1.8	1.8
Sucrose	100.0	100.0	100.0
AIN 93M Mineral mix	35.0	35.0	35.0
AIN 93M Vitamin mix	10.0	20.0	10.0
Corn starch	448.0	455.0	458.0
Cellulose	50.0	50.0	50.0
Choline bitartarate	2.5	2.5	2.5
Soybean oil	40.0	40.0	40.0
Maltodextrin	155.0	155.0	155.0

[1.] All diets were formulated and prepared at Test Diet, Richmond, IN

[2.] Curcumin (C1386) was purchased in powder form from Sigma-Aldrich, St. Louis, MO and sent to Test Diets to be added to CURAL diet

Supplemental Table 1: Composition of control (AL), CR and curcumin (CURAL) diets.

	AL	CR	CURAL
Adipose tissue			
Visceral (g)	1.16±0.11	0.31±0.03[1]	1.01±0.09
Subcutaneous (g)	0.61±0.08	0.26±0.02[1]	0.54±0.07
Biochemical markers			
IL-6 (pg/ml)	13.0±3.38	31.2±7.21	21.0±5.73
CRP (µg/ml)	11.9±0.86	10.0±0.73[1]	9.18±0.44[1]
GSH (µmol/mg protein)	2.59±0.60	2.97±0.50	5.85±0.41[1]
GSSG (µmol/mg protein)	1.81±0.25	1.43±0.18	1.53±0.21
GSH:GSSG	1.72±0.50	2.11±0.30	4.38±0.75[1]
Memory			
Learning Index (cm)	549±32.7	591±42.2	569±41.7
Acquisition (trials)	19.8±1.13	15.7±1.14[1]	19.2±1.07
Reversal (trials)	12.3±0.53	10.5±0.71[1]	10.3±0.43[1]

[1] $P < 0.05$ compared with AL

Supplemental Table 2: Effect of diet on adipose tissue, inflammation, oxidative stress and memory

FIGURE 3: Proposed shared anti-oxidant mechanism of action for CR and curcumin for a robust additive effect to prevent or ameliorate normal aging cognitive decline.

Curcumin: conformational change of Keap 1

Caloric restriction: mild nutrition deprivation causes stress

Caloric restriction + Curcumin Possible additive effect?

Keap1

cytoplasm

P Nrf2 P

Nrf2

AREs

nucleus

ROS → IKKβ

NF-κB

NF-κB

Transcription of inflammatory markers and mediators

Antioxidant defense: Up regulation of glutathione synthesis and glutathione S-transferase

Dorsolateral

Orbitofrontal

Prefront Cortex

Decreased oxidative stress
Decreased inflammation
Improved fronto-cortical function

CHAPTER III

Dietary curcumin and caloric restriction improved age associated neurobehavioral functionality in mice in a sex dependent manner

Marjana Rahman Sarker[1], Susan Franks[2], Nathalie Sumien[1], Michael Forster[1,3]

[1] Department of Pharmacology and Neuroscience, Institute for Aging and Alzheimer's disease Research University of North Texas Health Science Center, Fort Worth, TX 76107

[2] Family Medicine, Texas College of Osteopathic Medicine, University of North Texas Health Science Center, Fort Worth, TX 76107

[3] Corresponding author, mailing address: CBH 512, 3500 Camp Bowie, Fort Worth TX 76107, Tel no: 8177352092 Fax no: 8177350408, email: Michael.Forster@unthsc.edu

Abstract

Preclinical studies of curcumin and caloric restriction addressed whether or not these interventions could ameliorate age-associated cognitive or psychomotor impairment and if they could be combined for additive or synergistic benefit. C57BL/6J male and female mice received testing for functional end points during late middle age (MAG) (15 months) or early senescence (AG) (20 months) after assignment to one of 4 diet groups: (i) base diet ad libitum (AL), (ii) weight stable caloric restriction (CR), (iii) curcumin in the base diet (7200 mg/kg diet) (CURAL) or (iv) curcumin plus CR (CURCR). The curcumin dose in the CURCR group included a small adjustment to equate curcumin intake in CURCR and CURAL groups on the basis of body weight. When tested after 8 weeks of treatment, cognitive flexibility, measured as reversal performance, was significantly better for MAG males CURAL compared to AL, but not under MAG males CURCR, suggesting an antagonistic interaction. On the other hand, MAG females under CR, CURAL and CURCR did significantly better than AL, suggesting that a negative interaction was not present in this sex. Curcumin alone (CUR) improved reversal performance in AG females, whereas CR was ineffective in the aged mice. None of the treatments led to significant improvement in navigation efficiency on a water maze test of spatial memory, however, MAG CURCR males performed poorly in a probe test, further suggesting a negative interaction of these treatments in MAG males. There was no apparent effect of diet on spontaneous locomotion and rearing, although mice on CURCR and CR for both sexes and ages displayed improved performance in psychomotor tests of coordinated running, bridge walking and wire suspension. These results suggest that, when implemented separately, CR and CURAL have an ameliorative effect on impaired frontal cortical function present in late middle age. These effects were similar across different behavioral tasks and were non-interactive or antagonistic, suggesting that they could involve similar mechanisms. It is noteworthy that the combination treatment has a significant antagonistic effect on cognitive performance on MAG males but not females, and that both interventions alone and combined became less effective when implemented during early senescence when compared with middle

age. Overall, the results indicate that curcumin intake mimics some of the beneficial effects of

CR in the absence of diminished energy intake.

Introduction

Clinical data support the hypothesis that the accrual and persistence of chronic inflammation and oxidative stress during middle age are significant factors in the development of cognitive and psychomotor dysfunction during aging. Epidemiological evidence has further suggested that modifiable lifestyle-related factors such as diet and lack of exercise are associated with cognitive and motor decline, opening new avenues for prevention (Everitt et al. 2006; Caracciolo et al. 2014; Vel Szic et al. 2015). Diet, in particular, has become the object of intense research in relation to cognitive aging and neurodegenerative diseases (Everitt et al. 2006; Koh et al. 2015; Harrison et al. 2015; Bun et al. 2015). Caloric restriction, an extensively studied dietary intervention has widely been investigated as an anti-aging therapy; its downstream main effects far exceed just the simple body weight reduction, including a lowering of chronic inflammation and oxidative stress, both of which have been independently implicated as causative factors for age associated cardiovascular and neurodegenerative diseases (McGeer and McGeer 1999; Carmeli et al. 2002; Balaban et al. 2005; De la Fuente et al. 2005). Several studies have reported an improvement in behavioral tests in different Alzheimer's disease (AD) and Parkinson's disease (PD) mouse models when rodents were subjected to 30-40% CR (Martin et al. 2006; Murphy et al. 2014). However, the CR paradigm has been difficult to translate to a clinical setting due to the requirements of changing eating behavior and modifying food choices (Thomas et al. 2014; Allison et al. 2014). Based on our previous observation that curcumin had CR-like beneficial effects on cognition, markers of inflammation and redox stress without lowering food intake or body weight (Sarker et al. 2013), intake of curcumin would seem to be a highly desirable alternative to CR. The current study was conducted to determine if dietary curcumin, a polyphenolic compound derived from the naturally available Indian spice turmeric: (i) can improve both spatial and executive aspects of cognition, and (ii) improve age-associated motor and sensory impairment via attenuation of inflammation and oxidative stress. A significant anti-inflammatory action of curcumin has been well established in studies of obesity and arthritis

as well as neurodegenerative disorders such as Alzheimer's (AD) and Hungtington's (Anand et al. 2008; Begum et al. 2008; Hickey et al. 2012; Wang et al. 2014a; Wang et al. 2014b). Curcumin's strong anti-oxidant effects have also been well documented (Trujillo et al. 2014; Tang and Chen 2014). However, there are no reports on whether curcumin can prevent or reverse normal age-associated neurobehavioral dysfunction. Curcumin was previously tested in our laboratory as a dietary intervention to prevent obesity-associated cognitive impairment in middle-aged male mice and has recently been suggested to be a mimetic for caloric restriction (Madeo et al. 2014).

Existing literature on cognitive status in the elderly suggests mild cognitive impairment during late mid-life to be a strong predictor for developing late onset dementia. Given the long preclinical phase of AD, the diagnosis of non-cognitive impairments such as motor deficits as well as other subtle cognitive discrepancies during that period can lead to earlier detection of pathological cognitive decline (Buchman and Bennett 2011). This early detection can also provide a potentially bigger window for therapeutic intervention. Interestingly, with normal aging, significant cognitive deficits in executive and long-term memory manifest at early senescence, around 65-70 years (Buckner 2004), which possibly is the result of chronic redox stress and inflammation suffered throughout middle age. Further, studies have suggested pharmacological and other interventions to lose or have minimal beneficial effects when implemented later in life (Means et al. 1993; Kayo et al. 2001). In order to be able to better translate the findings from the current preclinical study, the age of mice at which the interventions were implemented were based on data that indicate middle age to be the ideal period to intervene to prevent the development of dementia (Caracciolo et al. 2014). Thus, the current study included 15- and 20-month-old mice that roughly translate to 55 and 70 human years, to model treatment starting at late middle age or early senescence.

An important goal of the current study was to provide pivotal information on sex differences related to normal aging and the neurobehavioral functional effects of dietary interventions. There

is increasing attention to differences between sexes and sexual dimorphism in the causes, manifestations, response to treatments, and outcomes of neurological diseases (Pletzer et al. 2013; Pletzer 2014; Rocca et al. 2014). However historically, this attention to sexual dimorphism influenced medicine has been stronger in fields like cancer, cardiovascular diseases, and endocrine diseases (Ober et al. 2008; Miller 2014). There is now a growing awareness of differences in brain structure and function between sexes throughout the entire life course. It is now evident that various neural mechanisms and susceptibility to diseases such as AD and PD differ for sexes. Further, sex steroids such as estrogen and testosterone not only reduce with aging but lower levels of it have different downstream effects related to neurobehavioral function(Wise 2002; Choi and Silverman 2002; Sanz et al. 2007; Golden et al. 2007; Cunningham et al. 2014). Therefore, in addition to using appropriate ages for the implementation of the dietary interventions, this study also included both males and females, addressing a clear deficiency in the number of CR studies, and preclinical intervention studies in general, that include female subjects.

Although there is no curative treatment at the moment for age associated neurodegenerative disorders, epidemiological research provides a substantial amount of evidence for modifiable risk factors, which if addressed during mid-life can prevent or delay the onset of serious functional decline (Akbaraly et al. 2013). While earlier studies have established positive effects of CR on age associated functional decline (Gemma et al. 2007; Spangler et al. 2010; Yang et al. 2014) (reviewed by Spangler et al., 2010), there is a lack of research on the effect of curcumin on a normal aging model. Furthermore, it is unclear whether curcumin and CR implemented concurrently would have additive or synergistic beneficial effects and also lead to greater improvements than either intervention alone. The current study was therefore conducted to address whether these dietary interventions implemented during middle age and late life can prevent or ameliorate cognitive and motor dysfunction respectively.

Materials and methods

Animals and diets

Fifteen and twenty-month-old C57BL/6J male (M) and female (F) mice, maintained at the National Institute on Aging (NIA) colony under ad libitum (AL) feeding were assigned as middle aged and aged (senescent). After shipment, each age group was assigned (in groups of 10 per sex) to (i) remain on AL, (ii) receive 30% caloric restriction gradually until weight stability (CR) or (iii) receive curcumin (*C1386*, Sigma Aldrich, St. Louis, MO) in their AL diet (CURAL) for 12 weeks. The control group (AL) was fed a purified maintenance diet (AIN 93M) AL (*58M1*, Test Diets, Richmond, IN). The CURAL group was fed the same maintenance diet supplemented with 7200mg of curcumin/kg of diet. Another calorically restricted curcumin group received a fortified diet with vitamins and minerals supplemented with 7274 mg of curcumin (CURCR). The curcumin dose in the CURCR group included a small adjustment to equate curcumin intake in CURCR and CURAL groups on the basis of body weight. On the other hand, the vitamin/mineral fortification was targeted to equate CR groups (both CR and CURCR) with AL (CURAL, AL) on a per mouse basis. The mice were maintained under a normal light/dark cycle beginning at 7:00 a.m., with access to water ad libitum and all testing was performed during the light phase of the cycle. All animals were fed AL (AIN 93M) for 2 weeks to acclimate to a purified diet since they were fed a grain-based diet at the NIA contract colonies (Charles River), following which they were randomly assigned to their treatment groups. Body weight was measured once every week. Cognitive and psychomotor tests were conducted after 8 weeks of treatment. Middle aged male mice were not assigned to the CR group to avoid duplication of a recent report from this laboratory (add reference). All animal handling procedures and experiments were approved by the Institutional Animal Care and Use Committee at UNTHSC.

Behavioral measures

The mice were maintained on the diets for a period of 8 weeks, following which they remained on the diets during a 10-week series of behavioral tests which included assessments

102

of: locomotor activity (spontaneous activity), simple reflexes, psychomotor function which included coordinated running, wire suspension and bridge walking, cognitive function which include spatial learning and memory (visuospatial capacity), and discriminated active avoidance (cognitive flexibility). By the end of behavioral testing period, the mice were 19 and 24 months of age respectively. Previous studies using this test battery have suggested that they represent independent functional domains of neurocognitive, psychomotor and reflexive capacity that are sensitive to dysfunction in different systems of the brain (Sumien et al. 2006; de, Fiebre, N,C. et al. 2006).

Locomotor activity (LMA).

Spontaneous locomotor activity was measured using a Digiscan apparatus (Omnitech Electronics, model RXYZCM-16), as described previously (Forster and Lal 1992). Each mouse was placed in a clear acrylic test cage (40.5 x 40.5 x 30.5 cm) that was surrounded by a metal frame lined with photocells. The test cage was enclosed in a dimly-lit, sound-attenuating chamber equipped with a fan that provided background noise (80 dB). During a 16-min period, movements in the horizontal plane as well as a vertical plane 7.6 cm above the floor were detected by the photocells and processed by software to yield 4 different variables describing horizontal, vertical, and spatial components (center and margin) of spontaneous activity in the apparatus.

