ABSTRACT

Title of Document: VARIABILITY IN COGNITIVE PERFORMANCE AND LEARNING IN YOUNGER AND OLDER ADULTS EXPLAINED BY CARDIOVASCULAR FITNESS, PHYSICAL ACTIVITY, AND *APOE* GENOTYPE

Maureen Kellogg Kayes, 2013

Directed By: Dr. Professor Bradley D. Hatfield, Professor and Chair, Department of Kinesiology

This dissertation investigated the association of physical activity with cognition in two cross-sectional studies. Physical activity has been positively associated with cognitive function, and in older adult populations has shown an additional benefit for carriers of the ApoE- ε4 allele. Cognitive training has also revealed a benefit for improved cognitive performance. Questions remain, however, about the interaction of these factors in their relation with cognition. One study addressed the relationship between physical activity and cognitive performance during executive function and working memory challenges in adults ages 50-70, and the other explored the role that physical activity plays in learning in adults ages 22-50 undergoing an online cognitive training intervention. In both studies, regard for influence of the ApoE genotype was considered, and the concept of specificity of physical activity was explored through employment of measures of both cardiovascular fitness and weekly physical activity kilocalorie expenditure.

The study of older adults revealed that performance on a working-memory task was positively related to weekly kilocalorie expenditure in *APOE*-ε4 carriers, with no

such benefit for non-carriers during a moderate challenge condition of the task, while a positive relationship was revealed for both ε4 carriers and non-carriers during a more challenging condition, but the magnitude of the relationship was greater in ε4 carriers. The study of younger adults revealed no transfer benefits for cognitive training; however, cardiovascular fitness was positively related to performance after the intervention on a transfer task of proactive interference, and a positive trend was also found for cardiovascular fitness on a divided-attention language vocabulary learning task. No association was observed with regard to *APOE*-ε4 genotype for any post-intervention task or learning transfer challenge. Taken together, these studies reveal that physical activity is associated with improved cognition in younger and older adults alike, but with specificity as to volume or intensity of physical activity mediating the relationship, cognitive processes benefited, and the role that the *APOE*-ε4 genotype plays.

VARIABILITY IN COGNITIVE PERFORMANCE AND LEARNING IN YOUNGER AND OLDER ADULTS EXPLAINED BY CARDIOVASCULAR FITNESS, PHYSICAL ACTIVITY, AND *APOE* GENOTYPE

By

Maureen Kellogg Kayes

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Advisory Committee:
Professor Bradley D. Hatfield, Chair
Professor Ben F. Hurley
Associate Professor Stephen M. Roth
Associate Professor Michael R. Dougherty, Dean's Representative
Assistant Professor Donald J. Bolger

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have gained understanding that is going to be most helpful for future research, enabling me to knowledgeably select appropriate cognition-related instruments, going beyond the method of choosing a measure because “others have used it”.

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Chapter 1: Introduction

This dissertation is composed of two studies examining the relation of physical activity with cognition – one examining a battery of cognitive challenges in areas of inhibitory control, working memory, and cognitive flexibility in older adults ages 50-70, and the other exploring the physical activity/cognition relationship in younger adults ages 22 -50 involved in a cognitive training intervention. An aim of this work is to study the differences in cognitive performance and learning in these two groups in order to more clearly define the role of physical activity throughout the lifespan in relation to cognition. This research is of great importance as maintenance of cognitive function throughout life is a major concern, both in terms of cognitive decline and dementia in older adults, and in maximizing performance in school, employment and personal life for younger adults. Looking at five decades of cognitive performance in relation to both cardiovascular fitness and total energy expenditure offers a chance to find clues into how an investment in physical fitness may promote brain fitness.

The role of genetics is also an important factor in the physical activity/cognition relationship. The *APOE*-ε4 genotype is of particular interest because it is a known risk factor for Alzheimer's type dementia and is negatively associated with cognitive function in healthy older adults, and there are also questions as to its role in relation to cognition earlier in life. Previous research has shown that physical activity may be of special benefit to slow cognitive decline for individuals possessing the ε4 allele, and in both studies consideration will be given to the interactive role of physical activity with this gene in relation to cognitive performance.

This work will be introduced with a chapter explaining neurobiological benefits of exercise and reviewing the concept of cognitive reserve and how physical activity may counteract both normal and pathological cognitive aging. The next chapter follows up on the theoretical discussion with a report of the results of a study of older adults. A second literature review regarding the relationship of physical activity and cognition in younger adults is presented in the following chapter, followed by a chapter reporting findings about the role of physical activity in relation to cognitive learning in a younger adult population.

Chapter 2: Influence of Physical Activity on Brain Aging and Cognition: the Role of Cognitive Reserve, Thresholds for Decline, Genetic Influence, and the Investment Hypothesis

Maureen K. Kayes and Bradley D. Hatfield

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Introduction

The purpose of this paper is to highlight how physical activity (i.e., gross bodily movement) and exercise (i.e., engagement in purposeful physical activity to improve physiological capacity) improve cognitive function in the areas of thinking and memory**.** Just as physical activity benefits cardiovascular and musculoskeletal function, it also exerts remarkable effects on the brain resulting in the enhancement of cognitive function through the lifespan and the delay of cognitive decline with aging. Positive effects have been observed in the brains of animals and humans with benefits accruing in processing speed, working memory, learning, attention, executive and inhibitory control, as well as mental flexibility. Exercise has been shown to increase oxygen delivery to the brain and to expand blood vessels and mitochondria, resulting in improved metabolic capacity. Exercise also improves uptake of neurotransmitters and increases levels of neurotrophic factors, enabling more efficient brain communication, better maintenance and repair of neurons, and improved dendritic strength and connectivity. Evidence has even been provided in animal studies for the generation of new neurons in an area of the brain susceptible to deterioration with age, the hippocampal region that mediates the formation of new memories. All of these neurobiological effects contribute to a better working brain that is more efficient and can withstand insult from injury, stress, or aging, with greater integrity, ability to focus, networking between brain regions, and improved ability to adapt to change**.**

The chapter is organized around a three-part conceptual framework in which the first component considered is that of central nervous system decline as related to both normal aging and pathological conditions such as Alzheimer's disease. A reasonable

assumption is that the alterations in the anatomy and physiology of the brain with age and disease translate or present as cognitive decline in the form of memory loss and executive dysfunction. The second component considered is that related to the evidence for the benefits of physical activity and exercise to the brain, which has been revealed in both animal-based and human studies. In essence, these neurobiological benefits serve as a non-pharmacological prescription that can counteract the neural degeneration that is incurred over time. The third component considered is the promotion of resilience to age-related and pathological degeneration by building biological reserve of the brain. In essence, participation in physical activity serves to 'vaccinate' the brain such that it can tolerate insult, injury, or cognitive decline to a greater degree as compared to the sedentary lifestyle. This model of exercise-induced tolerance to age and diseases of the central nervous system applies to the entire brain and is the central concept offered in this chapter. The principle provides a reasonable explanation of the benefits of exercise to cognitive function in older men and women. In addition, the masking of degenerative processes may be achieved through active compensatory strategies by which alternative brain networks are activated to respond to a given cognitive challenge when typical brain responses fail to meet the challenge. Interestingly, the foundation for this benefit during older age may well be laid during childhood and young and middle adulthood, as the benefits accruing to the brain may not reveal until significant decline has occurred such as experienced with advanced age and the presence of pathology. In this manner, a physically active lifestyle at a younger age could be conceived as an 'investment' that provides apparent benefits to cognition and emotion in older age. Furthermore, genetic influence on the brain may yield different magnitudes of benefit from physical activity

such that those who are at greater risk of age-related decline or accelerated aging, like carriers of the *APOE* ε4 allele who appear less resilient to neuropathology, have an apparent benefit relative to those who practice a sedentary lifestyle, thus underscoring the notion that exercise is essential medicine for some.

A Brief History of the Field

A number of individuals have made outstanding contribution to the study of physical activity and neurocognitive processes with some providing truly pioneering investigations. Seminal research on physical activity and cognitive function appeared in the 1970's with animal and human investigations into the role of dopamine and reaction time, with comparisons of young and older subjects (Spirduso, 1983). More specifically, Waneen Spirsuso reported faster reactivity (i.e. shortened reaction times) in older men and women who engaged in regular physical activity and sport activities while advancing the notion that reaction time was an index of general integrity of the central nervous system. As such, Spirduso believed that an active lifestyle promoted the health of the brain and provided biological plausibility for such a benefit in humans by the employment of animal models (rats) with demonstration of maintenance of central dopamine levels and increased receptor binding affinity with exercise across the lifespan. The research evolved beyond the consideration of reactivity with assessment of exercise-induced effects on perceptual and basic decision-making abilities in the first reported randomized clinical trial (RCT) with older men and women (Dustman et al., 1984). This classic work, reported by Robert Dustman and colleagues in Neurobiology of Aging, provides the initial causal evidence of the benefit of physical activity on neurocognitive function in light of the rigorous experimental design. Subsequently, identification of

specific cognitive tasks that benefitted most from exercise participation were identified by Chodzko-Zajko and Moore (1994), who described the difference between crystallized (i.e., vocabulary and accumulated knowledge) and fluid intelligence (i.e., effortful analyses and reasoning) with the former revealing little benefit from physical activity while, conversely, the latter revealed significant positive influence. This was the first time that specificity of the effect of exercise was reported, and determination that tasks that were more attentionally demanding, or more novel, were the most likely to benefit from physical fitness**.** Kramer and colleagues (1999) advanced this concept further with the publication of a landmark study that further supported cognitive specificity in the response to exercise such that executive function tasks were particularly improved by engagement in physical activity.

Early in the 1990's the psychophysiological approach to study in this area was introduced to the field, with use of event-related potentials (ERPs) derived from electroencephalography (EEG) to determine workings of the brain in both young and older men who were characterized by low and high levels of cardiovascular fitness. Specifically, Dustman et al. (1990) examined the latencies and amplitudes of ERPs to determine the speed and engagement, respectively, of cerebral cortical responses to various stimuli as well as assessment of cortico-cortical communication between brain regions, and reported that high-fit older individuals exhibited relatively efficient neural responses and integrative processing when compared to low-fit older men**.** In fact, the high-fit older men were more similar to younger men than they were to their age-matched counterparts – in essence, it appeared that physical fitness had arrested the aging of the human brain. The neurobiological basis of such effects in the human brain were provided

by the first mechanistic studies on the neurogenic and neurotrophic effects of exercise that were observed in animal studies conducted under the leadership of Gage (van Praag, Kemperman, & Gage, 1999) and Cotman (Neeper, Gomez-Pinilla, Choi, & Cotman, 1995), respectively. These studies offered a robust and profound mechanism for the impact on cognition of a physically active lifestyle in children and men and women of all ages if such effects also occurred in the human brain. The first neuroimaging study, using structural magnetic resonance imaging (MRI), was reported by Colcombe at al. (2003) and revealed a positive relationship in brain tissue density and the integrity of white matter tracts with cardiovascular fitness in older men and women. These structural differences in the brains of individuals characterized by high and low fitness, suggested that a physically active lifestyle positively impacted the scaffolding of the human brain. This notion is based on the finding that older adults who were characterized by cardiovascular fitness exhibited resistance to atrophy of the brain with aging. Further evidence that exercise causally influenced the brain in a positive manner was provided by Colcombe et al. (2004) who reported the results of a randomized control trial of the effects of cardiovascular conditioning on cortical and subcortical activation during executive challenge. In effect, the exercise training promoted an enhanced hemodynamic response during challenge via assessment of functional magnetic resonance imaging (fMRI) blood-oxygen level dependent (BOLD) response suggesting improved concentration and focus as a result of training. The studies reported by Colcombe and Kramer were the first to employ a measure of high spatial resolution in their studies of exercise and the brain (e.g., MRI) thus increasing our understanding and confidence in the biological underpinnings of the impact of exercise on the human brain.