Simple reflexes

The mice were administered three simple reflex tests over four consecutive sessions. The first test consisted of placing the mouse on a flat smooth surface and recording the latency to move one body length (walk initiation). The second test measured the latency to reverse direction when the mouse was placed in a 3.5-cm wide, 14-cm long, dead-end alley (alley turning). For the third test, the mouse was placed facing downward on a flat surface that was tilted 45°, and the latency to turn 180° in either direction was measured (negative geotaxis).

103

Psychomotor function

Coordinated running.

Maximum running performance was measured using an accelerating rotorod test described previously (Forster and Lal, 1999). The apparatus was a motor-driven treadmill (Accuscan Instruments, Model # AIO411RRT525M) that consisted of a 3-cm diameter nylon cylinder mounted horizontally at a height of 35 cm above a padded surface. On each trial, the mouse was placed on the cylinder, which then began rotating with increasing speed until the animal fell to a well-padded surface. Ability of the mice to improve running performance was assessed in a series of training sessions (two per day), each session consisted of four trials at 10-min intervals. The training sessions continued until the running performance (the average latency to fall from the cylinder) failed to show improvement over three consecutive sessions, which represented each mouse's average stable level of performance (Sumien et al., 2004)

Wire suspension.

Mice were administered a wire suspension test for four consecutive days (2 trials/day). A horizontal steel wire was suspended 27cm above a padded surface. For each trial (lasting a maximum of 60 s), the mouse was suspended with their front paws and the latency (in seconds) for it to return at least one hind paw (tread) to the wire, as well as the time for it to fall was recorded (Sumien et al. 2006).

Bridge walking.

Each mouse was tested for the latency to fall or reach a safe platform after being placed on one of four acrylic bridges, each mounted 50 cm above a padded surface. The bridges differed in diameter (small or large) and shape (round or square), providing four levels of difficulty. Each bridge was presented three times, and the measure of performance was the average latency to fall (up to a maximum of 60 s) across all bridges. The reliability and age sensitivity of this measure has recently been described for large reference groups of C57BL/6 mice tested under

the conditions of this study (de Fiebre et al., 2006)

Cognitive function.

Morris water maze

Spatial learning and memory were measured using a Morris water maze (MWM) test similar to that described previously (de, Fiebre, N,C. et al. 2006; Sumien et al. 2006; Chaudhari et al. 2014). This test provided a measure of the efficiency of mice in swimming to a hidden, safe platform from different locations within a cylindrical tank filled with opacified water. A pre-training phase was conducted in two sessions during which mice did not have access to spatial information (cues) in the testing room, to ensure that the mice had learned the motor components of swimming and climbing onto the platform prior to testing for spatial performance. During subsequent testing, a computerized tracking system recorded the length of the path taken by the mouse to reach the platform, as well as the swimming speed (Any-maze; Stoelting Co., Wood Dale, IL, USA), with spatial cues available in the open tank and the hidden platform in a fixed location. Testing was conducted over nine sessions (1-4, Tuesday-Friday and 5-9, Monday-Friday the following week). Each session consisted of five trials separated by 2 min during which the mouse had to swim to the platform from one of four different starting points. A learning index was calculated as the average path length on sessions 2, 3 and 4, the initial learning phase. Probe trials were conducted immediately following sessions 2, 4, 5, 7, and 9, during which the platform was submerged to a depth that prevented the mice from climbing onto it. The platform was raised after 30 s, and the trial was ended when the mouse successfully located it. The percentage of time spent in a 40 cm diameter annulus around the platform location was calculated as a measure of spatial bias for the platform location. Memory retention for the platform location was tested during one additional session conducted one week after session 9.

Discriminated active avoidance

An acrylic T-maze (black on the sides with a clear top) was used for the discriminated avoidance task (Forster and Lal 1992; McDonald et al. 2005). The maze was divided into three compartments with a stem and goal arms that rested on a grid floor wired to deliver 0.67-mA scrambled shock to the feet of the mice. The test consisted of three sessions separated by 1 h. Each mouse was trained to leave the start box and run to a designated correct goal within 5 s after lifting of the start door. On the first trial of the first session (acquisition), the correct goal arm was designated as the one opposite from the mouse's first arm choice (determined on a separate preference trial). On subsequent trials, shock was initiated 5 s after the opening of the start door if the mouse had not entered the correct goal arm, or immediately upon entry into the incorrect arm. In both cases, shock was continued until the correct goal arm was entered or a maximum of 60 s had elapsed. A correct avoidance trial was recorded when the mouse entered the correct goal arm within 5s of the opening of the start door. The mouse was then allowed to stay in the correct goal arm for 10 s after which the mouse was removed and placed in a holding cage for 1 min. A session ended when the mouse had made a correct avoidance on the last two trials and on at least 4 of the last 5 trials. The second and third sessions of avoidance training were reversals such that the mice were required to run to the goal arm opposite that to which they had previously been trained. Ability to learn the avoidance problem was considered inversely proportional to the number of trials required to reach criterion in each of the sessions. Learning efficiency during the second and third sessions was considered indicative of cognitive flexibility (Thangthaeng et al. 2008; Chaudhari et al. 2014).

Statistical analysis

Based on the independence of the different components of the neurocognitive/psychomotor test battery, the effects of age, diet and sex on performance were considered in separate analyses of variance (ANOVA) in which age, diet and sex were considered as between-groups factors. Because one cell was missing in the overall study (MAG male CR), different ANOVAs were applied to assess main effects and interactions of diet with sex and age. For all

measurements, age, sex and diet were considered in an ANOVA that did not include the CR group, whereas age and all diet groups were considered as factors in a separate analysis for females. Additionally diet (all groups) and sex were included as factors in a separate analysis for the AG groups. The effects of test phase and trials were considered as repeated measures in these analyses where applicable. Planned individual comparisons of each diet group with age and sex matched AL control were conducted using single degree-of-freedom F tests based on the error term from a separate ANOVA for each sex x age group. The alpha level was set at 0.05 for all analyses.

Results

Body weight

Mice on CR and CURCR had notable weight loss compared to age-matched male and female mice on CURAL and AL beginning in week 3, and they continued to lose weight until week 6 after which they maintained stable weight for the rest of the treatment period (Fig.1). CURAL and AL for both sexes and age groups maintained stable body weight throughout their dietary treatment with no body weight difference between the groups at any time point. Females however, weighed less than males in each dietary and age group. Analyses of variance for body weight supported these observations, revealing main effects of diet and sex (all P's<0.01).

Locomotor activity

Total distance (in centimeters) (Fig 2a), rearing or vertical activity (counts) (Table 1&2) and center time (in seconds) (Fig 2b) were measured to analyze spontaneous locomotor activity. Females under both age groups traveled a greater distance and spent more time in the center compared to male mice. Mice under CURCR overall spent more time in the center. There was no effect of diet, age or sex in rearing. There was an overall main effect of sex and diet in the total distance and time spent in the center (all P's<0.05), and a two way ANOVA yielded a significant effect of sex for the aged group (P<0.05). However, there were no significant three way interactions for any of the measures.

Simple reflexes

Measures of reflexive capacity included walk initiation; alley turn and negative geotaxis are in table 1 and 2. There was no significant effect of diet or age in any of the tasks for the female mice, however the middle aged male mice under CURCR displayed a decrement in their ability to turn in the dead end alley task compared to their age matched controls (P<0.05).

Psychomotor function

Coordinated running (maximal performance)

There was an improvement in the performance capacity of both middle aged and aged mice of both sexes over a period of seven sessions; however, both CURCR and CR alone group outperformed the ad libitum fed groups regardless of age or gender. There was an overall main effect of diet, age and sex as a function of sessions (data not shown). Longest latency to fall for an individual mouse was accounted for as a measure of its maximal performance (Fig 3). The data for maximum performance is reflective of performance across sessions for all groups with a main effect of diet, age and sex ($P<0.01$). However, the main effects of diet and sex were lost when body weight was taken as covariate for ANCOVA (all P>0.20)

Wire suspension

Performance of each mouse on the wire suspension test was measured by the average latencies over a period of 4 sessions to tread (not shown) or fall (Fig 4). Aged male mice that underwent dietary interventions stayed on the wire longer and their latency to tread was shorter than their aged matched control. A three way ANOVA yielded a main effect of only diet and none of the 2 way interactions were significant. A one-way ANOVA on aged male mice yielded a significant effect of all three dietary interventions on the latency to fall compared to their age matched control (all P's<0.05). However, the main effects of diet, age and sex were lost when body weight was taken as covariate for ANCOVA (all P>0.10)

Bridge walking

Ability of the mouse to balance on a bridge was measured by the average latency to fall from 4 bridges of differing difficulty (Fig 5). There was no effect of age on the latency to fall. None of the dietary interventions improved balance for the females regardless of their age. For the aged males, both mice on CR and CURCR on average stayed on the bridge longer compared to their age matched control. Only CURCR middle-aged male mice had better performance compared

109

to their age matched controls ($P<0.005$). A three-way ANOVA confirmed a significant main effect of diet ($P < 0.002$), and yielded no main effect of age or interaction of age, sex and diet. (All P's > 0.264).

Cognitive function

Morris water maze

The distance taken to reach the platform, regardless of the swim speed of the mouse, was assessed to indicate efficiency of locating the hidden platform. Path lengths of middle aged and aged mice decreased as a function of sessions during the acquisition phase (Fig not shown). Analysis of the data confirmed the effect of testing session on path length as repeated measures for the acquisition phase being different from the performance during the second week ($P<0.05$). There was also an overall effect of sex, males of both ages had a shorter pathlength as a function of sessions compared to females ($P<0.05$). However, there was no difference in the spatial learning index (Table 1&2) between the groups. Performance during phase 2 or the 2nd week remained relatively stable for all the groups. Further analysis indicated a significant interaction of diet with age, phase or sessions ($P<0.001$). However, planned comparisons did not yield an interaction of diet in any of the sessions. Probe trial data calculated from percent time spent in the 40-cm annulus around the target site confirmed that mice in the different treatment groups had acquired a spatial bias for the platform location that was maintained through session 9, there was no effect of sex, age or diet as a function of probe sessions. A three way ANOVA of the average time spent in the 40cm annulus over 5 probe sessions indicated a significant main effect of diet ($P<0.005$), a further one way ANOVA suggested that middle aged male mice under CURCR significantly spent less time in the 40cm annulus compared to their age matched control ($P<0.01$) (Fig 6). Analysis of the swim speed, independent of path length, from sessions 2-4 and 5-9 did not display any interactions or main effects ($P>0.252$).

Discriminated active avoidance

110

Cognitive flexibility was tested by first establishing a discriminated avoidance response, followed by two additional sessions separated by 1 hour in which the required correct response was reversed. Figure shows the initial learning versus the average number of trials taken during sessions 2 and 3 to reach criterion (reversal) (Fig 7). A four way ANOVA indicated a main effect of diet and age as well as a significant interaction of phase, diet, age and sex ($P<0.01$). Planned comparisons following the four way ANOVA confirmed both middle aged male and female mice fed curcumin ad libitum took fewer trials to reach criterion during the acquisition phase. Mice kept on CR took fewer trials to reach criterion compared to AL and CURAL in the initial learning phase. During the reversal sessions, both the CR and CURAL groups took fewer trials to reach criterion when compared with AL. A two-way ANOVA with diet and phase as the independent variables on the sessions confirmed the mentioned observations, suggesting a main effect of diet (All Ps<0.03) (Fig. 7).

Discussion

The main findings of this study include: (1) there was no main effect of age on any of the cognitive tests, (2) there was no additive effect of the combination CR and curcumin treatment, however some behavioral tests indicated an antagonistic action, (3) CURCR and CR mice weighed significantly less than their age matched controls, (4) females spent more time in the center and traveled greater distance than their male counterparts, (5) mice on CR and CURCR had better motor function compared to their age matched controls, (6) middle aged males and females on curcumin had better cognitive flexibility than their aged matched controls, however CR failed to better reversal performance in the aged and (7) spatial learning was not affected by any of the dietary interventions however middle aged male mice under CURCR had a poor probe performance

An interesting gender difference was noted with spontaneous locomotor activity, wherein female mice regardless of age were more active than males; they traveled greater distances as well as spent more time in the center. These results are in conjunction with earlier reports of aged female mice displaying anxiolytic behavior compared to their male counterparts. Estrogen has been suggested to be one of the primary contributors to this behavior due to the presence of estrogen receptors in the amygdala. Estrogen has been reported to increase levels of brain derived neurotrophic factor (BDNF) in the amygdala and induce anxiolytic effects (Wolf and Kirschbaum 2002).

The current study also included tests for simple reflexes. Ideally, the experimental groups are expected to not display any difference in these tasks. Although decrements in reflexes have been noted in the past, it has normally been reported to be evident between young and older mice (Forster et al. 1996; Sumien et al. 2004). However, middle aged mice under CURCR spent longer time in the alley without turning unlike their age matched controls. This antagonistic impact of the combination treatment in middle aged males is also evident in some of the other tasks.

112

Mice under CR and CURCR displayed better motor function than their age matched controls, and there was no main effect of age. The lack of an age related difference maybe partially due to choosing early senescence (20 months) model than late senescence (24 months). Previous studies have reported age related differences between middle age and late senescence (de, Fiebre, N,C. et al. 2006). The rotorod, as mentioned earlier, is widely used to test motor coordination and learning and is sensitive to cerebellum function. According to aging studies conducted in mice, the cerebellum is the first area of the brain to sustain age-associated deficits. Deficits in the cerebellum have been demonstrated as early as 12-13 months of age and progressively decline from then on (Kennard and Woodruff-Pak 2011). Performance on the bridge test and wire suspension is also partially dependent on cerebellum function since the test requires proper balance and coordination (Forster et al. 1996). This may explain why the functional outcomes from these motor tests display similar trends. Since there was no additive effect detected with the combination treatment and with CR and CURCR having comparable motor performance; body weight may have significantly affected this outcome. Caloric restriction has previously been reported to rescue cerebellum function in normal aging by increasing the GSH: GSSG and thereby protecting it from further oxidative damage (Rebrin et al. 2011).

The results from this study confirm the improvement in cognitive flexibility with dietary curcumin detected in the pilot study conducted in our laboratory. Both middle aged and aged females as well as middle-aged males on curcumin displayed better reversal learning than their age matched controls. Spatial memory, although not affected by dietary intervention or age, certainly displayed a strong sex difference. The sex difference in spatial learning is consistent with other studies and human data where males are reported to have better spatial performance whereas females outperform in object location or recognition memory (Malinowski and Gillespie 2001; Li 2014). Further, the lack of an age-related difference in learning is congruent with findings from a previous study where 16 and 24 month old male mice did not differ in acquisition (Sumien et al. 2006).

The outcomes from this study line up with the critical period hypothesis or the window of opportunity i.e. during midlife during which interventions have the maximum potential of delaying late life decrease in functional capacity (Wilson et al. 2002; Lindstrom et al. 2005). Interestingly, the results from our study show that CR did not impact the cognitive capacity in the aged mice but had a beneficial effect on middle-aged females and males (pilot study) for reversal learning implying improved fronto-cortical function thus signifying the importance of an appropriate intervention period (Sarker et al. 2015). The combination diet in the current study yielded negative cognitive results for both middle aged and aged males, which may be associated to an increase in testosterone levels. Some reports have linked short-term caloric restriction to increased serum testosterone levels (Chen et al. 2005; Schulte et al. 2014; Wahab et al. 2014). Curcumin too has been reported to increase testosterone levels (Abarikwu et al. 2014). Although the effect of testosterone on cognitive function has been controversial, there have been some studies that have reported higher levels or more than optimal levels of endogenous testosterone to be inversely proportional to cognitive function (Wallin and Wood 2015). Results from the Florey Adelaide Male Ageing study, which included more than a thousand male subjects, reported higher levels of total testosterone to be associated with poorer cognitive function in middle to older aged men (Martin et al. 2007).

The differential effect of the combination treatment on females may partially be due to curcumin's stronger antioxidant effect on females as reported by Shen and colleagues (Shen et al. 2013). In addition, estrogen has anti-inflammatory effects (Straub 2007; Viña et al. 2013) and may have aided in the prevention of an antagonistic effect in fronto-cortical functioning. Although, there are only a few studies that have reported on the effects of testosterone therapy on post-menopausal women, they all have reported testosterone to improve different domains of memory which include semantic and verbal (Shah et al. 2006; Ryan et al. 2012). These reports suggest testosterone to have a protective effect on cognition in the absence of estrogen in post-menopausal women and may be responsible for the effect of the combination treatment and

114

curcumin alone on cognitive flexibility in older female mice.

Additionally, the lack of a robust additive effect in the present study can possibly be attributed to CR and curcumin sharing a common mechanism of action to reduce oxidative stress. Caloric restriction has been reported to up regulate endogenous antioxidant synthesis via the NF-E2 related factor (Nrf2) pathway. Curcumin too has been reported to do the same but it utilizes a different mode of Nrf2 activation. Whereas CR induces mild stress that changes the conformation of Keap1, which allows for the nuclear translocation of Nrf2; curcumin has been reported to disrupt the interaction of Keap 1 to Nrf2 (Balogun et al. 2003; Mattson et al. 2007). Although up-regulation of Nrf2 has strong antioxidant effects, a continuous up-regulation may create a cellular environment of reductive homeostatic imbalance that would potentially be conducive to the development of cancers. Indeed, several studies have reported a hyper activation of the Nrf2 pathway associated with tumors (Lau et al. 2008; Jiang et al. 2010; Jaramillo and Zhang 2013; van der Wijst et al. 2015). Further, below par cognitive performance and neurological disorders have also been associated with different types of cancer, independent of chemotherapy (Andreotti et al. 2014). However, this inference should be looked upon with caution because both cancer and aging have multifactorial etiologies and Nrf2 has been reported to have both anti and promutagenic actions (Lau et al. 2008; Geismann et al. 2014). Nonetheless, there have been other reports of clinical trials conducted on combination antioxidant supplementation treatments yielding deleterious cognitive and functional effects (Miller et al. 2005; Galasko et al. 2012), for which the mechanism of action leading to these results is still unknown.

The dose used in this study is roughly equivalent to a human dose of 3g/day for a 60kg person and has a safe toxicity profile (Reagan-Shaw et al. 2008). Doses higher than that used for the current study have been tested in clinical trials for cancer with no adverse side effects reported. Curcumin itself as well as several of its potent analogs are currently being tested for other ailments such as Alzheimer's disease (AD), inflammatory bowel disease, Parkinson's

115

disease where inflammation and oxidative stress have been implicated as causative factors(Goel et al. 2008; Anand et al. 2008; Gupta et al. 2013). Although the anti-inflammatory and anti-oxidant properties of dietary curcumin and CR are well documented and also supported from results of the previous study conducted in our laboratory, the mechanism of action of the combination treatment is still unclear and further exploration is warranted due to distinct gender differences on the functional outcomes in this study.

Advanced aging is accompanied by cognitive and motor decline even in the absence of diseases such as Alzheimer and Parkinson disease. In fact, in humans, older adults exhibit a reduced functional correlation with different brain regions compared to younger adults primarily due to decreased white matter integrity (Buckner 2004; Andrews-Hanna et al. 2007). Dietary curcumin and caloric restriction when implemented in late middle age can alleviate and delay the onset of cognitive deficits and can potentially improve white matter integrity. An earlier intervention period may have yielded better results for motor functioning since as mentioned earlier, cerebellar dysfunction can be detected at only 12-13 months in mice (Kennard and Woodruff-Pak 2011). Further, the inclusion of both genders provides an overall perspective on experimental interventions during normal aging that is very often overlooked during the pre-clinical stage.

In conclusion, results from the present study did not support the hypothesis that a combination treatment of CR and curcumin would have additive functional benefits. However, our findings reveal an interesting gender difference as a result of the combination treatment as well as suggest that curcumin and CR alone still have beneficial effects, which may be dependent on an earlier intervention period.

REFERENCES

Abarikwu SO, Akiri OF, Durojaiye MA, Alabi AF (2014) Combined administration of curcumin and gallic acid inhibits gallic acid-induced suppression of steroidogenesis, sperm output, antioxidant defenses and inflammatory responsive genes.. J Steroid Biochem Mol Biol 143:49-60. doi: 10.1016/j.jsbmb.2014.02.008.

Akbaraly T, Sabia S, Hagger-Johnson G, Tabak A,G., Shipley M,J., Jokela M, Brunner E,J., Hamer M, Batty G,D., Singh-Manoux A et al (2013) Does overall diet in midlife predict future aging phenotypes? A cohort study. Am J Med. 126:411-419. doi: 10.1016/j.amjmed.2012.10.028.

Allison DB, Thomas DM, Heymsfield SB (2014) Energy intake and weight loss. JAMA 312:2687-2688. doi: 10.1001/jama.2014.15513 [doi].

Anand P, Thomas S, Kunnumakkara A, Sundaram C, Harikumar K, Sung B, Tharakan S, Misra K, Priyadarsini I, Rajasekharan K et al (2008) Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol 76:1590-1611. doi: 10.1016/j.bcp.2008.08.008.

Andreotti C, Root JC, Ahles TA, McEwen BS, Compas BE (2014) Cancer, coping, and cognition: a model for the role of stress reactivity in cancer-related cognitive decline. Psychooncology. doi: 10.1002/pon.3683.

Andrews-Hanna J, Snyder A, Vincent J, Lustig C, Head D, Raichle M, Buckner R (2007) Disruption of large-scale brain system in advanced aging. Neuron 56:924-935.

Balogun E, Hoque M, Gong P, Killeen E, Green CJ, Foresti R, Alam J, Motterlini R (2003) Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element.. Biochem J 371:887-895.

Begum A,N., Jones M,R., Lim G,P., Morihara T, Kim P, Heath D,D., Rock C,L., Pruitt M,A., Yang F, Hudspeth B et al (2008) Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease.. J Pharmacol Exp Ther 326:196-208. doi: 10.1124/jpet.108.137455.

Buchman AS, Bennett DA (2011) Loss of motor function in preclinical Alzheimer's disease.. Expert Rev Neurother 11:665-676.

Buckner R (2004) Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate.. Neuron 44:195-208.

Bun S, Ikejima C, Kida J, Yoshimura A, Lebowitz AJ, Kakuma T, Asada T (2015) A combination of supplements may reduce the risk of Alzheimer's disease in elderly Japanese with normal cognition. J Alzheimers Dis 45:15-25. doi: 10.3233/JAD-142232 [doi].

Caracciolo B, Xu W, Collins S, Fratiglioni L (2014) Cognitive decline, dietary factors and gut-brain interactions. Mech Ageing Dev:59-69.

Chaudhari K, Wong M, J., Vann H, P., Sumien N (2014) Exercise training and antioxidant supplementation independently improve cognitive function in adult male and female GFAP-APOE mice. J Sport Health Sci 3:196-205. doi: dx.doi.org/10.1016/j.jshs.2014.04.004.

Chen H, Luo L, Liu J, Brown T, Zirkin BR (2005) Aging and caloric restriction: effects on Leydig cell steroidogenesis. Exp Gerontol 40:498-505. doi: S0531-5565(05)00062-8 [pii].

Choi J, Silverman I (2002) The relationship between testosterone and route-learning strategies in humans. Brain Cogn 50:116-120. doi: S0278262602000155 [pii].

Cunningham RL, Singh M, O'Bryant SE, Hall JR, Barber RC (2014) Oxidative stress, testosterone, and cognition among Caucasian and Mexican-American men with and without Alzheimer's disease. J Alzheimers Dis 40:563-573. doi: 10.3233/JAD-131994 [doi].

de, Fiebre, N,C., Sumien N, Forster M,J., de, Fiebre, C,M. (2006) Spatial learning and psychomotor performance of C57BL/6 mice: age sensitivity and reliability of individual differences.. Age 28:235. doi: 10.1007/s11357-006-9027-3.

Everitt A, Hilmer S, Brand-Miller J, Jamieson H, Truswell A, Sharma A, Mason R, Morris B, Le Couteur D (2006) Dietary approaches that delay age-related diseases.. Clin Interv Aging 1:11-31.

Forster M,J., Lal H (1992) Within-subject behavioral analysis of recent memory in aging mice.. Behav Pharmacol 3:337-349.

Forster M, Dubey A, Dawson K, Stutts W, Lal H, Sohal R (1996) Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain.. Proc Natl Acad Sci U S A 93:4765-4769.

Galasko DR, Peskind E, Clark CM, Quinn JF, Ringman JM, Jicha GA, Cotman C, Cottrell B, Montine TJ, Thomas RG et al (2012) Antioxidants for Alzheimer disease: a randomized clinical trial with cerebrospinal fluid biomarker measures. Arch Neurol 69:836-841. doi: archneurol.2012.85 [pii].

Geismann C, Arlt A, Sebens S, Schäfer H (2014) Cytoprotection "gone astray": Nrf2 and its role in cancer.. Onco Targets Ther:1497-1518.

Gemma C, Vila J, Bachstetter A, Bickford P,C. (2007) Oxidative Stress and the Aging Brain: From Theory to Prevention. Riddle D,R. Brain Aging: Models, Methods, and Mechanisms. Boca Raton (FL), CRC Press.

Goel A, Kunnumakkara A,B., Aggarwal B,B. (2008) Curcumin as "Curecumin": from kitchen to clinic.. Biochem Pharmacol 75:787-809.

Golden SH, Dobs AS, Vaidya D, Szklo M, Gapstur S, Kopp P, Liu K, Ouyang P (2007) Endogenous sex hormones and glucose tolerance status in postmenopausal women. J Clin Endocrinol Metab 92:1289-1295. doi: jc.2006-1895 [pii].

Gupta S,C., Kismali G, Aggarwal B,B. (2013) Curcumin, a component of turmeric: from farm to pharmacy.. Biofactors 39:2-13. doi: 10.1002/biof.1079.

Harrison SL, Sajjad A, Bramer WM, Ikram MA, Tiemeier H, Stephan BC (2015) Exploring strategies to operationalize cognitive reserve: A systematic review of reviews. J Clin Exp Neuropsychol 37:253-264. doi: 10.1080/13803395.2014.1002759 [doi].

Hickey M, Zhu C, Medvedeva V, Lerner R, Patassini S, Franich N, Maiti P, Frautschy S, Zeitlin S, Levine M et al (2012)
Improvement of neuropathology and transcriptional deficits in CAG 140 knock-in mice supports a beneficial effect of dietary curcumin in Huntington's disease.. Mol Neurodegener 7.

Jaramillo MC, Zhang DD (2013) The emerging role of the Nrf2-Keap1 signaling pathway in cancer.. Genes Dev 27:2179-2191. doi: 10.1101/gad.225680.113.

Jiang T, Chen N, Zhao F, Wang XJ, Kong B, Zheng W, Zhang DD (2010) High levels of Nrf2 determine chemoresistance in type II endometrial cancer. Cancer Res 70:5486-5496. doi: 10.1158/0008-5472.CAN-10-0713 [doi].

Kayo T, Allison DB, Weindruch R, Prolla TA (2001) Influences of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. Proc Natl Acad Sci U S A 98:5093-5098. doi: 10.1073/pnas.081061898 [doi].

Kennard JA, Woodruff-Pak DS (2011)

Age sensitivity of behavioral tests and brain substrates of normal aging in mice.. Front Aging Neurosci 3.

Koh F, Charlton K, Walton K, McMahon AT (2015) Role of dietary protein and thiamine intakes on cognitive function in healthy older people: a systematic review. Nutrients 7:2415-2439. doi: 10.3390/nu7042415 [doi].