The consideration of individual differences through the role of genetics was brought into the picture in 2001 with a landmark study that showed particularly strong cognitive benefits of physical activity for carriers of the *APOE*-ε4 genotype, which has been associated with a higher risk of Alzheimer's dementia (AD) (Schuit et al., 2001)**.** More recently, a randomized clinical trial with employment of MRI in middle-aged men and women was reported by Erikson and colleagues (2011) that specifically examined the impact of exercise on the hippocampal region, which is the brain region primarily attacked by the pathology (e.g., plaques and tangles) that is responsible for AD. The work holds profound implications for the prophylactic effect of exercise as a prescription for this terrible affliction. Research has continued to evolve in the aging literature, with neuroimaging studies investigating possible reversal of cognitive aging processes through exercise intervention with mid-life and older adults (Colcombe et al. 2004; Erikson et al., 2011), and detailed looks at the connectivity, or networking, of the brain, which appears to be more efficient with physical activity (Burdette et al., 2010; Voss, Erikson, et al., 2010; Voss, Prakash, et al., 2010)**.** Investigations of the effects of physical activity in children have recently appeared in the literature as well, with consensus in the findings that aerobic fitness promotes superior executive functioning, improved academic performance, and increased brain volume of specific areas (Chaddock, Erikson, Prakash, Van Patter ,et al., 2010; Chaddock, Erikson, Prakish, Kim, et al., 2010; Chaddock, Hillman, Buck, & Cohen, 2011; Hillman, Castelli, & Buck, 2005)**.** Collectively, both prospective and retrospective epidemiological studies, as well as the experimental studies with animal and human subjects cited above, strongly support the lifelong effects of a physically active lifestyle, with promising results for "cognitive reserve" suggesting that

an investment of physical activity and other healthy lifestyle factors throughout life may promote resilience to cognitive decline in old age (Scarmeas & Stern, 2003; Rovio et al., 2005).

It is truly amazing to consider the remarkable advances in our understanding of the influence of physical activity and exercise on the brain and cognition in a period spanning just 35 years from the seminal work of Spirduso in the mid-seventies to the present time with sophisticated molecular biology and advanced neuroimaging studies. In essence, modern science is providing confirmatory evidence for the ancient notion of Mens Sana in Corpore Sano – that is a "sound/healthy mind within a sound/healthy body."

Exercise and Aging - Normal Versus Pathologic

The beneficial influence of exercise on brain processes and cognition is pronounced when the opportunity for benefit is significant. Such is the case in older men and women (e.g., typically over the age of 60 years) who are likely to incur age-related decline of the central nervous system (CNS), promoted by such processes as inflammation, oxidative stress, and apoptosis (Whalley, Deary, Appleton, & Starr, 2004) thus allowing 'room for improvement' from physical activity participation compared to those who are characterized by relative integrity of brain processes as would be the case with young men and women. As such, investigations with older adults have been in the forefront of exercise and cognition research because this population appears to show some of the strongest benefits for physical activity in terms of a protective effect against both normal cognitive decline with aging as well as dementia-related pathology and deterioration.

It is helpful to understand the differences between "normal" or "demented" aging in order to understand the effects of physical activity more clearly. Individuals change over a lifetime, and as one ages, change in the brain can occur either as a normal process or, alternatively, as pathological aging manifested as various forms of dementia such as Alzheimer's disease. The effects of normal aging, currently understood as a reduced ability for synaptic plasticity as opposed to a significant loss of neurons (Trachtenberg et al., 2002)**,** reveal in the cognitive-behavioral domain as slower processing speed, less efficient working and episodic memory, as well as difficulties with executive function – a set of behaviors typically described as organizing, goal direction, and inhibition (Park & Lorenz-Reuter, 2009). The loss of executive function can present in several ways such as perseveration or persistence in cognitive set as opposed to adaptive set shifting. This kind of age-related behavioral change can be revealed with challenge such as presentation of the Wisconsin Card Sort Test to older individuals, which requires mental flexibility. Typically, such individuals exhibit difficulty in recognition and adoption of a new rule to guide behavior relative to younger men and women and this likely due to frontally-mediated executive decline where such processes are largely mediated (West, 1996). Interestingly, knowledge and expertise tend to be maintained in normal aging (Park & Lorenz-Reuter, 2009) and it likely due to the relative independence of such processes in relation to the frontal lobes**.**

The cognitive decline associated with age is typically accompanied by structural changes in the brain. There is a decrease in brain volume in normal aging that occurs by the seventh decade (West, 1996) with major decreases found primarily in caudate, cerebellum, frontal gray matter, and hippocampal regions (Head, Snyder, Girton, Morris,

& Buckner, 2005; Park & Lorenz-Reuter, 2009; Raz et al., 2005). There are also changes in the characteristics of white matter, which is composed of the axonal projections and tracts in the brain, with the greatest loss of integrity concentrated in the frontal area (Park & Lorenz-Reuter, 2009). Since white matter is associated with loss of myelin, this change may be correlated with the 'slowing' of perceptual, cognitive, and motor processes typically observed in older adults (Park & Lorenz-Reuter, 2009). White matter hyperintensities, revealed as abnormal alterations of healthy structure in structural magnetic resonance images, increase with aging (Burns et al., 2005; Sonntag, Lynch, Cooney, & Hutchens, 1997; Wen & Sachdev, 2004). Loss of dendritic complexity, with reduction in synapses, is another structural change with normal aging (West, 1996), as is reduction of blood vessel density and blood flow (Sontagg et al., 1997), both of which contribute to the alterations in memory, focus, and mental flexibility.

One common feature of non-pathological aging is a tendency towards reduced levels of regional specialization in neural processes when negotiating a cognitive challenge as compared to the neural activity seen in younger individuals. This difference is exemplified by bilateral cerebral hemispheric activation during problem solving (i.e., heightened activation of the brain when compared to younger adults who exhibit specificity of hemispheric activation) (Park & Lorenz-Reuter, 2009). Such bilateral activation of the prefrontal cortex has been observed consistently in older adults, and suggests an adaptive compensatory mechanism, allowing for the requisite recruitment of neural resources to accomplish a task. While this adaptation, known as hemispheric asymmetry reduction in older men and women (Cabeza, 2002), has traditionally been considered indicative of diminished cognitive ability, current research suggests that it

may be a positive and dynamic adaptation to a normally aging (i.e., changing) brain. Accordingly, individuals who are able to adjust to such changes are likely to maintain performance for a longer period through the aging process. In support of this notion compensation has been shown to be associated with individuals with higher intellectual and physical activity (Stern, 2002; Voss, Erikson, et al., 2010)**,** who retain performance in attention- and memory-related functions. As a general proposition, it appears that normal aging is associated with accelerated frontal decline, relative to other brain regions, and that compensatory activation patterns are engaged as a coping strategy, particularly in the frontal region, in order to maintain executive function. Failure to maintain such an adaptive strategy can then reveal as a loss, to some degree, of executive function such as focus or working memory.

The characterization of demented aging is less clear than that of normal cognitive aging. In terms of development toward dementia, there are questions as to whether Alzheimer's disease is simply an exaggerated form of natural aging or whether it is a distinct disease process. Although there is no definitive anatomic or physiological landmark available that allows for a clinical diagnosis, there are data from neuroimaging investigations that appear to show distinct patterns of brain processes progressing towards dementia. Differences in brain activation and structural atrophy have been employed successfully to identify pathological aging. In this manner Head et al. (2005) examined brain volume in young compared to older adults who were classified as cognitively healthy or who were diagnosed with dementia. They reported an overall loss in frontal volume for all older adults regardless of status when compared to younger

adults. However, the hippocampal structure of those with dementia revealed significant atrophy.

In another series of investigations, Sperling (2007) employed a face-name recognition task during fMRI to assess the BOLD response, in order to compare young and older adults, the latter who were cognitively healthy versus those diagnosed with Alzheimer's disease (AD) and mild cognitive impairment (MCI). MCI is characterized by memory dysfunction for which the magnitude does not impair the ability to maintain activities of independent daily living. MCI does not always convert to AD, but is considered by some as a prodromal stage. The AD patients exhibited a pattern of hypoactivation in the hippocampal region that was not present in older healthy adults. Sperling's investigation with MCI patients revealed a period of hyperactivation of the hippocampus in early MCI, which changed to hypoactivation with late MCI. This dynamic evolution of brain activation could indicate a threshold whereby compensation described in normal aging is no longer able to work as deterioration of brain structure goes beyond the ability to function normally, rendering adaptation no longer possible (Sperling, 2007). The failure to maintain compensation can then reveal as memory impairment.

The critical physical change in the brain associated with pathologic aging is the significant development of neurotoxic extracellular amyloid plaques and neurofibrillary tangles. Plaques are formed from amyloid peptides that are released from the amyloid precursor protein (APP), which is cleaved by beta and gamma secretases. In the most destructive form APP is cleaved to a longer B42 form that has a greater tendency to aggregate destructively (Bu, 2009). In addition, neurofibrillary tangles are formed from

intracellular hyperphosphorylated tau protein. Tau protein is important to the cell, aiding in scaffolding of microtubules, but when the pathologic process occurs it becomes hyperphosphorylated, forming tangles that disturb cell structure and function. The amyloid cascade theory postulates that these two processes are responsible for the beginning of the disease process of dementia, and that the destruction that follows is due to the degenerative properties of the plaques and tangles (Hardy & Selkoe, 2002). Heightened activation of critical brain regions during challenge may provide for maintenance of cognitive function, but may also serve as a "forecast" of cognitive decline to come. In the future, if the cost of such neuroimaging (e.g., fMRI) is reduced, routine examination of brain activation during medical exams over time may serve to predict forthcoming cognitive decline.

Although few studies have been conducted on the efficacy of exercise to influence neurocognitive processes in the elderly suffering from dementia, there is ample evidence of benefit in those who are normally aging, albeit somewhat more apparent for frontal regions and executive functions relative to other brain regions and non-executive tasks (Colcombe & Kramer, 2003). That is, cognition is maintained over time or even improved in several domains, but the magnitude of benefit is greatest with frontally mediated executive tasks. A brief summary follows as to the biological plausibility of 'exercise as good medicine for the mind'.

Neuorobiological Benefits of Exercise on Brain and Cognition

While there is robust evidence for the benefits of physical activity for cognition in humans, the mechanisms that determine these effects are not fully understood. However, animal studies have revealed several factors that appear to contribute positively to

cognitive function. Examination of learning and memory in animals exposed to either to an enriched environment (ladders, tubes, ropes, toys) or aerobic exercise regimens (running wheels or treadmill running), has consistently revealed beneficial effects of exercise on cognitive function in young, adult, and aged animals.

In humans, it is challenging to determine specific effects of physical activity on brain function in light of ethical constraints, but noninvasive neuroimaging in tandem with cognitive-behavioral testing is informative of how exercise may impact the human brain. Overall, the use of animal studies to complement investigations involving human subjects suggests pathways and mechanisms for the beneficial effect of physical activity on the human brain. Neuroimaging studies, such as those described previously, have offered insight into brain mechanisms underlying cognition, such as compensatory activity, and these tools can be used to examine the effects of exercise on the brain. The benefits described below in this section are not likely to reverse any pathology such as plaques and tangles in the brain, but they may help to build the reserve or resilience to both normal brain aging and pathology so that the individual may tolerate such degradation, be it largely concentrated in the frontal region as in normal aging or in the hippocampal region as in demented aging.

In this manner, we maintain that the primary role of physical activity and exercise on the human brain is to build reserve. Specifically, cognitive reserve is the hypothesized capacity of the mature adult brain to sustain the effects of disease or injury sufficient to cause clinical dementia in an individual possessing less cognitive reserve (Whalley et al., 2004). This important element of brain integrity can be classified as both active and passive reserve. The former refers to the efficiency and adaptability of neural circuits to

respond to cognitive challenge exemplified by compensation, as described above, while the latter refers to brain tissue density, white matter integrity, and vascularity. All of the neurobiological benefits of exercise likely contribute to a healthier cognitive reserve throughout life (note; most likely passive reserve), and are particularly beneficial in older adults to delay cognitive decline.

The following elements of the neurobiological response to exercise are likely contributors to cognitive reserve:

Neurotransmitters

Physical activity elevates a number of neurotransmitters that enable communication between neurons underlying cognitive, motor, and affective processes. Dopamine in particular exerts a significant effect on the quality of cognitive function, particularly on motor and executive processes in light of the relevant anatomical structures in these regions (i.e., basal ganglia and frontal lobes, respectively). Animal research by Meeusen et al. (1997) revealed that six weeks of running increased dopamine release in the striatum in rats, and Goekint et al. (2012) found that a 60 minute acute bout of treadmill running in rats resulted in a twofold increase in hippocampal dopamine release when compared to sedentary controls. Running has also shown to reduce inflammation injury-induced degeneration of dopamine in the substantia nigra through the upregulation of brain-derived neurotrophic factor (BDNF) in mice (Wu, Wang, Jen, Chuang, & Wu, 2011). Other dopamine-related investigations have shown that exercise offers dopaminergic neuroprotection. Zigmond, Cameron, Hoffer, & Smeyne, (2012) revealed that physical exercise reduced neurobiological and behavioral deficits induced by administration of toxins known to cause dopamine deficiency, and O'Dell, Galvez,

Ball, & Marshall (2012) found that physical exercise ameliorated dopamine transporter damage in the striatum in rats after administration of methamphetamine.

Acetylcholine is also affected by exercise. Park, Pappas, Murtha, and Ally (1992) reported that an enriched environment (including running wheel) for rats resulted in higher acetylcholine synthesis in the caudate, cortex, and hippocampus. The significance of such effects was revealed in a lesion study on cholinergic neural tracts in rats as the lesions resulted in diminished neurogenesis in the dentate gyrus and increased cell death (Cooper-Kuhn, Winkler, & Kuhn, 2004). The importance of the finding rests on the fact that acetylcholine is critical to memory, and administration of acetylcholine after its depletion results in reversal of memory deficits (Parent & Baxter, 2004).

Norepinephrine adaptations also occur with exercise in animals. Brown et al. (1979) determined that norepinephrine levels were greater in the cerebral cortex after exercise training in rats and Morishima et al. (2006) observed elevated norepinephrine in the hippocampus after wheel running in rats. The effect of noradrenaline in relation to BDNF was explored by Garcia, Chen, Garza, Cotman, & Russo-Neustadt (2003) who found that noradrenergic blockade inhibited exercise-induced BDNF transcription in the hippocampus. The importance of these findings relates to this neurotransmitter function in hippocampal-related memory (Murchison et al., 2004) and a role in attention regulation in prefrontal cortex (Arnsten & Li, 2005). Regarding serotonin (5HT), there is evidence that exercise results in increased 5HT levels in several areas of the brain, including the hippocampus (Blomstrand, Perrett, Parry-Billings, & Newsholme, 1989; Lista & Sorrentino, 2010). Gomez-Merino, Béquet, Berthelot, Chennaoui, & Guezennec (2001) found increased 5-HT levels in both the hippocampus and cortex after 90 minutes

of exercise in rats, with a rapid decrease in cortical area but longer retention in the hippocampus. Of note, serotonin is closely associated with brain-derived neurotrophic factor (BDNF) (Martinowich & Lu, 2008; Mattson, Maudsley, & Martin, 2004)) and neurogenesis (Brezun & Daszuta, 2000) in the hippocampal region, and depletion of serotonin in the hippocampus has been shown to result in a depletion of neurogenesis (Brezun & Daszuta, 1999).

Neurotrophic Factors

Another way that physical activity likely benefits cognitive function in the human brain is through increased expression of neurotrophins. Neurotrophins are a family of growth factors that are responsible for the survival and maintenance of neurons and work towards growth and development of dendritic connections. The main neurotrophin that has shown a strong connection to exercise's role in cognitive function is BDNF, which supports neuronal maintenance and repair in many areas of the brain and is particularly active in the hippocampus and cerebral cortex (Neeper et al., 1995). It is associated with long term potentiation (LTP) that is believed to underlie learning and memory (Farmer et al., 2004), and facilitates synaptic plasticity. The blockage of BDNF uptake has been shown to reduce LTP and degrade learning and memory relative to animals not receiving BDNF blockade, (Korte et al., 1995; Lista & Sorrentino, 2010). Conversely, increased brain BNDF levels are associated with dendritic complexity in the dentate gyrus, greater arborization, and increased dendritic length (Redila & Christie, 2006).

Studies reveal that BDNF upload is regulated through activity such as exercise as well as an enriched environment (Cotman, Berchtold, & Christie, 2007; van Praag, Kemperman, & Gage, 2000). Uptake of BDNF in the hippocampus has been shown to be

greatly increased with physical activity in animals. In fact, BNDF mRNA has increased as high as 20% with running wheel activity (Neeper et al., 1995). Tong, Shen, Perreau, Balaz, & Cotman (2001) showed in a genetic study that physical activity resulted in an elevation of numerous proteins related to BDNF and synaptogenesis, LTP, and synaptic plasticity, all providing additional support for findings of BDNF and downstream effects. In addition, a recent clinical trial with human subjects examined memory performance on the Auditory Verbal Learning Test (AVLT) after a 6-month exercise period in older adults and revealed significant improvement in cognition as well as a positive effect for peripheral BDNF (Ruschweyh et al., 2009), reinforcing the notion that the neurotrophic effects of exercise in animals likely occur in the human brain as well. There is a polymorphism of the BDNF gene (val^{66}met) that has been shown to have effects on cognitive function, with individuals with BDNF Met revealing relatively impaired memory and hippocampal differences (Hariri et al., 2003). While there has been no published physical activity research to date in relation to cognition and this genetic relation, this may be important research in the future.

Other studies have revealed that different neurotrophic factors such as insulin-like growth factor-1 (IGF-1), a neurotrophin with structural similarities to insulin**,** may also be involved in the beneficial effects of exercise on cognition. Carro et al. (2000) observed that serum IGF-I is upregulated from peripheral sources to several areas of the brain such as the cortex, hippocampus, striatum, cerebellum, thalamus, hypothalamus and brain stem nuclei as a result of physical activity. Ding, Zhou, Rafols, Clark, & Ding (2006) also reported that exercise elevated hippocampal IGF-I and that blocking the uptake of IGF-I reversed the exercise-induced elevation of hippocampal BDNF, eliminated the benefits of

exercise on recall in the Morris Water Maze memory task, and abolished the effects of proteins downstream in the BDNF pathway related to synaptic function such as synapsin I (Ding et al., 2006). IGF-I is also known to promote both neurogenesis and angiogenesis in animals, with blockage of receptor uptake by IGF-I completely eliminating exercise effects on neurogenesis (Trejo, Carro, & Torres-Aleman, 2001) and blood vessel remodeling (Lopez-Lopez, LeRoith, & Torres-Aleman, 2004)**.**

Vascular endothelial growth factor (VEGF) is a protein that also appears relevant to the benefit of exercise on cognition. VEGF is involved in angiogenesis and neurogenesis, suggesting an interaction between the two processes (Cao et al., 2004). Experimental administration of VEGF has been shown to result in improvement in learning on a passive-avoidance task and as well as memory processes during the performance of the Morris Water Maze in rats (Cao et al., 2004) while the blockade of peripheral VEGF results in elimination of exercise-related increased neurogenesis. Blockage also affects generation of new blood vessels in the brain, while not affecting the existing vasculature (Fabel et al., 2003)**.** Overall, VEGF acts in concert with both IGF-I and BDNF, and appears to play an important modulatory role on the effects of physical activity on brain processes.

Synaptogenesis

There is evidence for synaptogenesis as a result of exposure to enriched environments as animal studies have revealed that such exposure positively contributes to dendritic branching and synaptic density. Studies have demonstrated elevations in synapse number, with more synapses per neuron, as well as the strengthening of existing synaptic connections (Bruel-Jungerman, Davis, & Laroche, 2007; Markham &

Greenough, 2004). For example, **s**ynaptogenesis has been observed in mice exposed to complex obstacle courses requiring "acrobatic" activity, with specific adaptation in the motor and cerebellar cortices when compared to wheel running or sedentary controls (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990). The protein synapsin I, which acts to enhance neuronal communication through neurite outgrowth and synaptogenesis, is elevated with exercise and appears to be upregulated by the exercise-induced elevation of BDNF (Vaynman & Gomez-Pinilla, 2005)**.** The relevance of this work to the exercise-cognition relationship in humans is that engagement in exercise typically occurs in complex settings such as sport activities and outdoor settings that require navigation and memory of complex running routes. It seems reasonable then that regular participation in physical activity would positively impact the vascular and neuronal architecture of the human brain thus contributing to the resilience of active and fit individual to age-related and disease-related degenerative processes.