Lau A, Villeneuve NF, Sun Z, Wong PK, Zhang DD (2008) Dual roles of Nrf2 in cancer. Pharmacol Res 58:262-270. doi: 10.1016/j.phrs.2008.09.003 [doi].

Li R (2014) Why women see differently from the way men see? A review of sex differences in cognition and sports. J Sport Health Sci 3:155-162.

Lindstrom H, Fritsch T, Petot G, Smyth K, Chen C, Debanne S, Lerner A, Friedland R (2005) The relationships between television viewing in midlife and
the development of Alzheimer's disease in a case-control study.. Brain Cogn 58:157-165.

Madeo F, Pietrocola F, Eisenberg T, Kroemer G (2014)
Caloric restriction mimetics: towards a molecular definition.. Nat Rev Drug Discov 13:727-740. doi: 10.1038/nrd4391.

Malinowski J, Gillespie W (2001) Individual differences in performance on a large-scale, real-world wayfinding task. . J Environ Psychol 21:73-82.

Martin DM, Wittert G, Burns NR, Haren MT, Sugarman R (2007) Testosterone and cognitive function in ageing men: data from the Florey Adelaide Male Ageing Study (FAMAS).. Maturitas 57:182-194.

Mattson MP, Son TG, Camandola S (2007)

Viewpoint: mechanisms of action and therapeutic potential of neurohormetic phytochemicals.. Dose Response 5:174-186. doi: 10.2203/dose-response.07-004.Mattson.

McDonald S,R., Sohal R,S., Forster M,J. (2005) Concurrent administration of coenzyme Q10 and alpha-tocopherol improves learning in aged mice.. Free Radic Biol Med 38:729-736.

Means LW, Higgins JL, Fernandez TJ (1993) Mid-life onset of dietary restriction extends life and prolongs cognitive functioning. Physiol Behav 54:503-508. doi: 0031-9384(93)90243-9 [pii].

Miller ER,3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E (2005) Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 142:37-46. doi: 0000605-200501040-00110 [pii].

Miller VM (2014) Why are sex and gender important to basic physiology and translational and individualized medicine? Am J Physiol Heart Circ Physiol 306:H781-8. doi: 10.1152/ajpheart.00994.2013 [doi].

Ober C, Loisel DA, Gilad Y (2008) Sex-specific genetic architecture of human disease. Nat Rev Genet 9:911-922. doi: 10.1038/nrg2415 [doi].

Pletzer B (2014) Sex-specific strategy use and global-local processing: a perspective toward integrating sex differences in cognition. Front Neurosci 8:425. doi: 10.3389/fnins.2014.00425 [doi].

Pletzer B, Kronbichler M, Nuerk HC, Kerschbaum H (2013) Sex differences in the processing of global vs. local stimulus aspects in a two-digit number comparison task--an fMRI study. PLoS One 8:e53824. doi: 10.1371/journal.pone.0053824 [doi].

Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited.. FASEB J 22:659-661.

Rebrin I, Forster M,J., Sohal R,S. (2011) Association between life-span extension by caloric restriction and thiol redox state in two different strains of mice.. Free Radic Biol Med 51:225-233. doi: 10.1016/j.freeradbiomed.2011.04.006.

Rocca WA, Mielke MM, Vemuri P, Miller VM (2014) Sex and gender differences in the causes of dementia: a narrative review. Maturitas 79:196-201. doi: 10.1016/j.maturitas.2014.05.008 [doi].

Ryan J, Stanczyk F, Dennerstein L, Mack W, Clark M, Szoeke C, Kildea D, Henderson V (2012) Hormone levels and cognitive function in postmenopausal midlife women.. Neurobiol Aging 33:e11-e22.

Sanz A, Hiona A, Kujoth GC, Seo AY, Hofer T, Kouwenhoven E, Kalani R, Prolla TA, Barja G, Leeuwenburgh C (2007) Evaluation of sex differences on mitochondrial bioenergetics and apoptosis in mice. Exp Gerontol 42:173-182. doi: S0531-5565(06)00317-2 [pii].

Sarker M, Franks S, Sumien N, Forster M (2015) Caloric Restriction and Dietary Curcumin Improve Functional Outcomes of Aging in Mice. The FASEB Journal 29.

Schulte DM, Hahn M, Oberhäuser F, Malchau G, Schubert M, Heppner C, Müller N, Güdelhöfer H, Faust M, Krone W et al (2014)
Caloric restriction increases serum testosterone concentrations in obese male subjects by two distinct mechanisms.. Horm Metab Res 46:283-286. doi: 10.1055/s-0033-1358678.

Shah S, Bell R, Savage G, Goldstat R, Papalia M, Kulkarni J, Donath S, Davis S (2006) Testosterone aromatization and cognition in women: a randomized, placebo-controlled trial.. Menopause 13:600-608.

Shen L,R., Xiao F, Yuan P, Chen Y, Gao Q,K., Parnell L,D., Meydani M, Ordovas J,M., Li D, Lai C,Q. (2013) Curcumin-supplemented diets increase superoxide dismutase activity and mean lifespan in Drosophila.. Age (Dordr) 35:1133-1142. doi: 10.1007/s11357-012-9438-2.

Spangler E, Long J, Bennett K, Marshall M, Minor R, de Cabo R (2010) Will Calorie restriction stave off age-related brain dysfunction, specifically to learning and memory? A review and critique of the rodent literature. Everitt A, Rattan S, Couteur D, de Cabo R. Calorie restriction, Aging and Longevity. London, Springer, pp 177-190.

Straub RH (2007) The complex role of estrogens in inflammation. Endocr Rev 28:521-574.

Sumien N, Heinrich K, Sohal R, Forster M (2004) Short-term vitamin E intake fails to improve cognitive or psychomotor performance of aged mice.. Free Radic Biol Med 36:1424-1433.

Sumien N, Sims M,N., Taylor H,J., Forster M,J. (2006) Profiling psychomotor and cognitive aging in four-way cross mice.. Age (Dodr) 28:265-282. doi: 10.1007/s11357-006-9015-7.

Tang Y, Chen A (2014) Curcumin eliminates the effect of advanced glycation end-products (AGEs) on the divergent regulation of gene expression of receptors of AGEs by interrupting leptin signaling.. Lab Invest 94:503-516. doi: 10.1038/labinvest.2014.

Thangthaeng N, Sumien N, Forster M,J. (2008) Dissociation of functional status from accrual of CML and RAGE in the aged mouse brain. Exp Gerontol 43:1077-1085. doi: 10.1016/j.exger.2008.08.045.

Thomas DM, Martin CK, Redman LM, Heymsfield SB, Lettieri S, Levine JA, Bouchard C, Schoeller DA (2014) Effect of dietary adherence on the body weight plateau: a mathematical model incorporating intermittent compliance with energy intake prescription. Am J Clin Nutr 100:787-795. doi: 10.3945/ajcn.113.079822 [doi].

Trujillo J, Granados-Castro L,F., Zazueta C, Andérica-Romero A,C., Chirino Y,I., Pedraza-Chaverrí J (2014) Mitochondria as a target in the therapeutic properties of curcumin.. Arch Pharm (Weinheim) 347:873-884. doi: 10.1002/ardp.201400266.

van der Wijst MG, Huisman C, Mposhi A, Roelfes G, Rots MG (2015) Targeting Nrf2 in healthy and malignant ovarian epithelial cells: Protection versus promotion. Mol Oncol. doi: S1574-7891(15)00052-6 [pii].

Vel Szic K, Declerck K, Vidaković M, Vanden-Berghe W (2015) From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition?. Clin Epigenetics 7.

Viña J, Gambini J, García-García FJ, Rodriguez-Mañas L, Borrás C (2013) Role of oestrogens on oxidative stress and inflammation in ageing. Horm Mol Biol Clin Investig 16:65-72. doi: 10.1515/hmbci-2013-0039.

Wahab F, Atika B, Huma T, Shahab M (2014) Primate HPT axis response to the peripheral kisspeptin challenge under different time periods of food restriction in monkeys. Horm Metab Res 46:187-192. doi: 10.1055/s-0033-1363263 [doi].

Wallin KG, Wood RI (2015) Anabolic-androgenic steroids impair set-shifting and reversal learning in male rats.. Eur Neuropsychopharmacol. doi: 10.1016/j.euroneuro.2015.01.002.

Wang P, Su C, Li R, Wang H, Ren Y, Sun H, Yang J, Sun J, Shi J, Schaffer M (2014a) Mechanisms and effects of curcumin on spatial learning and memory improvement in APPswe/PS1dE9 mice.. Neurosci Res 92:218-231. doi: 10.1002/jnr.23322.

Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P, Kwun I, Shen C (2014b) Novel insights of dietary polyphenols and obesity.. J Nutr Biochem 25:1-18. doi: 10.1016/j.jnutbio.2013.09.001.

Wilson R, Mendes De Leon C, Barnes L, Schneider J, Bienias J, Evans D, Bennett D (2002) Participation in cognitively stimulating activities and risk of incident Alzheimer disease.. JAMA 287:742-748.

Wise P (2002) Estrogens and neuroprotection. Trends Endocrinol Metab 13:229-230.

Wolf OT, Kirschbaum C (2002) Endogenous estradiol and testosterone levels are associated with cognitive performance in older women andmen.. Horm Behav 41:259-266.

Yang F, Chu X, Yin M, Liu X, Yuan H, Niu Y, Fu L (2014) mTOR and autophagy in normal brain aging and caloric restriction ameliorating age-related cognition deficits. Behav Brain Res 264:82-90. doi: 10.1016/j.bbr.2014.02.005.

Figure 1: Body weight over a span of 15 weeks of dietary treatment. Data are means ± SEM, for

surviving mice for AL, base diet fed ad libitum; CR, base diet restricted to 70% AL; CURAL,

curcumin in base diet fed AL; CURCR, curcumin in base diet restricted to 70%. Experiments

began with 12-14 mice. Mortality during the course of the study was 70% in the aged males

CURCR, 87% in the aged male AL, and 97.3% in the remaining groups.
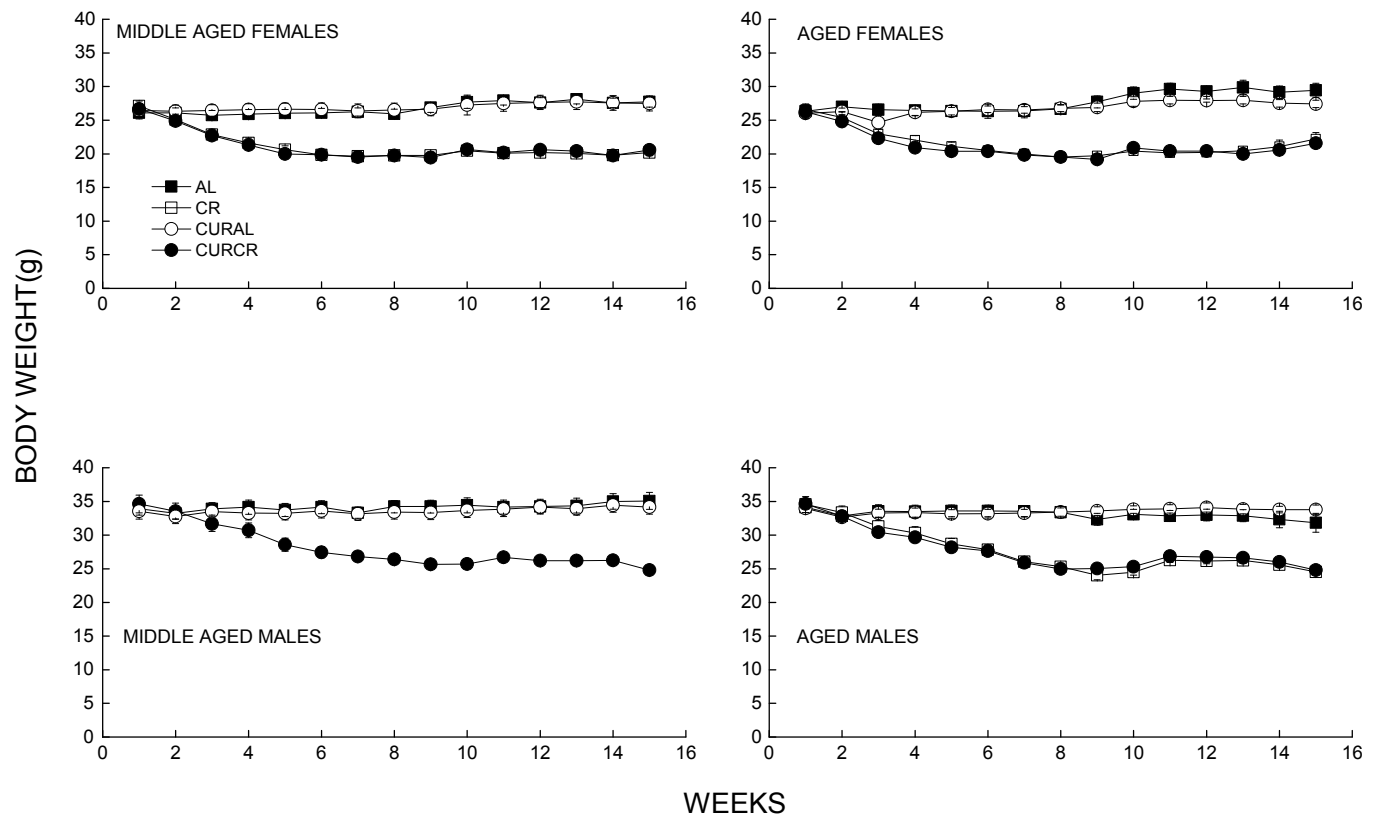
Figure 2: Effect of diet, age and sex on (a) total distance in cm and (b) total time in center in seconds; that are different components of spontaneous locomotor activity. The dashed line is to compare the difference between sexes. Bars represent means ± SEM, n=10-13, for AL, CR, CURAL and CURCR