Neurogenesis

Beyond the neurotrophic effects of exercise described above, neurogenesis, the growth of new neurons in the brain, as revealed in the dentate gyrus in animals, represents an exciting finding that holds great promise for medicine since the appearance of new neurons in the human hippocampus–the brain region most affected by AD dementia – would suggest resilience to the pathology (i.e., plaques and tangles) through participation in physical activity. In a classic study van Pragg, Kemperman, and Gage (1999) reported that neurogenesis in adult mice occurred in response to wheel running activity. After controlling for the effects of environment, they determined that physical activity, alone, was responsible for the observed proliferation of neurons in the dentate

gyrus. In addition, van Praag, Christie, Sejnowski, & Gage (1999) determined that the appearance of new hippocampal cells in adult mice housed with running wheels was associated with improved cognition, as shown in performance on the Morris Water Maze, and enhanced LTP, when compared to controls in standard housing .

Although neurogenesis is reduced in older adult animals (Kim et al., 2004), it has been shown that exercise can offer benefits for the creation of new neurons even during advanced age. van Praag, Shubert, Zhao, & Gage (2005) extended the study of neurogenesis and enhanced learning in response to physical activity with aged mice who were sedentary until they were 19 months old. After a 1-month running wheel intervention, they observed that aged mice exhibited improved water- maze performance compared to age-matched controls, which coincided with a reversal of decline in neurogenic activity. Comparison of the older exercisers to young adult mice revealed that the new neurons from enhanced neurogenesis in the older group were similar in quality to those of the younger mice. The investigators observed equivalent dendritic length and spine density, revealing that neurons in aged mice were functionally equivalent to those of younger animals. Such an effect in older humans would underscore the notion that it is never too late to accrue the benefits of a physically active lifestyle.

Neurogenesis cannot be determined directly in humans, although one study did confirm the process through postmortem examination (Eriksson et al., 1998). A recent 12-month exercise intervention in older adults by Erikson et al. (2011) revealed a specific increase in anterior hippocampal volume of 2% for an aerobic exercise group, while a decline in volume of 1.4% was seen over the course of the study in a comparison group that did stretching and toning activities. The change was accompanied by increases in

circulating serum BDNF. Importantly, this increase in hippocampal volume was in the area where the dentate gyrus is located – the area where neurogenesis occurs. These researchers commented that normal hippocampal decline with aging averages 1-2% per year, so this increase in volume in essence offered up to two years of reversal of deterioration for one year of exercise. While the study results can not pinpoint that the hippocampal volume increases are indicative of neuronal cell proliferation and could alternatively be indicative of increases in dendritic branching or angiogenesis as well, the findings do show that the hippocampus is subject to positive change in aging individuals.

Angiogenesis

An additional manner by which physical activity may offer improvement in cognitive function in men and women is through improved vasculature and increased blood flow in the brain. Cerebral blood flow is normally decreased with age and in dementia (Ajmani, Metter, Jaykumar, & Ingram, 2000; Anderson, Greenwood, & McCloskey, 2010), and exercise has been shown to improve both blood volume and the vasculature of the brain. Angiogenesis, the development of new blood vessels through sprouting of existing endothelial cells, results from an enriched environment and physical activity interventions in animals (Black et al., 1990; Isaacs, Anderson, Alcantara, Black, & Greenough, 1992). Angiogenesis takes place in several areas of the brain. Ding et al. (2006) observed increases in both angiogenesis and vascular endothelial growth factor (VEGF) in the motor cortex and striatum of aged rats exercised on treadmills for 3 weeks. These changes contributed to improved oxygen and nutrient delivery, as well as increased neurotrophin uptake, which provides for brain maintenance and repair (van Praag et al., 2000; Isaacs et al., 1992).

More specific association of angiogenesis to improved cognitive function and interconnections with neurogenesis have been reported in several studies. Pereira et al. (2007) investigated the relationship between angiogenesis and neurogenesis in the dentate gyrus in exercising mice and humans. A 2-week voluntary running wheel intervention (compared to a non-exercising control group) was conducted with 7-week old mice. Magnetic resonance imaging (MRI) was conducted at baseline, at cessation of the exercise period, and at four and six weeks after exercise to determine cerebral blood volume in regions of the hippocampus. Thymidine analog BrdU marker was administered during the second week of exercise to examine for evidence of neurogenesis. The results revealed a selective increase in cerebral blood volume in the dentate gyrus for the exercising group, and these findings also correlated with increased neurogenesis. Pereira et al. also conducted a 3-month aerobic exercise intervention with men and women (mean age 33, range of 21-45), measuring cerebral blood volume (CBV) before and after training with MRI, changes in fitness via assessment of VO_2max, and memory function with the Auditory Verbal Learning Test (AVLT). As with the mice, there was increased CBV in the dentate gyrus post exercise, which correlated with increased VO_2max and improved learning in a short-term memory portion of the AVLT. Additional animal studies support Peireia et al's findings. van Der Borght et al. (2009) observed increases in blood vessel density after three to ten days of wheel running in mice, which was correlated with cell proliferation in the dentate gyrus. These findings were reversed upon cessation of the exercise regimen. Palmer, Willhoite, & Gage (2000) also observed that neurogenesis in mice was associated with clusters of capillaries and speculated that the angiogenic effects of exercise provide the critical adaptation that promotes neurogenesis.

The role of angiogenesis to improved cognitive function in older adults was revealed in a longitudinal study by Rogers, Meyer, and Mortel (1990). They examined the role of cerebral blood perfusion of cognitively healthy older adults. Lifestyle and work status were assessed, cerebrovascular function was obtained using positron emission tomography (PET), and cognitive function was determined through a Cognitive Capacity Screening Examination annually over a period of four years. Rogers et al. observed that individuals who retired and lived sedentary lives exhibited reduced cerebral oxygen perfusion over time that correlated with poorer cognitive function while older adults who continued to work or who retired, but maintained a physically active lifestyle, showed maintenance of cerebrovascular function and cognition.

Mitochondrial S**ynthesis**

Exercise training in mice also results in mitochondrial synthesis in many brain regions (Steiner, Murphy, McClellan, Carmichael, & Davis, 2011)**.** An enriched environment has been shown to improve mitochondrial function in rats in the cerebral cortex and hippocampus (Lores-Arnaiz, Arnaiz, Czerniczyniec, Cuello, & Bustamante, 2010). In addition, synaptogenesis may be dependent on mitochondrial adaptations in the hippocampus; a voluntary exercise intervention in mice that increased mitochondrial number was associated with increased synaptic density (Dietrich, Andrews, & Horvath, 2008), and an *in vitro* experiment by Li et al. (2004) also revealed that mitochondria in the dendrites of hippocampal cells were associated with synaptic strengthening. While there have not been direct findings in relation to mitochondria, exercise, and cognition, these findings illustrate additional links and interdependencies in the neurobiological effects that may contribute to improved cognitive function.

Attenuation of Glucocortocoids

Beyond the direct effects of exercise on the brain, exercise can mitigate the impact of chronic mental stress throughout life. Such stress contributes to cognitive dysfunction, possibly through degradative action on neurotrophins such as BDNF, and through structural and functional deterioration in the hippocampus. Mental stress activates the hypothalamic-pituitary-adrenal (HPA) axis, which results in elevation of corticosteroid hormones from the adrenal cortex. Elevated levels of adrenal steroid hormones in animals are known to decrease levels of BDNF (Adlard, Perreau, & Cotman, 2005)**,** reduce neurogenesis (Cameron & McKay, 1999), alter dendritic branching in the amygdala, hippocampus, and frontal cortex (Krugers, Lucassen, & Jöels, 2010)**,** and impair learning and memory tasks (Krugers et al., 1997)**.** In fact, experiments with marmoset monkeys revealed decreased cell proliferation in the dentate gyrus after a single exposure to stress (Gould, Tanapat, McEwen, Flugges, & Fuchs, 1998). A study of 4-month old rats found that social stress contributed to reduced reference and working memory (Krugers et al., 1997). Cameron & McKay (1999) observed that while decline in neurogenesis in aged rats was associated with high corticosteroid levels, eliminating these steroid hormones through adrenalectomy returned dentate gyrus cell proliferation to that of younger animals.

Studies of older men and women have revealed a positive relationship between cortisol and hippocampal atrophy, memory deficits, and general cognitive decline. Lupien et al. (1998) reported that older adults with extended elevations of cortisol exhibited 14% lower hippocampal volumes than controls with normal cortisol levels, and that the reduced volumes correlated with diminished performance on hippocampal-

dependent tasks of delayed memory recall and spatial memory (time to finding way through simple and complex maze). These relationships have been found to be of particular significance for individuals who are carriers of the *APOE*-ε4 gene, which is a known risk factor for AD. In this manner, Lee et al. (2008) tested midlife and older adults (ages 50-70) with or without the *APOE*-ε4 genotype on several cognitive procedures and measured salivary cortisol levels. Poorer performance in all participants was correlated with elevated cortisol levels, and homozygous ε4 carriers who also exhibited high cortisol levels performed significantly worse than non-carriers in processing speed, executive function, and verbal memory. As such, the impact of stress on some individuals is profound and it follows that exercise may be essential medicine for them.

Accordingly, exercise reverses some of the effects of elevated corticosterone in animals**.** Adlard and Cotman (2004) studied four groups of adult mice–stressed or non-stressed sedentary or exercising groups. After 3 weeks during which half were given running wheels, restraint stress was applied to half of the sedentary and half of the exercising animals that were then sacrificed for testing. Sedentary stressed mice showed elevated corticosterone and decreased hippocampal BDNF compared to control groups, Exercised animals, on the other hand, exhibited increased corticosterone, but also showed increased hippocampal BDNF levels compared with controls, suggesting that exercise had countered the effect of stress exhibited in sedentary stressed mice. When animals were subjected to adrenalectomy in a second experiment, neither exercised nor sedentary stressed mice showed a decrease in BDNF. In related work, Nakajima, Ohsawa, Ohta, Ohno, & Mikami (2010) examined cognitive function in mice subjected to restraint stress (12 hours per day) for 8 weeks. An exercising group was allowed free running-wheel

access during the hours not restrained. After five weeks of stress, the Morris Water Maze was administered and the exercising group showed superior performance compared to control sedentary stressed group. This was accompanied by increased cell proliferation in dentate gyrus and increases in IGF-1 in the cerebral cortex. Again, such findings, if also relevant to humans, suggest an additional mechanism by which exercise would build the reserve of the brain and the tolerance to stress, aging, and pathology.

How Does Physical Activity Counteract Cognitive Aging?

It appears that exercise builds biological reserve as one distinguishing effect of physical activity appears to be a structural benefit, with evidence for a reduction in normal age-related atrophy and even reversal of structural decline. For example, MRI evaluation of older adults classified by cardiovascular fitness level found that higher fitness resulted in increased brain volume of white and gray matter (Colcombe et al., 2006). Further investigations of physical activity interventions have confirmed that some structural deterioration may be reversed with exercise, with findings of significant increases in brain volume in both gray and white matter after a 6-month aerobic exercise intervention in older adults (Smiley-Oyen, Lowry, Francois, Kohut, & Ekkekakis, 2008). Another study revealed specific improvements in hippocampal volume after a 12-month intervention with an aerobic exercise intervention, but not with a stretching and toning group (Erikson et al., 2011)**.**

Executive functions of inhibitory control, mental flexibility, and memory encoding have all shown benefit from physical activity. Aerobic exercise interventions with older adults have resulted in improvements in frontally mediated executive functions of task switching and response inhibition (Colcombe et al., 2006; Kramer et al., 1999)**.**

Neuroimaging of older adult participants in a 6-month aerobic exercise intervention revealed greater post-intervention activation in prefrontal and parietal regions, with an accompanying decrease in activation of the anterior cingulate cortex (ACC), an area associated with conflict monitoring (Colcombe et al., 2004). Collectively, the results of the study suggest improved function of the cortical attentional networks thereby reducing the demand on the ACC. The finding is also significant in that there was regional specificity in the impact of exercise on the brain and not a generalized directional change – this finding illustrates the importance of examination of functional neural networks and not limited regions. Otherwise the results of various studies that examine different brain regions may appear contradictory in the effect of exercise.

Of essential importance to the basic theme of this chapter, it appears that physical activity may also delay the symptoms associated with dementia and the normal aging process. Importantly, the increase in hippocampal volume reported in the study described above may offset the known hippocampal decline associated with progression to dementia (Erikson et al., 2011). More specifically, cardiovascular fitness in early-stage Alzheimer's patients was negatively related to brain atrophy and positively related to white matter (Burns et al, 2005). In addition, biomarkers for amyloid plaque and tau protein tangles obtained through cerebrospinal fluid samples and amyloid imaging revealed that older adults engaging in higher levels of physical activity exhibited less accumulation of neurotoxic pathology (Liang et al., 2010).

Also, in a recent study, Baker et al. (2010) observed a significant improvement in executive functioning as a result of a vigorous exercise intervention with individuals diagnosed with mild cognitive impairment (MCI), which is believed to be a prodromal

stage for AD. Importantly, the observed improvement in cognitive function was more pronounced in women than in men. This gender-selective association was also related to improvement in insulin sensitivity and reduced cortisol levels in women. The observed benefit to cognitive function in MCI patients suggests a reversal of neuropathological processes. However, the notion of a synchronous benefit (owing to neurobiological benefit of exercise that outweighs the presence of pathology) is equally tenable and even a more likely explanation as there is little evidence in humans of a reversal of brain pathology as a result of exercise participation. The notion of synchronous benefit is consistent with that of the building of cognitive reserve through regular participation in physical activity.

Cognitive Reserve – Epidemiological Evidence

The studies discussed above highlight some effects of physical activity interventions, and provide a "window" on the state of brain and cognitive health through cross-sectional observation and intervention studies with humans as well as those with animals. Beyond such investigations, large-scale epidemiological studies offer a broader look at the exercise-cognition / brain health relationship across the lifespan and pose answers to the question of how choices made throughout life, including participation in physical activity, affect cognitive aging.

The theory of cognitive reserve suggests that an individual may be able to partially offset the effects of brain aging by nature of the resources he or she possesses or develops throughout life. It is believed that factors such as intellectual capacity, education, socioeconomic class, and intellectual, social, or physical lifestyle choices may affect how one is able to resist brain injury or insult. This theory is relevant, in particular,

to the development of dementia, allowing one with superior reserve to operate cognitively at a more efficient level for many years of aging, or to function through use of alternative brain regions during early stages of pathology, possibly delaying decline into clinical dementia (Stern, 2002).

Cognitive reserve is classified as either passive or active. Passive reserve describes a model in which an individual is able to withstand disease by nature of superior brain "hardware" - such as greater brain volume, increased neural density, improved blood vessel organization, increased neurotransmitter uptake or neurotrophin expression. These physical characteristics allow for the brain to sustain degenerative insult to a greater extent without revealing clinical symptoms, allowing for a greater threshold to be passed before exhibiting cognitive decline or dementia. An individual with passive reserve is also able to engage cognitive function with more efficiency. Alternatively, active cognitive reserve involves brain "software" and utilizes the concept of compensation, with which one who has compromised brain networks and processes may employ greater brain resources (neuronal recruitment) to solve a problem, using alternative brain networks for function when original regions or networks have been compromised through damage from aging or injury. Active reserve implies that deterioration in the brain can be tolerated, and that the symptoms of cognitive decline are not exhibiting clinically since the individual is able to compensate for the loss (Stern, 2002). In this manner, the threshold for cognitive decline is elevated and the symptoms delayed.

Cognitive reserve is examined most commonly through epidemiological investigations conducted prospectively or retrospectively to determine characteristics that

may affect cognitive aging. Studies of early life factors have revealed that IQ or intellectual ability may correlate with later life cognitive ability. Researchers examining neuropathology post-mortem in a study of Catholic nuns were able to correlate their dementia-related findings to the quality of writing exhibited by the young women upon entering the convent at an early stage of life – approximately 20 years of age. Linguistic ability at a young age was positively associated with total brain volume and negatively related to clinical evidence of Alzheimer's disease. Those with low idea density in early life showed significantly greater impairment in old age and pathology at post-mortem analysis (Riley & Snowdon, 2005). Another study that examined IQ, educational level, and intellectual, social, and physical activities during early life found a strong positive correlation for IQ and education level to cognitive functioning in later life, but no long-lasting effects of social or physical activities (Fritsch et al., 2007). Different researchers, however, have found a positive correlation for early life physical activity and processing speed in later life for men, but not women (Dik, Deeg, Visser, & Jonker, 2003)**.** These varied findings may indicate different mechanisms by which various reserve factors act, with some factors working from childhood and others being more responsive to adult manipulation. Limitations of retrospective studies may also affect outcomes for physical activity, since it is extremely difficult to obtain detailed and accurate information about lifestyle choices that occurred several decades in the past.

Examination of midlife activities has revealed more about the relation of physical activity to cognitive functioning in older adults. Scarmeas and Stern (2003), in a review article examining midlife activities and education and their relation to later cognitive decline, revealed that there was a synergistic effect of high education and high levels of

intellectual, social, and physical activities in helping older adults maintain cognitive function and resist cognitive decline or dementia**.** Physical activity performed in midlife has shown to be protective against dementia decades later. Rovio et al. (2005) examined 1449 survivors from a cohort study conducted with individuals at midlife (average interval to follow-up was 21 years). They discovered that those who engaged in leisure-time physical activity at least twice a week for 20-30 minutes that "made them breathless or caused them to sweat" exhibited 50% lower odds of developing dementia of any type, and were 60% less likely to experience AD, even after controlling for age, education, *APOE* genotype, vascular and other physical conditions, smoking, and alcohol. Two other studies with follow-up times of five or six years showed similar findings (Larson et al., 2006; Laurin, Verreault, Lindsey, MacPherson, & Rockwood, 2001). While these findings do not indicate any "prevention" of AD and other forms of dementia, they do lead one to believe that physical activity can provide the means to delay onset of dementia by providing a reserve that enables a greater threshold to be passed before pathology is exhibited. The underlying basis of such an effect (i.e., building reserve) is likely due to the neurobiological mechanisms described in animals above.

Physical Activity and Brain Connectivity

Neuroimaging studies with humans provide more insight to the basis of cognitive reserve. Recent interventions examining connectivity between brain regions have allowed for investigation of how physical activity is related to both passive and active reserve, revealing that exercise can facilitate brain networks by working efficiently with elevated coherence between regions. This adaptation would likely promote resilience to aging or dementia, enabling one with good connectivity to achieve greater neuronal activation or

strategically employ alternative networks as required by the age-related or pathology-related changes in the brain. Voss, Prakash, et al. (2010) observed that one year of aerobic training resulted in greater functional connectivity in the brain's default mode network (DMN) and frontal executive networks. One year of stretching and toning (that involved learning of new routines) also resulted in greater connectivity, with increased DMN connectivity after six months and improved frontal-parietal network connectivity after 12 months. These neural changes were reflected in improved executive function in the participants and provide strong evidence of exercise-related brain plasticity in men and women. Another study of networking by Burdette et al. (2010) reported a comparison of brisk walking to a health education treatment and a "light stretching" control group (four months duration for the interventions). The researchers observed that the anterior cingulate cortex was better connected to the hippocampus in the walking group when compared to the sedentary control group and accompanied by improved cerebral blood flow. These improvements show the power of physical activity to keep the brain operating efficiently and ready to meet cognitive challenges that could not be met if disconnectivity was occurring.

Genetic Influence on Cognition and the Mediating Role of Physical Activity

Although the study of genetic factors and cognition is in its infancy, there is evidence that genetics plays a role in the typical cognitive decline of older adults, both in terms of normal aging and in the development of AD. Autosomal dominant genes play a role in the small proportion of early-onset Alzheimer's (before age 65) accounting for about 5% of the disease (Bookheimer & Burggren, 2009; Parasuraman & Greenwood, 2002). The Apolipoprotein E (*APOE*) gene is notable in its relation to development of

late-onset AD, but it appears as a risk factor rather than showing direct causality. *APOE* is characterized by three variants or alleles - ε2, ε3, and ε4, with the ε4 allele present in about 15% of the population, but associated with about 40% of late-onset AD (Bu, 2009). The risk for possession of one ε4 allele increases the risk of developing late-onset AD by three times, with two copies of the allele increasing risk to eight times compared to the general population (Corder et al., 1993). However about 50% of ε4 carriers at age 90 will not have AD (Henderson et al., 1995), so it is indeed a complex issue and raises the question about protective factors that enable cognitive reserve to occur in these genetically at-risk individuals, increasing the threshold to defend against dementia-related pathology.

The ε4 allele has a different orientation with amino acid substitutions that contribute to a more unstable protein with higher likelihood of fragmentation of the protein (Harris et al., 2003). As a result of these differences from ε2 or ε3, the ε4 genotype is associated with greater amyloid buildup, reduced amyloid clearance, increased neurofibrillary tangles, decreased glucose metabolism is some areas of the brain, reduced brain volume, lysosome leakage, translocation and damage to cytoskeleton, mitochondrial impairment, differences in brain activation, and cognitive impairment, particularly in regards to memory and executive function tasks in healthy adults (Mahley, Weisgraber, & Huang, 2006). In this manner, the ε4 carrier would garner greater neurobiological benefit from exercise owing to the lessened ability to maintain and repair aging and insult to the human brain.