TOTAL DISTANCE (cm)

MIDDLE AGED MALES

MIDDLE AGED FEMALES

AGED MALES

AGED FEMALES

TIME IN CENTER (s)

MIDDLE AGED MALES

MIDDLE AGED FEMALES

AGED MALES

AGED FEMALES
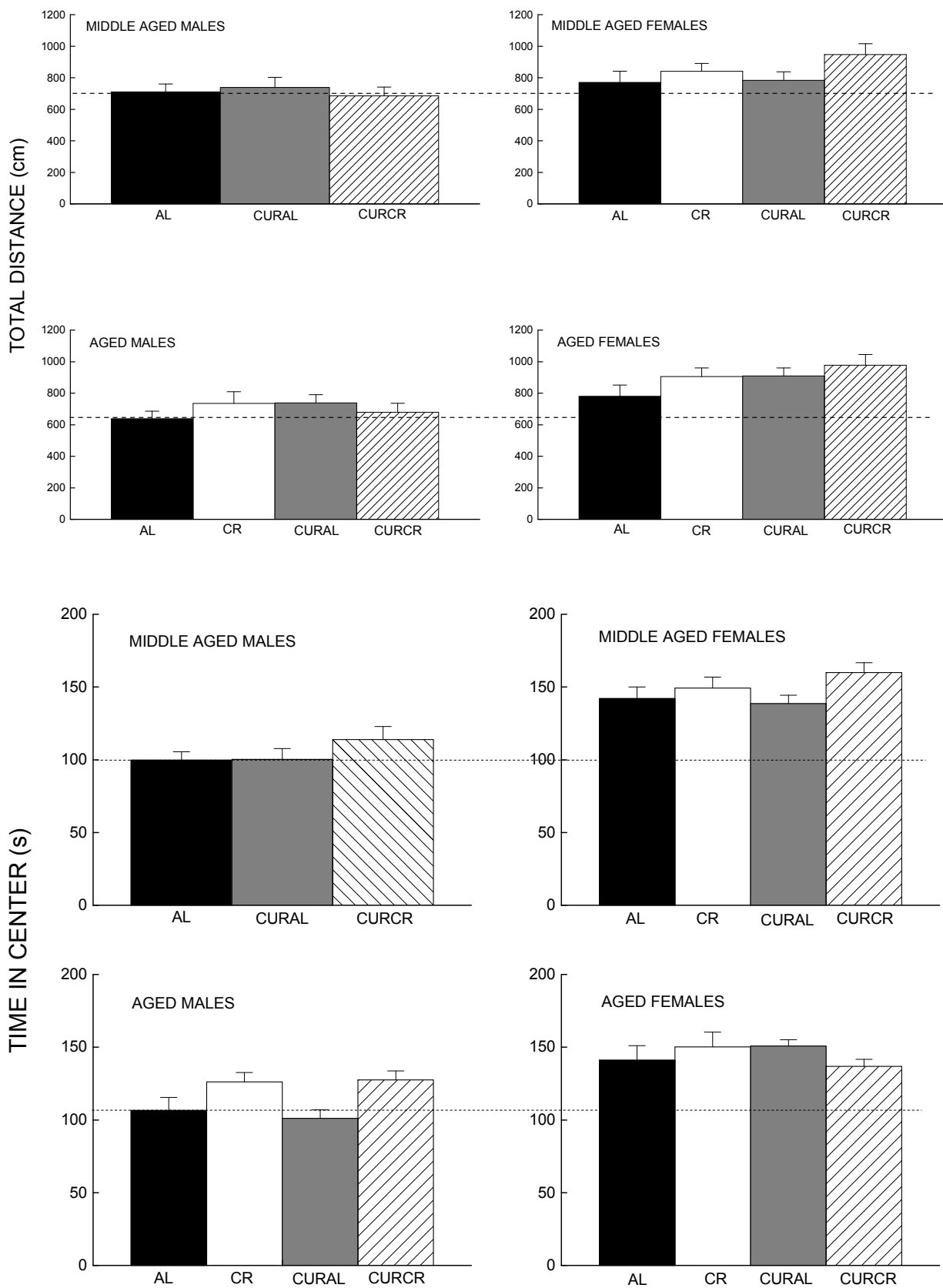
Figure 3: Maximal performance measured as longest latency to fall amongst sessions on the rotorod as a function of diet, age and sex. Bars represent means ± SEM, n= 10-13. * Significant difference compared to age matched control (*P*<0.01)

LATENCY TO FALL (s)

MIDDLE AGED FEMALES

AL CR CURAL CURCR

AGED FEMALES

AL CR CURAL CURCR

MIDDLE AGED MALES

AL CURAL CURCR

AGED MALES

AL CR CURAL CURCR

Figure 4: Strength, balance and coordination as measured by latency to fall on a wire

suspension test as a function of diet, age and sex. * Significant difference from age matched
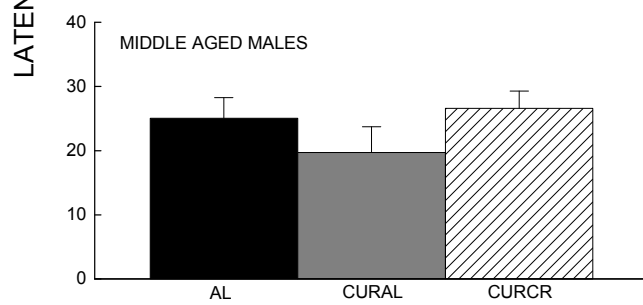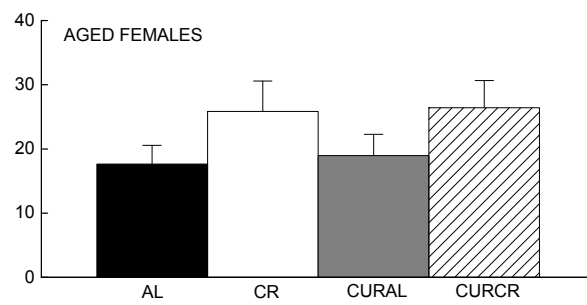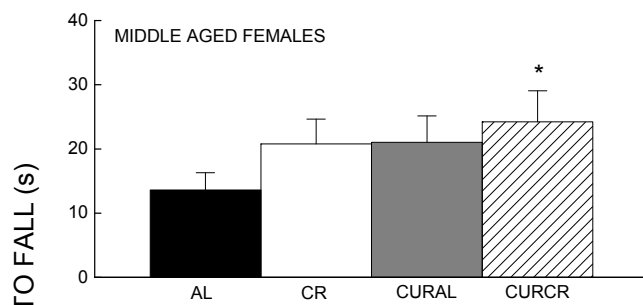
control (P<0.05).

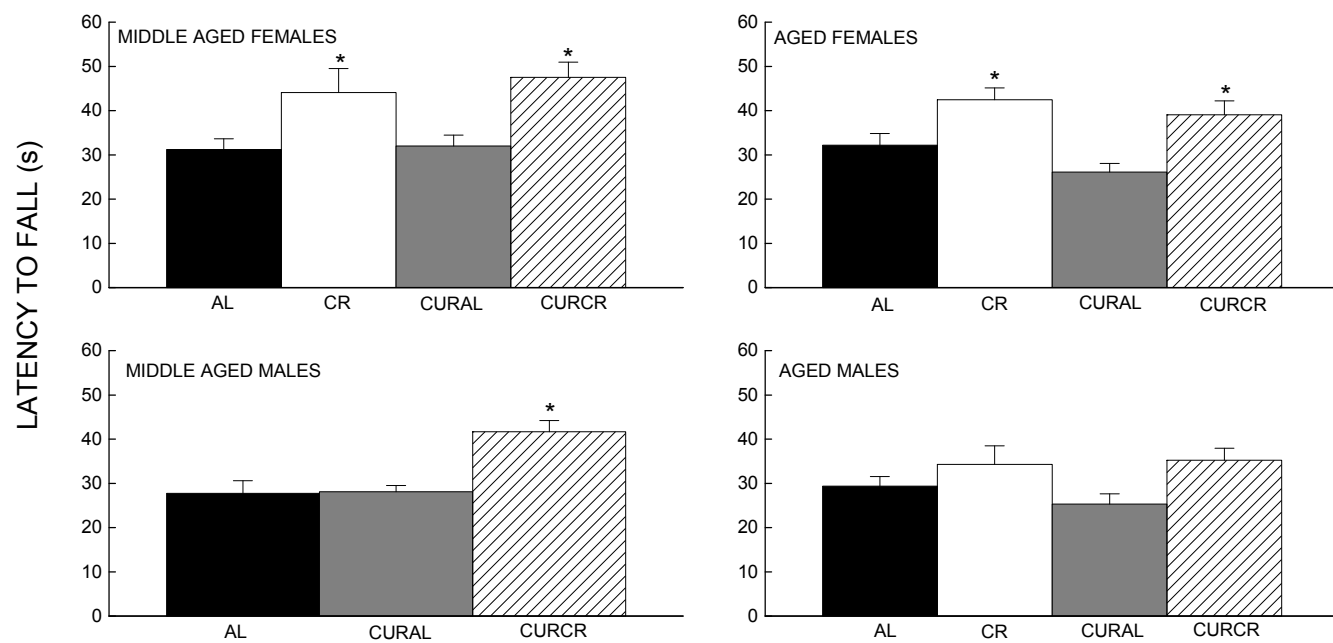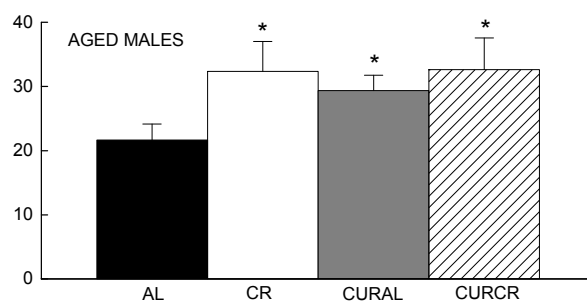Figure 5: Strength, balance and coordination as measured by latency to fall on a bridge test as a function of diet, age and sex. * Significant difference from age matched control ($P<0.05$).

LATENCY TO FALL (s)