Characteristic Differences in *APOE*-ε4 Carriers

The characteristics of brain activity in ε4 carriers throughout life show some distinct differences from non-carriers. Hypometabolism at rest has been shown to be a distinct aspect of ε4 carriers' brain function even in healthy young adulthood, with reduced metabolism and lower oxygen delivery to the frontal, parietal, medial temporal lobes, and posterior cingulate cortex (Reiman et al., 2004). Differences in brain structure in *APOE* ε4 carriers compared to non-carriers also exist. For example, older adult ε4 carriers frequently show more brain atrophy compared to non-carriers even in cognitively healthy individuals (Chen et al., 2007) and this difference is pronounced in the hippocampal area (Lind et al., 2006). With regards to brain activity, cognitively healthy *APOE*-ε4 carriers show altered brain activation under memory challenge tasks (Bookheimer et al., 2000; Sperling, 2007). Sperling reported that ε4 carriers displayed increased hippocampal activation that converted to hypoactivation as memory became more impaired, possibly forecasting a path toward dementia. Older adult carriers of the *APOE*-ε4 allele have been observed in numerous studies to exhibit lower cognitive function in areas of executive function, working memory, episodic memory, and perceptual processing, and reveal cognitive deficits even in the absence of pathology (O'Hara et al., 1998; Small, Rosnick, Fratiglioni, & Bäckman, 2004; Wisdom, Callahan, & Hawkins, 2009). Some recent findings in younger adults have shed some new light on possible effects of the ε4 allele. One study that examined postmortem gene transcripts from brain cortical samples of young adults who were either ε3 or ε4 revealed difference in mitochondrial function with ε4 carriers that was occurring before evidence of plaque

or tangle formation (Conejero-Goldberg et al., 2010). Another study revealed mitochondrial damage postmortem in the posterior cingulate cortex in young adults, again found without plaque (Valla et al., 2010). Both of these studies offer support for a theory of mitochondrial dysfunction mediating, at least in part, the role the *APOE*-ε4 allele in cognitive aging and development of AD. The mitochondrial synthetic effect of exercise reported above may mitigate this process.

Physical Activity and Cognition in Relation to *APOE* Genotype

With all of the findings showing differences in brain structure and function between *APOE* genotypes, as well as poorer cognitive performance in older adult ε4 carriers and a higher rate of development of Alzheimer's disease, one may wonder about the benefit of physical activity for ε4 carriers in comparison to the general population. While such studies are few at this time, there may be particular benefit for physical activity for *APOE*-ε4 carriers.

In light of the known risk of *APOE* genotype for AD, Schuit et al. (2001) conducted a prospective study of the effects of physical activity participation on cognitive decline over a three-year period in 347 older men ages 65 - 84. Participants were characterized for presence of *APOE*-ε4, exercise behavior at baseline (less than or more than 1 hour per day of walking, bicycling, gardening, odd jobs, and sport), and cognitive performance both at study inception and follow-up on the Mini-Mental Status Exam (MMSE). Incidence of cognitive decline indicated by a drop of three or more points on the MMSE over the three-year period was related to activity level and genotype. Specifically, the risk of cognitive decline was 13.7 times higher in sedentary ε4 carriers

relative to physically active non-carriers. Higher physical activity involvement was associated with remarkable attenuation of cognitive decline in the ε4 carriers.

Deeny et al. (2008) investigated the effect of exercise on working memory in adults ages 50-70 with regard to *APOE* genotype. Using both behavioral and magnetoencephalographic (MEG) brain imaging evidence, these researchers examined cortical dynamics and cognitive performance on a behavioral Sternberg memory task, comparing carriers and non-carriers of the *APOE*-ε4 allele. Highly active ε4 carriers showed faster reaction time in the memory task compared to sedentary carriers. Sedentary ε4 carriers also showed a lower level of activation in the right medial temporal lobe compared to highly active ε4 carriers, who were not differentiated from non-carriers in activation. This lower activation may be forecasting that a change to hypoactivation may be occurring even at midlife for sedentary ε4 carriers, while physical activity is providing cognitive protection. In addition, Etnier et al. (2007) examined working and episodic memory in relation to aerobic capacity (VO_2 max) in older women who were classified according to *APOE*-ε4 carrier or non-carrier groups. They reported that the cognitive behavioral effects of ε4 showed evidence of a dose response, for sedentary ε4 carriers, with ε4 homozygotes of lower aerobic fitness exhibiting poorer response to a delayed memory task compared to ε4 heterozygotes or non-carriers.

In terms of the neurobiological mechanisms provided through physical activity, it appears reasonable why exercise would be particularly beneficial to carriers of the *APOE*-ε4 allele. As previously described, physical activity increases angiogenesis in the brain and improves mitochondrial function, which could provide a direct benefit for the hypometabolism or mitochondrial dysfunction exhibited by ε4 carriers. The fact that

physical activity increases uptake of brain derived neurotrophic factor (BDNF) may indicate possible repair to damage caused by the unstable conformation of ε4, and neurogenesis as reported in animal studies (and implied in at least one human study that showed improved hippocampal volume after an exercise intervention) could delay the destruction toward AD caused by this genotype if hippocampal regions show reversal of damage with aging due to the effects of physical activity. It should be noted that *APOE*-ε4 carriers are subject both to normal cognitive decline and pathological dementia-related decline, so there is a double insult to these individuals. Studies have revealed that even cognitively healthy ε4 carriers experience lower cognitive function in areas of working and episodic memory, processing speed, and executive function, and they may benefit from exercise in ways to improve frontal as well as hippocampal function (O'Hara et al., 1998; Small et al., 2004; Wisdom et al., 2011). Overall, exercise may offer benefits to *APOE*-ε4 carriers to extend cognitive reserve, preserving brain structure and function, offering ability to repair damage, and improving resilience to effects of aging and dementia pathology.

Benefits of Physical Activity in Children and Young Adults: An Investment Hypothesis

The notion that lifestyle choices in youth or young adulthood could affect cognitive aging decades later is intriguing, and studies in children have shown that physical activity may improve cognitive function. Some studies are revealing distinct effects of increased brain volume and improved executive function or working memory in children as young as nine years of age. Greater relational memory in high fit nine and ten year olds compared to lower fit children has been reported in one study, with the

difference mediated by increases in hippocampal volume (Chaddock, Erikson, Prakash, Kim, et al., 2010). Another study revealed superior performance on an inhibitory control measure (Eriksen Flanker) in high-fit children compared to lower-fit children, with greater activation of the dorsal striatum that was positively related to inhibitory control (Chaddock, Erikson, Prakash, VanPatter, et al., 2010). Davis, Tomporowski, McDowell et al. (2010) conducted a 3-month exercise intervention with 7-11 year old children with two exercise groups (one 20-minutes three times per week; one 40-minutes) and observed improvement in executive function based on the Cognitive Assessment System and significant improvements in mathematics scores on the Woodcock-Johnson Test of Achievement. Both of these improvements showed a dose-response effect with the 40-minute exercise group achieving significantly better than the 20-minute exercise group. In addition, the exercise groups (combined) showed increased bilateral activation in the prefrontal cortex and decreased activation in bilateral posterior parietal cortex when compared to non-exercising controls. Hillman et al. (2005) further reported improved P300 amplitude and reduced latency in children who are "high fit", indicating that they exhibit enhanced attention resource allocation when challenged.

There is less research with young adults, but there are interesting results that add credence to the investment reserve theory. Woo et al. (unpublished abstract, 2008) employed an oddball and go/no-go executive task in college-aged men with consideration of cardiovascular fitness and *APOE* genotype, and reported that fitness proved to be especially beneficial for *APOE*-ε4 carriers. Further, a retrospective study of military records of young Swedish males born between 1950-1976, including comparisons in monzygotic twin pairs, found that cardiovascular fitness in adolescent years predicted

intelligence in several areas (global, verbal, visuospatial, logical, and technical)(Aberg et al., 2009). A cohort study of adults revealed that physical activity at age 36 was associated with verbal memory performance at age 43 years, and was protective against memory decline from ages 43-53. This study reflects that effects of cognitive aging begin at surprisingly young ages for sedentary individuals, and underscores the importance of physical activity in preserving memory (Richards, Hardy, & Wadsworth, 2003). The work of Woo et al. suggests that the preservative effects of fitness on cognition in later life may be particularly important for *APOE* ε4 carriers. Even the prenatal environment may contribute to lifelong cognitive development. A study of guinea pigs in which nutrient and blood supply was reduced in utero showed a strong effect on hippocampal development, with lower volume and suboptimal dendritic outgrowth (Gomez-Pinilla & Vaynman, 2005). On a positive note, other studies have shown that when the mother is physically active during pregnancy, there is improved brain development (Lee et al., 2006). While there is synaptic remodeling throughout life and evidence that enriched environment may offset prenatal insufficiencies, these findings do lead to questions about lifelong influences of cognitive reserve.

Additional Considerations:

Exercise Intensity and Modality Versus Skillful Movement

One common question concerning the role of exercise in cognition is that of exercise prescription. Unfortunately, there is no definitive answer as to what type, intensity, frequency, or duration of physical activity extends the greatest benefit to cognitive function, but there are hints that can be gleaned from animal and human investigations that may provide a basis for future research in this area. Most of the

research has involved aerobic exercise, and studies have consistently revealed associations between vigorous physical activity and improved executive function. However, animal research has shown that there are also important effects from enriched environment i.e. – social housing, tubes, toys, ladders, nesting material, and running wheel - that involves more learning or spatial activity when compared to running-wheel activity, alone. For example, Olsen et al (2006) reported that elements of enriched environments offered more specific effects in memory improvement or in increasing synaptic strength. In terms of neurogenesis, it appears that high-intensity running in animals is important for proliferation of progenitor cells in the dentate gyrus, but that an enriched environment is more related to survival of new cells (Fabel & Kemperman, 2008; van Praag et al., 2000). A study that investigated running and enriched environment in tandem revealed that 10 days of wheel running followed by 35 days of enriched environment resulted in a 30% increase in neurogenesis compared to either alone. These authors suggested that aerobic exercise provides a "priming" mechanism for learning, with more complex physical activity from an enriched environment leading to more long-lasting benefits (Fabel et al., 2009). A study conducted with older rats revealed that an enriched environment preserved memory and synaptic plasticity in aging compared to sedentary controls (Lores-Anraiz et al., 2006). Interestingly, a study that controlled for the social effects of enriched environment did not reveal the benefits for the socially enriched group that were shown when complex motor activities were present (van Praag et al., 2000)**.**

Translating to human research, several epidemiogical studies have revealed that a variety of activities provides the most impressive cognitive benefit in older adults. Chan

et al. (2005) reported on a cross-sectional study that mind-body exercises involving more cognitive processing (e.g.-Tai Chi) and cardiovascular exercise performed together resulted in significantly improved memory scores for participants. Another study revealed that overall physical activity, including leisure, household, and occupational activities as measured by Actigraphy predicted improved global cognition in older adults, whereas a self-report of physical activity that was more limited to exercise-type activities did not (Buchman, Wilson, & Bennett, 2008). Interestingly, these improvements were associated with increases in peripheral IGF-1. Even intervention studies that revealed impressive effects of aerobic exercise on improved hippocampal volume or improved networking also revealed positive effects on cognition from moderate physical activity relative to control groups whose intervention involved learning routines. Overall, it appears that both high-intensity aerobic activity and more moderate activity that involves cognitive challenge may be important for different aspects of cognition.

The issue of aerobic fitness versus motor fitness areas of strength, balance, or flexibility in humans is also of interest, and several studies of strength training have shown that these programs alone can provide cognitive effects. A moderate 6-month resistance and balance intervention predicted individual improvement in memory in older adults when compared to a wait-list control (Lachman, Neupert, Bertrand, & Jette, 2006). A 24-week intervention revealed that both moderate and high-intensity resistance training improved cognitive performance in older adults in attention and memory compared to a stretching control (Cassilhas et al., 2007). A recent fMRI study by Voelcker-Rehage (2010) examined motor fitness (strength, balance, flexibility, and fine motor control) as well as aerobic fitness, and found distinct areas where each type of fitness was optimal

for cognitive improvement. These researchers reported that inhibitory executive function accrued a selective benefit for aerobic fitness. Visuo-spatial tasks that required more attentional control were found to be more related to motor fitness. These findings underscore that at this point in time, understanding of effects of different modalities and duration of exercise is in its infancy - the puzzle has only begun to be solved. Future kinesiology/cognitive neuroscience research with both animal and human research may be productive as it tries to elucidate the subtleties of movement and or aerobic fitness to find more specialized answers.

Challenges for Future Research

It appears that a reasonable role for physical activity participation and exercise is the building of cognitive reserve, both through active and passive mechanisms. In this manner the neurobiological benefits of exercise observed in animals, if also present in the human brain, would result in tolerance to age-related decline and pathology such as AD. The work on neurotrophic influence in animals and the observed benefit of exercise on hippocampal volume in humans suggest a powerful remediation effect that could reasonably delay the aging and the symptoms associated with the pathology of dementia. In this manner the threshold for cognitive decline would be increased with a physically active lifestyle. In addition, it may be that physical activity early in life and during midlife exerts a profound benefit to cognitive function in later life. This type of an investment would underscore the importance of a physically active lifestyle throughout the entire developmental sequence. Finally, it appears that there are individual differences in genotype that may underscore a higher need for exercise in some individuals relative to others such as ε4 carriers and non-carriers. In this manner,

exercise may be essential medicine for some. The insight that has been accrued over the last 35 years in this area is remarkable and lends strong scientific support for the ancient notion of a sound mind with a sound body (mens sana in acorpore sano).

Chapter 3: Cognitive Performance in Older Adults in Relation to *APOE* Genotype, Weekly Energy Expenditure, and Cardiovascular Fitness.

Abstract

Physical activity has been associated with improved cognitive performance in older adults, both for the general population and with regards to the *APOE*- ε4 genotype, but questions remain as to what type of physical activity preferentially mediates this relationship, and what type of cognitive processes are most likely to benefit. To explore these issues, this study evaluated performance across a battery of cognitive tasks relating to inhibition control, cognitive flexibility, and working memory capacity, while employing measures of physical activity that emphasized either high-aerobic intensity exercise or weekly kilocalorie expenditure without regard to intensity. Additional consideration of the influence of the *APOE* genotype was given in examination of this relationship. Hierarchical regression analyses of 117 healthy individuals age 50-70 revealed that working memory performance was positively related to weekly kilocalorie expenditure in *APOE*-ε4 carriers when challenged at a moderate level of difficulty, while no such relationship was observed in non-carriers. This benefit was accounted for by differences observed in those individuals with higher kilocalorie expenditure, as shown in an upper median split. In addition, a positive relationship between working memory performance and kilocalorie expenditure was observed in both ε4 carriers and non-carriers, but magnitude of the relationship was greater in ε4 carriers. The results support specificity in the relationship between physical activity and cognitive performance in an older population based on genetic, type of cognitive task, and physical activity considerations. The positive association of physical activity with working memory suggests that cognitive processes subserved by frontally mediated complex neural proceses benefit, in particular, from a physically active lifestyle.

Introduction

Maintenance of cognitive function is a major concern for older adults. As early as midlife, decline in memory, inhibition, and interference control can be observed (West, 1996). Cognitive decline can occur both pathologically, related to development of dementia, and as a normal part of aging (Albert, 1997) and these deficits can greatly affect quality of life and interfere with ability to function normally in activities of daily living. An important consideration in neurocognitive function is that of genetic influence. *APOE* is a gene known as a risk factor in development of Alzheimer's disease (Strittmatter et al., 1993) and is also related to cognitive decline in normal, non-pathological aging (Small, 2004; Wisdom, Callahan, & Hawkins, 2009). There are three variations, or alleles, within the *APOE* genotype, of which one allele, *APOE* -ε4, that occurs in about 15% of the population, is negatively associated with cognitive function in older adults(Corder et al., 1993). In a meta-analysis, Wisdom et al. concluded that cognitively intact adult carriers of ε4 exhibited poorer performance in areas of global cognitive ability, episodic memory, executive functioning, and perceptual speed. O'Hara et al. (2008) observed that ε4 carriers performed cognitive tasks more slowly than non-carriers. Reinvang, Winjevoll, Rootwelt, and Espeseth (2010) observed relatively poor working memory in older adult *APOE*-ε4 carriers, while Hofer et al. (2002) reported accelerated decline in memory and processing speed in non-demented older adult ε4 carriers compared to non-carriers over a seven year period.

Despite the relationship between *APOE*-ε4 and cognitive decline or dementia, there is evidence that many who carry the allele do not suffer cognitive decline, with almost half of homozygote ε4 carriers at age 80 showing no signs of dementia (Myers et

al., 1996). As such, other factors may be protecting individuals from the effects associated with *APOE*-ε4. The theory of cognitive reserve proposes that lifestyle factors may offer benefit against cognitive decline for individuals at risk for dementia (Scarmeas & Stern, 2003). Education, intellectual, social, and physical activities throughout life have been shown to be associated with slower cognitive decline in the general population (Fritsch et al., 2007) and reduction in development of Alzheimer's disease (Scarmeas et al., 2001; Scarmeas & Stern,2003).

Physical activity, in particular, is associated with robust effects for preserving cognitive function in older adults. With regard to *APOE* and physical activity, several cross-sectional and longitudinal studies have revealed cognitive benefit of physical activity and aerobic fitness for *APOE*-ε4 carriers, as well as reduction in risk for development of dementia. For example, Schuit et al. (2001) observed that physical activity of more than one hour per day was associated with a four-fold decrease in the risk of cognitive decline over a three year period for older male ε4 carriers when compared to sedentary ε4 carriers, while no such benefit was observed in non-carriers. Furthermore, Rovio et al. (2005) reported that physical activity was associated with reduced development of dementia two decades after initial assessment in *APOE*-ε4 carriers, but not for non-carriers. Etnier et al. (2007) reported that aerobic fitness, as measured by VO_2 peak in healthy older female ε4 carriers, was associated with elevated performance on several cognitive tasks for ε4 homozygotes, but not for heterozygous ε4 carriers or ε4 non-carriers. Niti, Yap, Tan, and Ng (2008) observed a benefit for physical activity for *APOE*-ε4 carriers in a Chinese population of adults over age 55 as

measured by decline in Mini-Mental Status exams (MMSE) scores over one year, whereas non-carriers showed no effect.

However, some studies have not revealed a benefit for physical activity for *APOE*-ε4 carriers. Based on a 5-year longitudinal study, Podewils et al. (2005) reported a lower level of incident dementia only in physically active non-carriers of ε4, and no reduction of risk shown in ε4 carriers, while Lautenschlager et al. (2008) noted a benefit of moderate physical activity in *APOE* -ε4 non-carriers, which was absent in ε4 carriers, as a result of a 6-month interventional study. It should be noted, however, that differences in study design may have affected outcomes. Whereas Rovio et al., Schuit et al., and Niti et al. examined cognitively healthy individuals and assessed general cognitive decline, Lautenschlager et al. examined individuals with self-reported memory impairment. Podelwils et al. employed a different endpoint of cognitive performance then typically used by others, that is the development of clinically diagnosed AD within five years of initial assessment. Interestingly, Niti et al. employed the same physical activity measure as Podewils et al., but with a cognitive endpoint outcome of decline on the MMSE, as opposed to clinical diagnosis of Alzheimer's, and observed a stronger association with reduced cognitive decline with *APOE*-ε4 carriers than with non-carriers.

While a positive association with cognitive function has been reported for physical activity in *APOE*-ε4 carriers, there is still confusion as to what mode of exercise may be responsible for the benefit. Findings from several studies have indicated divergent results relating to intensity of exercise for select cognitive tasks in older adults. Some investigators have reported a benefit of aerobic exercise for executive function tasks (Colcombe et al., 2004; Kramer et al., 1999), but others have reported no benefit of

aerobic exercise for working memory tasks (Flöel et al., 2010; Madden, Blumenthal, Allen, and Emery, 1989), while other investigators have reported positive relationship between cognitive performance and total physical activity level, with no differences regarding exercise intensity (Buchman et al., 2008; Ruschweyh et al., 2009). Although cardiovascular fitness, as indexed by aerobic capacity, offers an objective biomarker, it may not be the critical mediator of movement and cognitive benefit in all cases. Barenbeerg, Berse, & Dutke (2011) based on a review of several interventional studies, reported that aerobic exercise was most likely to benefit inhibitory-related executive function, with less certainty for other executive processes. Different cognitive processes may benefit from different aspects of physical activity, and complex, thoughtful movement that may occur in moderate as well as high-intensity physical activity could be an important component in the physical activity/cognition association. Therefore, it is important to evaluate measures of physical activity that highlight both high aerobic intensity and total energy expenditure. As described previously, it is also important to evaluate a battery of diverse cognitive tasks to determine specificity of the relationships between physical activity and cognitive performance.

With the previous findings in mind, this study aimed to evaluate the association of cognitive function in a population of older adults with weekly kilocalorie expenditure, high-aerobic intensity exercise, and estimated aerobic capacity, with additional regard for the *APOE*-ε4 allele. The present study assessed performance across a range of cognitive challenges related to inhibitory control, cognitive flexibility, and working memory, controlling for age, sex, education, and intellectual activity. We predict that energy expenditure will be positively related to cognitive performance across the battery of

cognitive tasks in carriers and non-carriers of the *APOE*-ε4 allele, but that the magnitude of the relationship will be higher in carriers. The examination of these relationships across tasks from simple inhibition to working memory will be conducted in an exploratory manner.

Methods

Participants

Individuals were recruited for this study through flyers distributed at local endurance running events, newspaper advertising, outreach at health fairs or with community groups, and through enrollment of University of Maryland faculty and staff. In response to recruitment, 133 individuals ages 50-70 responded and presented for screening appointments. Individuals who responded represented a broad range of physical fitness levels.