MIDDLE AGED FEMALES

AGED FEMALES

MIDDLE AGED MALES

AGED MALES

AL   CR   CURAL   CURCR

AL   CURAL   CURCR

AL   CR   CURAL   CURCR
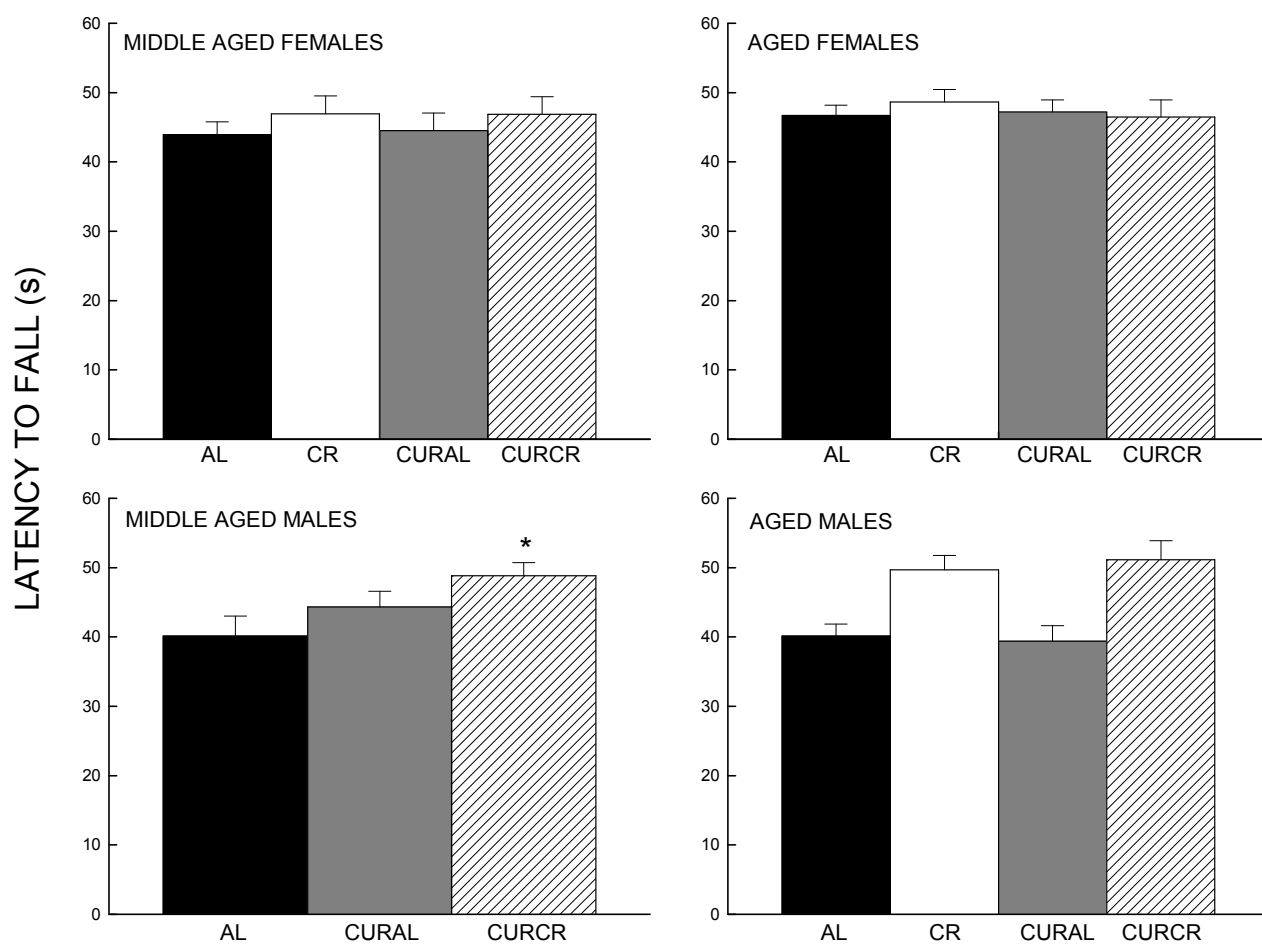
Figure 6: Learning and memory for spatial discrimination as a function of diet, age and sex as measured by performance in the water maze which is described as time spent in a 40cm annulus over the center of the platform position. The dashed lines represent the percentage of time mice would spend in the 40cm annulus due to chance. Each value represents the mean ± SEM, n=10-13. * Significant difference compared to age matched control ($P<0.01$)
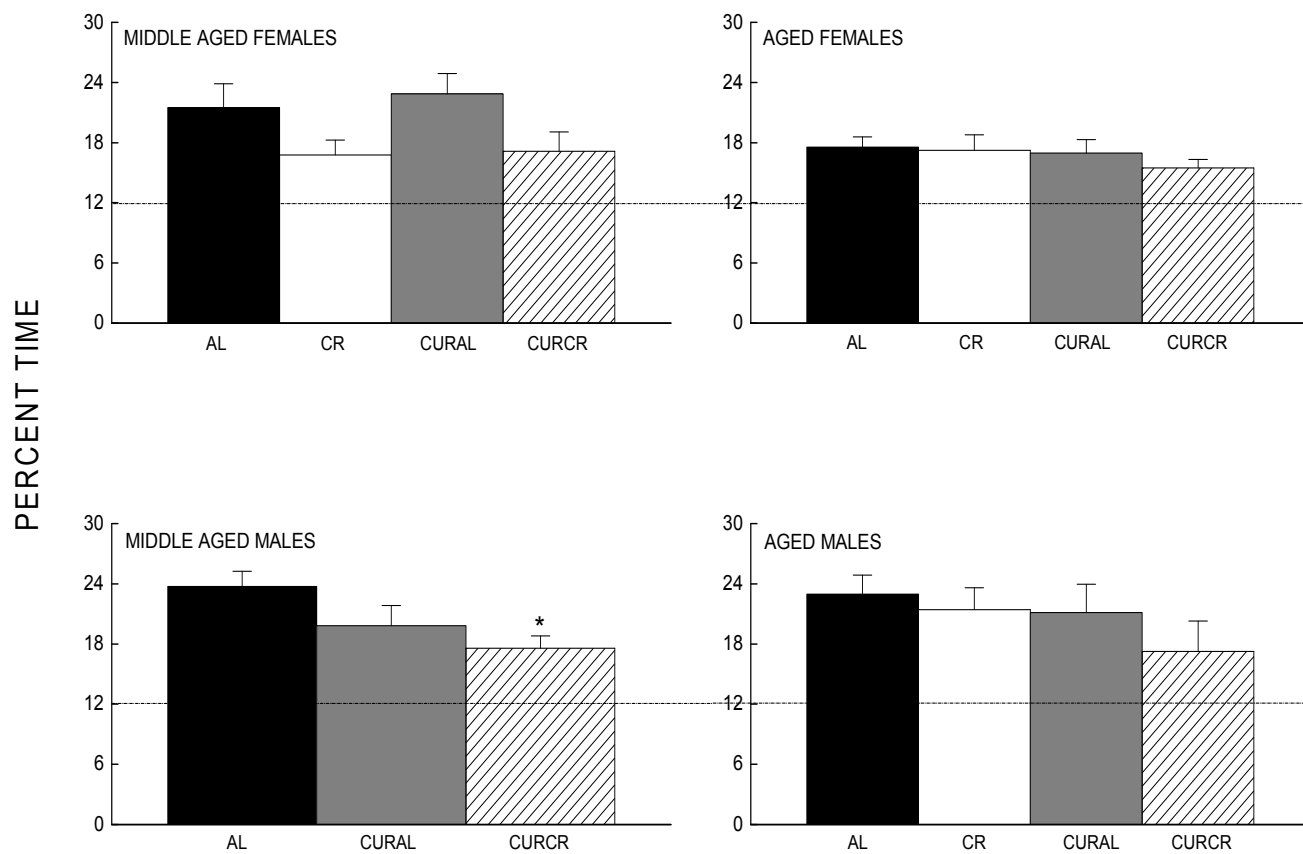
Figure 7: Effect of diet, age and sex on learning and reversal of an active avoidance response to one of two locations in a T-maze. During the first session mice learned the avoidance response, and reversals of the correct location occurred during two subsequent sessions which were combined (AVG) to assess cognitive flexibility. All data are expressed as the mean no. of trials ± SEM needed to meet the correct avoidance criterion, n-10-13. * Significant difference compared to age-matched control.
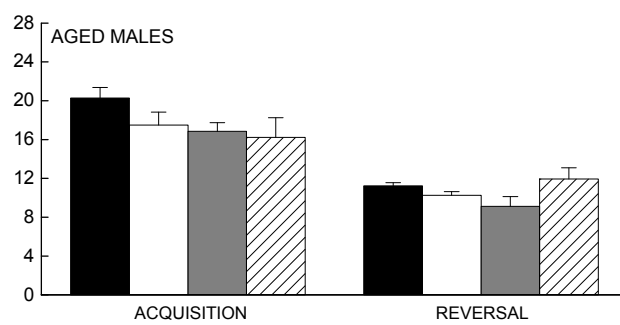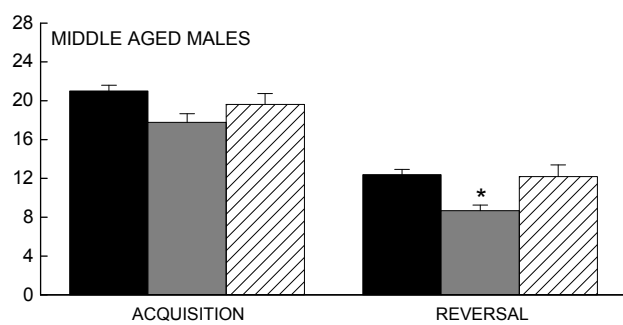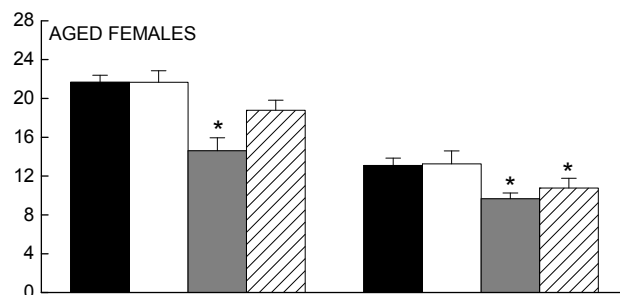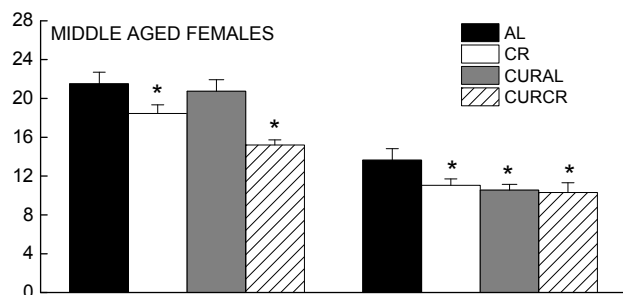
Table 1: Effect of dietary interventions on functional outcomes in middle aged and aged male mice. All values are the group means±SE. *Significant difference compared to age matched control.

Table 2: Effect of dietary interventions on functional outcomes in middle aged and aged female mice. All values are the group mean±SE. *Significant difference compared to age matched control.

Table 1

|	MALES						
	15 months			20 months			
	AL	CURAL	CURCR	AL	CR	CURAL	CURCR
Locomotor activity							
Total distance (cm)	709.6±51	685.6±55	738.3±64	638.3±48	734.6±75	737.8±53	678.2±58
Vertical activity (counts)	28.1±2.8	31.9±3.6	32.3±2.7	26.6±3.5	21.8±1.7	32.4±2.5	20.9±2.7
Center time (s)	99.6±5.9	100.4±7.4	113.9±9.0	106.6±8.8	126.2±6.3	101.1±5.9	127.4±6.2
Reflexes							
Walk initiation (s)	2.2±0.2	3.0±0.6	3.1±0.3	2.4±0.3	3.3±0.6	1.8±0.4	2.4±0.4
Alley turn (s)	12.3±1.3	16.6±2.7	24.3±3.3*	15.1±1.7	23±3.6	14.7±1.4	20.8±2.7
Negative geotaxis 180°	5.4±0.7	7.1±1.2	3.5±0.4	6.0±1.1	6.1±1.3	5.3±0.7	4.0±0.5
Psychomotor function							
Rotorod fall (s)	27.8±2.8	28.1±1.4	41.7±2.5*	29.4±2.1	34.3±4.2	25.3±2.4	35.2±2.8
Wire fall (s)	23.9±2.8	19±3.2	27.1±2.1	14.9±2.2	28.3±3.0*	23.13±2.18	27.1±4.0*
Bridge fall (s)	40.1±2.1	44.4±2.2	48.8±1.9	40.1±1.7	49.7±21	39.4±2.3	51.2±2.7
Swim speed (cm/s)	17.5±0.5	16.9±0.6	17.5±0.5	15.5±0.6	15.5±0.6	16.9±0.5	17±3.0
Learning/memory							
Learning index (cm)	567.2±69	538.1±52	585.7±50	589.9±75	551.9±57	538.1±53	585.7±50
Probe A40 (%)	23.7±1.5	19.8±2	15.5±0.6*	22.3±1.9	21.43±2.2	21.13±2.8	17.25±3.0
Avoidance: acquisition (trials)	21±0.6	17.8±.0.9	19.6±1.1	20.3±1.1	17.5±1.3	16.83±0.9	16.22±2.0
Avoidance: reversal (trials)	12.4±0.5	8.69±0.6*	12.2±1.2	11.2±0.3	10.25±0.4	9.12±1.0	11.9±1.1

*significant difference compared to age matched control

Table 2

Behavioral measure					FEMALES			
	15 months				20 months			
	AL	CR	CURAL	CURCR	AL	CR	CURAL	CURCR
Locomotor activity								
Total distance (cm)	770.6±71	840.7±50	784.1±54	946.8±70	779.7±72	905.5±54	909.5±50.7	976.8±69
Vertical activity (counts)	23.9±4.0	28.7±2.7	29.9±2.7	30.1±1.9	29.1±3.4	26.7±2.7	32.5±2.7	28±3.5
Center time (s)	142.1±7.9	149.2±7.6	138.5±6.0	160±6.8	141.1±10	150.3±10	150.8±4.3	136.7±4.9
Reflexes								
Walk initiation (s)	2.2±0.2	3.3±0.4	3.1±0.4	3.2±0.3	3.2±0.4	3.0±0.3	2.6±0.4	2.9±0.3
Alley turn (s)	17.0±2.1	12.8±1.6	14.1±1.7	16.0±1.4	12.5±1.8	14.7±2.3	12.9±1.6	17.6±3.4
Negative geotaxis 180°	3.7±0.5	5.5±0.4	3.7±0.5	4.5±0.6	6.2±1.2	5.1±0.7	4.5±0.5	4.4±0.8
Psychomotor function								
Rotorod fall (s)	31.2±2.5	44.1±5.4*	31.2±2.5	47.5±3.4*	32.2±2.6	42.4±2.7*	26.1±1.9	39.1±3.1*
Wire fall (s)	13.6±2.0	20.8±3.9	21.0±4.1	24.2±4.9*	17.6±2.9	25.9±4.7	19.0±3.3	26.4±4.3
Bridge fall (s)	43.9±1.9	46.9±2.5	44.5±2.5	46.9±2.5	46.7±1.5	48.6±1.8	47.2±1.7	46.5±2.5
Swim speed (cm/s)	17.1±0.7	15.4±0.9	19.4±1.5	17.4±0.8	18.0±0.5	17.0±0.5	18.8±0.7	18.2±1.3
Learning/memory								
Learning index (cm)	624.4±81	630.6±68	757.8±82	565±67	727.8±82	540.1±71	673.5±103	535.9±50
Probe A40 (%)	21.5±2.4	16.8±1.5	22.9±2	17.1±1.9	18.0±0.54	17.0±0.5	18.8±0.7	18.2±1.3
Avoidance: acquisition (trials)	21.5±1.2	18.5±0.9*	20.7±1.2	15.2±0.5*	21.7±0.7	21.7±1.2	14.6±1.3*	18.8±1.0
Avoidance: reversal (trials)	13.6±1.2	11.0±0.6*	10.5±1.2*	10.3±1.0*	13.1±0.8	13.2±1.3	9.7±0.6*	10.8±1.0*

*significant difference compared to age matched control

CHAPTER IV

General Discussion

The exacerbation of oxidative stress and inflammation has long been accepted as a plausible explanation for age - associated neurobehavioral dysfunction. Due to the dearth of biomarkers of aging, it has been difficult to determine causative factors as well as the timeline for the progression of age associated neurobehavioral functional deficits. However, metabolic decline with aging is well documented and is evident in several species including rodents and humans (Houtkooper et al., 2011). One of the many outcomes of metabolic decline along with negligible food intake modifications is an increased susceptibility to obesity. Mid-life obesity in the United States has reached epidemic proportions; 78 million adults were reported to be obese in 2014, amongst which middle aged individuals accounted for 40% making them the largest age group in the overweight and obese category (Ogden et al., 2014; Sturm & Hattori, 2013). Obesity and its comorbidities have been implicated as accelerants for age-associated neurobehavioral disorders, which include cognitive and motor dysfunction (Anstey et al., 2011; Deckers et al., 2015; García-Ptacek et al., 2014; Gupta et al., 2015; Xu et al., 2011).

However, preclinical progress to understand and prevent obesity-associated cognitive decline has been hindered by a number of gaps in research. Firstly, the lack of a proper animal analog of human mid-life obesity has hindered the assessment of underlying mechanisms affecting obesity-associated cognitive decline. Secondly, despite a surge in the popularity of naturally available natural pharmacotherapeutic agents or nutraceuticals as anti-obesity and anti-aging therapies, the FDA does not regulate this industry. Smaller studies and anecdotal evidence have suggested these compounds to have anti-inflammatory and antioxidant properties, with the potential of delaying the onset of age-associated neurodegenerative disorders. Unfortunately, there is a lack of research on the effects of nutraceuticals (Hardy, 2000), not only when used in isolation but also when used concurrently with another mode of treatment such as dieting, which includes restricting calories. Furthermore, little research has been conducted on both males and females, leaving the question of potential gender effects largely unanswered.

The present experiments were therefore designed to bridge these critical gaps in knowledge, specifically in relation to the use of curcumin as a putative nutraceutical agent and a CR mimetic that would address causative factors of age-associated neurobehavioral dysfunction and determine an intervention period.

Gap 1: The need for a proper analog of human mid-life obesity to study the acceleration of late-life cognitive decline that is associated with obesity.

A majority of the animal models used to study obesity are either genetically manipulated or fed a high fat diet and include younger mice. These mice weigh more than double that of their age-matched controls, which when translated to a clinical setting would be considered Class 2 or 3 obese (Harrison et al., 1984). Class 2 and 3 obese are characterized as morbidly or severely obese where individuals are a 100% over their ideal body weight. Normally aging C57BL/6 mice gain around 36% body weight at midlife compared to their weight at 4 months (Sohal & Forster, 2014). Their weight at 4 months is considered a normal healthy weight for C57BL/6 mice based on caloric restriction studies. Interestingly, singly-housed, normally aging C57BL/6 mice also suffer from what has been termed as "late onset obesity". This term applies to mice that have gained a considerable amount of weight by 12 months of age due to lower activity and also suffer from glucose intolerance (Becskei et al., 2009; B. Martin et al., 2010). With reports from CDC suggesting that the majority of the obese population within the United States falls within the middle-aged range and in the overweight and Class 1 range. Class 1 obese individuals weigh more than 20% of their normal body weight making normal aging C57BL/6 mice an appropriate analog for the study of midlife obesity in humans. It is therefore important that this age and class of obesity group should be the target for preventive interventions for obesity-related cognitive dysfunction at the preclinical phase.

To incorporate the sedentary lifestyle based obesity experimental model, the first of the current studies used normally aged middle-aged male mice fed ad libitum, as a novel analog for overweight and Class 1 obese individuals in order to target obesity associated late life dementia. Curcumin and CR were implemented as dietary interventions to test their ability to decrease adiposity, alleviate inflammation and oxidative stress, as well as improve different aspects of cognitive function. Caloric restriction was used as a negative control in this study

as weight loss is one of the primary effects of this intervention. Results from the study indicated that short-term CR indeed decreased body weight and systemic inflammation, as well as yielded positive cognitive effects. However, there was no improvement in oxidative stress, which could have been attributed to the short time period of 3 to 4 months that these mice underwent CR (Kalani et al., 2006).

Inferences from the results of this experimental model of obesity can provide important information toward understanding the impact of weight loss in obese humans during midlife.

Gap 2: Data on age-associated neurobehavioral functionality from a mixed interventions study of a nutraceutical and dieting.

Overweight and obese individuals wanting to lose weight typically start with short-term CR. This paradigm is also popular due to studies reporting its beneficial effects on inflammation and oxidative stress (Spangler et al., 2010). The increased attention to the health benefits of reducing inflammation and oxidative stress in relation to both obesity and aging has also created a 150-billion-dollar industry focused on the sale of nutraceuticals with potential antioxidant and anti-inflammatory capacities for treating common health problems (Coppens et al., 2006). However, this industry is not FDA regulated, creating a distinct lack of knowledge about their long-term effects on functional capacity (Kesselheim et al., 2015). Further complicating this problem is the lack of information on combination treatments, for example when nutraceuticals are used in conjunction with dieting (Coppens et al., 2006).

Although beneficial effects of CR have previously been established, the current experiment addressed the effects of combining CR with curcumin on the reversal of obesity and age-related inflammation and oxidative stress developed during mid-life and the exacerbation of cognitive and motor dysfunction during early senescence. In doing so, the current projects aimed to determine whether these effects were additive, synergistic and/or dependent on ages at which these interventions were implemented. Finding additive effects could potentially lead to development of future interventions involving milder CR and a reduced curcumin dosage, and provide valuable information regarding drug interactions to the growing literature of polypharmacy

practiced by the middle-aged and aged populations. Contrary to the hypothesis for the experiment, results from the current study indicated CURCR to be neither beneficially synergistic nor additive. In fact, when tested for spatial learning via the probe index and for cognitive flexibility, CURCR middle-aged males performed more poorly than their age-matched controls. Although females also did not demonstrate any additive functional outcomes, they also did not demonstrate the antagonistic effect evident in the males. Whereas CURCR treatment failed to be of any cognitive benefit to male mice, it did enhance motor function in CURCR males when compared to their age-matched controls.

Conducting trials of and implementing them at different ages in combination is essential to further scientific knowledge on the long-term impact of dietary interventions on aging processes and disorders. According to the results from this study, the antagonistic action of the combination treatment on cognition would send a note of caution to individuals who practice polypharmacy with different nutraceuticals.

Gap 3: Effect of dietary interventions may be sex dependent.

The over reliance on male animals as subjects and sources for cells in preclinical studies prevents demonstrating what may be key gender differences that could potentially guide clinical studies. With the recent call of action by the NIH to balance sex in cell and animal studies (Clayton & Collins, 2014), it is clear that heeding this call and adding female mice to experimental designs could bridge the current gap in knowledge of the effects of nutritional interventions in aging females.

In the current study, there was a distinct sex difference in cognitive flexibility, such that males under CURCR had than females, regardless of age. This sex difference may be hypothesized as due to higher than optimal levels of testosterone levels in male mice (Golden et al., 2007; Hogervorst et al., 2010). Previous studies have indicated that both short-term CR and curcumin increase testosterone levels (Abarikwu et al., 2014; Pop et al., 2015; Schulte et al., 2014; Sitzmann et al., 2010). Although testosterone has been shown to have beneficial effects on cognition (Cherrier et al., 2001; Choi & Silverman, 2002), there may be a ceiling effect to these positive effects. As reported by Wallin et al. (2015), injecting anabolic steroids in younger male mice with regular

testosterone levels hinders their executive function (Wallin & Wood, 2015). Similarly, higher total testosterone in middle-aged men has also been associated with poorer executive function (D. M. Martin et al., 2007). Whereas CR in the present study had no independent effect on executive function in the aged mice of either gender, it was shown that aged females under CURCR had better cognitive flexibility compared to CR alone. This manifestation is possibly a protective effect of estrogen in aged female mice. Estrogen has been reported to up-regulate nuclear translocation of Nrf2 (Gorrini et al., 2014; Wu et al., 2014) whereas the effect of testosterone on Nrf2 remains inconclusive (Schultz et al., 2010) (Fig 4). The cognitive effect would otherwise not been reported, had the study only included male mice, leading to an incorrect conclusion that combination treatment would be harmful for the general population..

Evidence of sex-specific or gonadal hormone effects for different interventions is scant in both pre-clinical and clinical studies. Results from the current experiment illustrate the importance for studying gender differences in the pre-clinical phase, thus allowing for further investigation of gender-specific optimal dosages and mechanisms of action for newer therapeutic agents in order to cater to the whole population.

REFERENCES:

Abarikwu, S. O., Akiri, O. F., Durojaiye, M. A., & Alabi, A. F. 2014. Combined administration of curcumin and gallic acid inhibits gallic acid-induced suppression of steroidogenesis, sperm output, antioxidant defenses and inflammatory responsive genes. J Steroid Biochem Mol Biol., 143, 49-60.

Abete, I., Parra, M. D., Zulet, M. A., & Martínez, J. A. 2006. Different dietary strategies for weight loss in obesity: role of energy and macronutrient content. Nutr Res Rev, 19, 5-17.

Anstey, K., Cherbuin, N., Budge, M., & Young, J. 2011. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obes Rev, 12, 426-437.

Becskei, C., Lutz, T. A., & Riediger, T. 2009. Blunted fasting-induced hypothalamic activation and refeeding hyperphagia in late-onset obesity.. Neuroendocrinology, 90, 371-382.

Buckner, R. 2004. Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron, 44, 195-208.

Cherrier, M. M., Asthana, S., Plymate, S., Baker, L., Matsumoto, A. M., Peskind, E., Raskind, M. A., Brodkin, K., Bremner, W., Petrova, A., LaTendresse, S., & Craft, S. 2001. Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology, 57, 80-88.

Choi, J., & Silverman, I. 2002. The relationship between testosterone and route-learning strategies in humans. Brain Cogn. 50, 116-120.

Clayton, J.,A., & Collins, F.,S. 2014. Policy: NIH to balance sex in cell and animal studies.. Nature, 509, 282-283.

Coppens, P., da Silva, M., & Pettman, S. 2006. European regulations on nutraceuticals, dietary supplements and functional foods: a framework based on safety. Toxicology, 221, 59-74.

Deckers, K., van, Boxtel, M,P., Schiepers, O.,J., de, V., M., Muñoz, Sánchez, J,L., et al. 2015. Target risk factors for dementia prevention: a systematic review and Delphi consensus study on the evidence from observational studies. Int J Geriatr Psychiatry, 30, 234-246.

Floegel, A., & Pischon, T. 2012. Low carbohydrate-high protein diets. BMJ, 344.

Frodl, T., & Amico, F. 2014. Is there an association between peripheral immune markers and structural/functional neuroimaging findings? Prog Neuropsychopharmacol Biol Psychiatry., 295-303.

García-Ptacek, S., Faxén-Irving, G., Cermáková, P., Eriksdotter, M., & Religa, D. 2014. Body mass index in dementia.. Eur J Clin Nutr., 68, 1204-1209.

Golden, S. H., Dobs, A. S., Vaidya, D., Szklo, M., Gapstur, S., et al. 2007. Endogenous sex hormones and glucose tolerance status in postmenopausal women. J.Clin.Endocrinol.Metab., 92, 1289-1295.

Gorrini, C., Gang, B. P., Bassi, C., Wakeham, A., Baniasadi, S. P., et al. 2014. Estrogen controls the survival of BRCA1-deficient cells via a PI3K-NRF2-regulated pathway. Proc.Natl.Acad.Sci.U.S.A., 111, 4472-4477.

Gupta, A., Mayer, E.,A., Sanmiguel, C.,P., Van, Horn, J,D., Woodworth, D et al. 2015. Patterns of brain structural connectivity differentiate normal weight from overweight subjects. Neuroimage Clin, 7, 506-517.

Hardy, G. 2000. Nutraceuticals and functional foods: introduction and meaning. Nutrition, 16, 688-689.

Harrison, D. E., Archer, J. R., & Astle, C. M. 1984. Effects of food restriction on aging: separation of food intake and adiposity. Proc Natl Acad Sci U S A, 81, 1835-1838.

Hogervorst, E., Matthews, F. E., & Brayne, C. 2010. Are optimal levels of testosterone associated with better cognitive function in healthy older women and men? Biochim.Biophys.Acta, 1800, 1145-1152.

Houtkooper, R., Argmann, C., Houten, S., Cantó, C., Jeninga, E., et al. 2011. The metabolic footprint of aging in mice. Sci Rep.

Kalani, R., Judge, S., Carter, C., Pahor, M., & Leeuwenburgh, C. 2006. Effects of caloric restriction and exercise on age-related, chronic inflammation assessed by C-reactive protein and interleukin-6. J.Gerontol.A Biol.Sci.Med.Sci., 61, 211-217.

Kesselheim, A., Connolly, J., Rogers, J., & Avorn, J. 2015. Mandatory disclaimers on dietary supplements do not reliably communicate the intended issues. Health Aff (Millwood)., 34, 438-446.

Lagiou, P., Sandin, S., Lof, M., Trichopoulos, D., Adami, H. O., & Weiderpass, E. 2012. Low carbohydrate-high protein diet and incidence of cardiovascular diseases in Swedish women: prospective cohort study. BMJ, 344.

Lin, W. M., Chen, M. H., Wang, H. C., Lu, C. H., Chen, P. C., et al. 2014. Association between peripheral oxidative stress and white matter damage in acute traumatic brain injury. Biomed Res Int.

Martin, B., Ji, S., Maudsley, S., & Mattson, M.,P. 2010. "Control" laboratory rodents are metabolically morbid: why it matters. Proc Natl Acad Sci, 107, 6127-6133.

Martin, D. M., Wittert, G., Burns, N. R., Haren, M. T., & Sugarman, R. 2007. Testosterone and cognitive function in ageing men: data from the Florey Adelaide Male Ageing Study (FAMAS).. Maturitas, 57, 182-194.

Martinez, J. A., Navas-Carretero, S., Saris, W. H., & Astrup, A. 2014. Personalized weight loss strategies-the role of macronutrient distribution. Nat Rev Endocrinol, 10, 749-760.

McCall, A. L. 2010. Is there a magic diet? Studying the balance of macronutrients needed for best weight loss. Curr Diab Rep, 10, 165-169.

Ogden, C.,L., Carroll, M.,D., Kit, B.,K., & Flegal, K.,M. 2014. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA. 311, 806-814.

Pleasure, D., Soulika, A., Singh, S. K., Gallo, V., & Bannerman, P. 2006. Inflammation in white matter: clinical and pathophysiological aspects. Ment Retard Dev Disabil Res Rev, 12, 141-146.

Pop, L. C., Sukumar, D., Tomaino, K., Schlussel, Y., Schneider, S. H., et al. 2015. Moderate weight loss in obese and overweight men preserves bone quality. Am J Clin Nutr, 101, 659-667.

Roberts, R. O., Roberts, L. A., Geda, Y. E., Cha, R. H., Pankratz, V. S., et al. 2012. Relative intake of macronutrients impacts risk of mild cognitive impairment or dementia.. J Alzheimers Dis., 32, 329-339.

Ryan, J., Stanczyk, F., Dennerstein, L., Mack, W., Clark, M., et al. 2012. Hormone levels and cognitive function in postmenopausal midlife women. Neurobiol Aging, 33, e11-e22.

Schulte, D. M., Hahn, M., Oberhäuser, F., Malchau, G., Schubert, M., et al. 2014. Caloric restriction increases serum testosterone concentrations in obese male subjects by two distinct mechanisms. Horm Metab Res, 46, 283-286.

Schultz, M. A., Abdel-Mageed, A. B., & Mondal, D. 2010. The nrf1 and nrf2 balance in oxidative stress regulation and androgen signaling in prostate cancer cells. Cancers (Basel), 2, 1354-1378.

Shah, S., Bell, R., Savage, G., Goldstat, R., Papalia, M., et al. 2006. Testosterone aromatization and cognition in women: a randomized, placebo-controlled trial. Menopause. 13, 600-608.

Sitzmann, B. D., Leone, E. H., Mattison, J. A., Ingram, D. K., Roth, G. S et al. 2010. Effects of moderate calorie restriction on testosterone production and semen characteristics in young rhesus macaques (Macaca mulatta). Biol Reprod, 83, 635-640.

Sohal, R.,S., & Forster, M.,J. 2014. Caloric restriction and the aging process: a critique. Free Radic Biol Med., 73, 366-382.

Solon-Biet, S. M., McMahon, A. C., Ballard, J. W., Ruohonen, K., Wu, L. E., et al. 2014. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab, 19, 418-430.

Souza, M. F., Couto-Pereira, N. S., Freese, L., Costa, P. A., Caletti, G., et al. 2014. Behavioral effects of endogenous or exogenous estradiol and progesterone on cocaine sensitization in femalerats.. Braz J Med Biol Res, 47, 505-514.

Spangler, E., Long, J., Bennett, K., Marshall, M., Minor, R., & de Cabo, R. 2010. Will Calorie restriction stave off age-related brain dysfunction, specifically to learning and memory? A review and critique of the rodent literature, in: A. Everitt, S. Rattan, D. Couteur, & R. de Cabo (Eds.), Calorie restriction, Aging and Longevity Springer, London, pp. 177-190.

Sturm, R., & Hattori, A. 2013. Morbid obesity rates continue to rise rapidly in the United States.. Int J Obes (Lond), 37, 889-891.

Wallin, K. G., & Wood, R. I. 2015. Anabolic-androgenic steroids impair set-shifting and reversal learning in male rats. Eur Neuropsychopharmacol.

Wu, J., Williams, D., Walter, G. A., Thompson, W. E., & Sidell, N. 2014. Estrogen increases Nrf2 activity through activation of the PI3K pathway in MCF-7 breast cancer cells. Exp.Cell Res., 328, 351-360.

Xu, W.,L., Atti, A.,R., Gatz, M., Pedersen, N.,L., Johansson, B., & Fratiglioni, L. 2011. Midlife overweight and obesity increase late-life dementia risk: a population-based twin study.. Neurology, 76, 1568-1574.

Yon, M. A., Mauger, S. L., & Pickavance, L. C. 2013. Relationships between dietary macronutrients and adult neurogenesis in the regulation of energy metabolism.. Br J Nutr, 109, 1573-1589.

FIGURE 4: Overall schematic and future hypotheses based on results from current projects

Curcumin + Caloric restriction
gonadal hormones:
Protective/antagonistic?

Proposed hypotheses:
Males: more than optimal levels of testosterone – cognitive dysfunction?
Females: protective effect of estrogen rescues potential antagonistic effects of combination treatment?

Curcumin:
conformational change of Keap 1

?

Caloric restriction:
mild nutrition deprivation causes stress

Keap1   Nrf2

cytoplasm

ROS → IKKβ

P Nrf2 P

NF-κB

- Delayed onset of neurobehavioral dysfunction when implemented during mid-life
- Amelioration of neurobehavioral dysfunction implemented at early senescence

AREs

nucleus

NF-κB

Antioxidant defense: Up regulation of glutathione synthesis and glutathione S-transferase

Transcription of **inflammatory markers and mediators**

Chapter V

Conclusions and future directions

Fulfillment of the specific aims with the current project has confirmed curcumin not only to have CR like properties but also suggests curcumin to be more beneficial than CR. This holds particularly true in the aged, where CR fails to produce any noticeable cognitive benefits. The current experimental design also introduced a new model for obesity associated cognitive decline, which was an appropriate analog for midlife human obesity. One of the more outstanding outcomes from both of these studies was the significant improvement in fronto-cortical function in both middle-aged and aged mice that were fed curcumin ad libitum. In both of these studies, curcumin significantly improved the executive function compared to their respective age matched controls. Interestingly, even though our hypotheses for the combination treatment did not hold, an antagonistic effect on cognitive outcomes was noted in males. The antagonistic nature of the combination treatment on males warrants future tests on hormones and dietary interventions. The comprehensive nature of the completed project fills in some critical gaps on the current understanding of aging, accelerants of age associated functional decline, and dietary interventions to treat these deficits. Based on the results and inferences from these results, future experimental designs may:

Incorporate varying ratios of macronutrients to test on the sedentary lifestyle midlife obesity model.

Obesity in humans is a heterogeneous condition and encompasses factors other than just excessive intake of calories relative to activity. Studies have shown that adiposity and its comorbidities are also dependent on macronutrient intake. For example, Solon-Biet et al. 2014 reported that a low protein: low carbohydrate diet was associated with increased body fat in late middle-aged mice, whereas mice on a low protein: high carbohydrate diet not only weighed less but had high autophagic activity, improved cardiovascular health, and a marked increase in longevity (Solon-Biet et al., 2014). A review of the literature provides an array of studies that were conducted to understand the effects of varying macronutrients on body weight and adiposity (Abete et al., 2006; Martinez et al., 2014; McCall, 2010). However, there has been minimal research directed towards understanding how macronutrient ratios may affect some of the secondary comorbidities of obesity, such as late life dementia.

Whereas the model of obesity used for the present study appears to be an adequate analog to human mid-life obesity, it does not take into account the variance of macronutrient intake in humans that can influence physiological outcomes. Several macronutrients tested alone have been shown to result in varying effects on cognition (Roberts et al., 2012; Yon et al., 2013). However, many of the current obesity models have focused on only one aspect of diet, either fat content or sugar content, which makes the results more difficult to understand in conjunction to human food intake. Since humans have a variable macronutrient intake that would likely influence their body weight, it would seem beneficial to include macronutrient ratios as a variable to study obesity in animal models. Indeed, epidemiological studies have shown that low protein, high carbohydrate diets are associated with improved health (Floegel & Pischon, 2012; Lagiou et al., 2012). Future preclinical studies on cognitive decline associated with mid-life obesity should include obese controls and a diet that mimics both the energy imbalance and macronutrient ratio of the middle-aged human obese population to aid with better translation of results to a clinical setting.

Test different doses of curcumin to elucidate the possibility of non-linear dose response effects

Based on the outcomes from both the studies, it would have been ideal to test a dose in between the 1000 and 7000 ppm range to detect whether curcumin has an inverted U-shaped dose-response function and whether this curve is gender dependent. Since the combination treatment produced an antagonistic impact on cognition, this warrants testing lower doses to clarify whether the effect was a result of combining two interventions or if a higher dose of curcumin negates the beneficial effect of caloric restriction. This information would be pivotal in providing information for a translational dose of curcumin if it were to be tested as an anti-aging therapeutic clincally. Further, an additional CURCR group with a dose of 1000 ppm of curcumin would have provided valuable insight on whether this concurrent application has an inverted U shaped dose response response in males, which could potentially explain the antagonistic effect at the high dose of curcumin. The hypothesis that curcumin plus CR would induce an additive benefit was not supported for either gender in this study. However, the combination therapy still yielded several compelling sex-dependent functional outcomes.

Androgen deficient or knockout experimental models

Since the combination treatment had distinct sex dependent functional outcomes, the optimal dose for beneficial effects on fronto-cortical functions maybe different for the sexes and dependent on gender specific hormone concentrations. The inclusion of testing for functional outcomes in female mice proved critical to reveal the gender dependent effects. The hormone specific effects should be further investigated by analyzing estrogen receptor regulation in CURCR mice or testing curcumin and CR in the presence of an estrogen blocker. Further, female mice in the present study were more active and displayed anxiolytic effects which was attributed to estrogen. Ovariectomized mice could serve as an important in vivo model to test these hypotheses (Souza et al., 2014). Additionally, testosterone deficient male mouse or androgen receptor knockouts could serve as an important model to test for antagonistic effects of the combination treatment or lack of and whether these effects are dependent on gonadal steroids. In fact, a handful of studies have also suggested exogenous testosterone therapy to be beneficial to post-menopausal women in terms of improvement in semantic and episodic memory (Ryan et al., 2012; Shah et al., 2006); suggesting beneficial effects or lack of an antagonistic effect of the CURCR treatment on females to be a result of increased testosterone.

One of the more profound findings from this study was the improvement in executive function and the lack of an effect on hippocampal dependent spatial learning. Both long term and working memory depend on proper executive functioning because strategic processes are required for task performance. Changes in the fronto-cortical and fronto-striatal regions of the brain are most likely the significant cause for reduced executive function in non-demented, normally aging older adults. Pathophysiological changes that occur with aging affecting the brain structure can cause age associated executive difficulties. One of the major changes that can impact executive function is damage to white matter and both oxidative stress and inflammation have been implicated in age associated white matter abnormalities (Frodl & Amico, 2014; Lin et al., 2014; Pleasure et al., 2006).

Based on the results from these studies, dietary curcumin holds potential as palliative care to delay the onset of fronto-cortical dysfunction commonly suffered by normal aging individuals (Buckner, 2004). Interestingly, curcumin also improved cognitive outcomes when implemented during early senescence where caloric

restriction failed to have a positive impact. Although the current experimental model provides an adequate demonstration of functional outcomes from the combination therapy and the individual interventions, the experiment would have benefited with a middle-aged male CR group to help with a comprehensive analysis. Nevertheless, results from this study offer important insights into the complexity of the several interacting systems and the differential outcomes from dietary interventions, implemented alone and concurrently.

References

Abete, I., Parra, M. D., Zulet, M. A., & Martínez, J. A. (2006). Different dietary strategies for weight loss in obesity: Role of energy and macronutrient content. *Nutr Res Rev, 19*, 5-17. doi:10.1079/NRR2006112

Buckner, R. (2004). Memory and executive function in aging and AD: Multiple factors that cause decline and reserve factors that compensate. *Neuron, 44*, 195-208.

Floegel, A., & Pischon, T. (2012). Low carbohydrate-high protein diets. *BMJ, 344* doi:10.1136/bmj.e3801

Frodl, T., & Amico, F. (2014). Is there an association between peripheral immune markers and structural/functional neuroimaging findings?. *Prog Neuropsychopharmacol Biol Psychiatry.,* 295-303. doi:10.1016/j.pnpbp.2012.12.013.

Lagiou, P., Sandin, S., Lof, M., Trichopoulos, D., Adami, H. O., & Weiderpass, E. (2012). Low carbohydrate-high protein diet and incidence of cardiovascular diseases in swedish women: Prospective cohort study. *BMJ, 344* doi:10.1136/bmj.e4026

Lin, W. M., Chen, M. H., Wang, H. C., Lu, C. H., Chen, P. C., Chen, H. L., Lin, W. C. (2014). Association between peripheral oxidative stress and white matter damage in acute traumatic brain injury. *Biomed Res Int.,* doi:10.1155/2014/340936

Martinez, J. A., Navas-Carretero, S., Saris, W. H., & Astrup, A. (2014). Personalized weight loss strategies-the role of macronutrient distribution. *Nat Rev Endocrinol, 10*, 749-760. doi:10.1038/nrendo.2014.175

McCall, A. L. (2010). Is there a magic diet? studying the balance of macronutrients needed for best weight loss.. *Curr Diab Rep, 10*, 165-169. doi:10.1007/s11892-010-0105-7.

Pleasure, D., Soulika, A., Singh, S. K., Gallo, V., & Bannerman, P. (2006). Inflammation in white matter: Clinical and pathophysiological aspects. *Ment Retard Dev Disabil Res Rev, 12*, 141-146.

Roberts, R. O., Roberts, L. A., Geda, Y. E., Cha, R. H., Pankratz, V. S., O'Connor, H. M., . . . Petersen, R. C. (2012). Relative intake of macronutrients impacts risk of mild cognitive impairment or dementia.. *J Alzheimers Dis., 32*, 329-339. doi:10.3233/JAD-2012-120862

Ryan, J., Stanczyk, F., Dennerstein, L., Mack, W., Clark, M., Szoeke, C., Henderson, V. (2012). Hormone levels and cognitive function in postmenopausal midlife women. *Neurobiol Aging, 33*, e11-e22.

Shah, S., Bell, R., Savage, G., Goldstat, R., Papalia, M., Kulkarni, J., Davis, S. (2006). Testosterone aromatization and cognition in women: A randomized, placebo-controlled trial.. *Menopause., 13*, 600-608.

Solon-Biet, S. M., McMahon, A. C., Ballard, J. W., Ruohonen, K., Wu, L. E., Cogger, V. C., Simpson, S. J. (2014). The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice.. *Cell Metab, 19*, 418-430. doi:10.1016/j.cmet.2014.02.009

Souza, M. F., Couto-Pereira, N. S., Freese, L., Costa, P. A., Caletti, G., Bisognin, K. M., Barros, H. M. (2014). Behavioral effects of endogenous or exogenous estradiol and progesterone on cocaine sensitization in femalerats.. *Braz J Med Biol Res, 47*, 505-514.

Yon, M. A., Mauger, S. L., & Pickavance, L. C. (2013). Relationships between dietary macronutrients and adult neurogenesis in the regulation of energy metabolism.. *Br J Nutr, 109*, 1573-1589.