Medical Considerations

Of those selected for participation in the study, one individual was a current smoker in the group, and no one reported excessive use of alcohol. Although no individual reported diagnosis of clinical depression in medical histories, 10 participants were taking antidepressants for various reasons. Three participants were receiving estrogen hormone replacement therapy. Eight participants were using Beta blockers. Separate statistical analyses were conducted with participants receiving either antidepressants or hormone replacement therapy included or excluded. No differences in significance were shown and these participants remained in dataset. Participants who were taking Beta blockers were excluded from analysis of estimated VO_2 since that medication is known to affect heart rate, a factor used in the calculation of this measure.

Procedures

Participants who responded to recruitment efforts were informed of the study requirements and were briefly screened in a telephone interview. They were then invited to attend an initial appointment visit, and were provided with medical history forms to complete. During the first session of approximately two hours, informed consent was obtained and medical histories were reviewed. Smoking status, alcohol consumption patterns, and use of medications, vitamins, or other supplements were recorded. DNA was collected by blood or salivary methods for genotype determination. The Salthouse Inventory, the Yale Physical Activity Survey, and the Cambridge Cognitive Examination (CAMCOG) were administered, and height and weight were determined and BMI calculated. The Rockport Walk test was administered to some participants during the first session, although others opted to complete fitness testing at a later date. Participants were scheduled for a second day of cognitive testing upon successful completion of screening.

Screening Instruments

CAMCOG

The CAMCOG-R questionnaire is a neuropsychological assessment of cognitive status and is used to aid in diagnosis of dementia or cognitive impairment (Williams, J. G., Huppert, F. A., Matthews, F. E. & Nickson, J., 2003). It contains107 questions and provides scores on several cognitive domain subscales, such as attention, memory, perception, language, orientation, and abstract thinking. For the purposes of this study, two questions deemed irrelevant or confusing with regard to the specific testing situation were omitted, resulting in a maximum possible score of 105. Individuals were excluded from the study if a score was below 80.

Medical History

All participants completed a medical history form detailing any medical problems, along with information on family history of medical conditions, including dementia. Participants were also asked to rate their physical activity history by decade to determine stability of their exercise patterns.

Cognitive tasks

Eriksen-Flanker task

This test is a measure of the executive function of inhibition. With this task, the participant is presented with a display of five arrows, either congruent (>>>>>) or incongruent (>><>>), in a visuospatial orientation on a computer screen and is told to respond to the direction in which the middle arrow is pointing, pressing a keyboard button designated for left or right directions (Eriksen & Eriksen, 1974). After completing a practice session, the participant performed 100 trials. The middle arrow was randomly presented with half shown in a congruent direction, and half incongruent. For each trial, a visual orientation cue (+) was presented for 1250-1700 ms before the imperative stimulus appeared. Arrows were presented for up to 3500 ms. An interstimulus interval (blank screen) appeared for 1250-1750 ms before presentation of the next cue**.** Scores were calculated for numbers correct and reaction times for Congruent and Incongruent conditions. An Interference score was calculated based on the difference in reaction time between the two conditions and served as an index of adaptive resistance to distraction ([Incongruent RT - Congruent RT/Congruent] x100).

Stroop Color and Word Test

This test, a measure of inhibitory control, was administered by a trained research assistant. Each participant was timed for 45 seconds while reading a list of colored words that represented the colors red, green, or blue. A second list was presented with a list of the same colors represented by XXXX instead of the written word, again timed for 45 seconds while participant stated the colors. A third timed presentation consisted of a list written with words of colors, but they were written in ink a different color than the color that was written (i.e.- the color "red" written in blue ink). Scores were determined for Word, Color, and Color-Word conditions, and an age adjusted Interference score was calculated to interpret the Color-Word score in relation to a predicted color-word score (Golden & Freshwater, 2002).

Sternberg memory task

This working memory task (Sternberg, 1966) was conducted via computer presentation. A visual orientation cue was presented on a black screen for 1000 ms, followed by string of white letters, 6 or 8 characters long, and displayed for 2000ms. A yellow prompt letter was then presented for 1000 ms. The participant was instructed to press a button on the right of the keyboard if the prompt matched a letter in the string previously shown, and a button on the left was pressed if the prompt was not in the set. After completing a practice session, the participant performed 100 trials, with 50% of the trials containing matching probes and 50% non-matching. Scores were determined for the number correct for “matches” and “non-match” trials and reaction times associated with the triasl for each condition.

Wisconsin Card Sorting Test

This task is considered a measure of cognitive flexibility (Heaton et al., 1993). Each participant was shown four cards across the computer screen and one card was presented below to be matched to the series according to the rules of color, number, or shape. A brief practice session was allowed. The matching rule was not defined and the participant was required to determine the rule through trial and error. After ten trials, the rule changed and the participant was scored on his or her ability to adopt the new matching rule, according to how many attempts it took to correctly match the card by the new category. A total of 64 to 128 presentations were made, depending on participant performance.

Predictors of Cognitive Performance

Yale Physical Activity Survey

The Yale Physical Activity Survey (YPAS) was employed to determine estimated weekly kilocalorie expenditure. An Index was also obtained from this survey that compiled activity defined as vigorous, moderate, or sedentary. This instrument is validated for use with older adults (Di Pietro, Caspersen, Ostfield, & Nadel, 1993) and includes energy expenditure calculation of activities of daily living (i.e- cooking, gardening, stair climbing) as well as that for exercise and recreational activities. Details of this instrument may be found in Appendix A.

Rockport Walk Test

Cardiovascular fitness was determined through the Rockport Walk test, developed and validated by Kline et al. (1987), which yields an estimated VO_2 max measure. This test consists of a timed one-mile walk while heart rate is monitored. The heart rate at the

end of the test is used along with time to completion, age, sex, and weight in a formula to determine estimated VO_2 max.

Salthouse Activity Inventory

This instrument provided an indication of intellectual activity, along with a self-reported measure of cognitive demand. Each participant was asked how much time each week was spent in activities such as book reading, computer use, card playing, classes, etc., and each activity was rated by participant on a scale of 1 to 5 according to how much cognitive demand that individual felt the task entailed, with a higher score reflecting increased activity. The score reflected the total number of hours engaged in activities with each score multiplied by the cognitive demand score. This instrument was developed and validated by Salthouse, Berish, & Miles (2002).

APOE genotyping

APOE genetic information was determined either from blood samples taken with a venous blood draw from a certified research assistant, or alternatively through mouthwash saliva-sampling technique (Lum & Marchand, 1998). With the saliva procedure, each participant was asked to rinse with 10 ml of mouthwash for 45 seconds and then expectorate into a 30 ml test tube. This procedure was conducted after abstinence from food or drink for one hour as dictated by procedure protocols. This method has been shown to be a reliable collection method for obtaining buccal cell DNA. Participants were classified as ε4 carriers ($\varepsilon4^+$) if their genotype was either heterozygous (ε3/ε4) or homozygous (ε4/ε4). Non-carriers ($\varepsilon4^-$) were classified as either ε2/ε3 or ε3/ε3. The ε2/ε4 genotype was excluded from analysis due to conflicting evidence of preventive versus increased risk of the two alleles (Corder et al., 1993). All researchers were blind to

genotype during the study. Details regarding DNA isolation and genotyping can be found in Appendix B.

Statistical Analysis

Descriptive characteristics were determined for age, sex, education, Salthouse Inventory, BMI, Yale kilocalorie expenditure, Yale Index, estimated VO_2 max, and CAMCOG cognitive screening, presented separately by *APOE* genotype status and sex. In addition, Pearson correlation coefficients were computed to determine the expected relationships between each of participant characteristics used in regression analysis.

Each of the five cognitive measures of interest (i.e., Eriksen Flanker, Stroop, Sternberg 6 and 8, and WCST) was regressed in a hierarchical manner on each of three models. The first model included 1) Age, 2) Sex, 3) Education, 4) *APOE* genotype, 5) Salthouse Activity Inventory, 6) Yale weekly kilocalorie expenditure, and 7) interaction of genotype with Yale weekly kilocalorie expenditure. Separate regressions were conducted using the same model, but substituting 1) Age, 2) Sex, 3) Education, 4) *APOE* genotype, 5) Salthouse Activity Inventory, 6) Yale Index and 7) interaction of genotype with Yale Index for the second model and 1) Age, 2) Sex, 3) Education, 4) *APOE* genotype, 5) Salthouse Activity Inventory, 6) estimated VO_2 max, and 7) interaction of genotype and estimated VO_2 max for the third model. Simple regressions were conducted separately when any interaction term achieved significance to determine which group exhibited a significant relationship between physical activity and cognitive performance. Regression diagnostics were conducted to determine effects of leverage, discrepancy, and global or local influence.

Results

In response to recruitment efforts, 133 individuals ages 50-70 responded and presented for screening appointments. Three individuals were excluded from testing due to CAMCOG scores below cutoff point of 80. Of those who remained seven did not successfully genotype, and four participants with *APOE*-ε2/4 status were removed from analysis. Loss of computer data resulted in missing data for three participants for the Wisconsin Card Sort Test, six for the Eriksen Flanker task, 10 for the Sternberg 6 and 9 for the Sternberg 8 task. One participant could not complete the Stroop test due to color-blindness. Because an analysis revealed a non-random pattern regarding missing data, regression imputation was conducted to provide predicted values for the missing dependent variable scores. Regression diagnostics were conducted and revealed two influential outliers whose values on either Yale Index or Yale Weekly Kilocalorie score affected significance on several cognitive tasks, and were removed from the dataset (Outlying values were three –four standard deviations from the mean, and inconsistent with related physical activity data for these participants.) The final study population number was 117. A subset of 77 participants additionally completed estimated VO_2 max cardiovascular fitness testing.

Descriptive Statistics

Descriptive statistics revealed that this study population was a highly educated group, with a mean education level of 17.6 years of formal education, with 86% of participants reporting at least a college degree and 52% with graduate degrees. No significant differences were noted of participant characteristics by genotype except for age, with *APOE* -ε4 carriers younger than non-carriers (p = .04). Females reported

significantly lower age (p = .01) and education levels (p = .02), and as would be expected, were characterized by lower mean estimated VO_2 max (p < .001). Only 17% of females carried the ε4 allele, compared to 38% of males who exhibited an *APOE*-ε4 genotype. Results for demographic characteristics are listed in Table 1.

Table 1

Descriptive Statistics by Sex and Genotype – Means and Standard Deviations

	Male $\varepsilon4^-$ (n=36)	Male $\varepsilon4^+$ (n=23)	Male Total (n=59)	Female $\varepsilon4^-$ n=48)	Female $\varepsilon4^+$ (n=10)	Female Total (n=58)	Total (n=117)
Age	61.2(5.0)	60.3(4.8)	60.8(4.9)	59.5(4.6)	53.1(4.0)	58.4(5.1)	59.6 (5.1)
Education	18.2(2.7)	18.2(2.5)	18.2(2.6)	17.0(2.5)	17.5(1.7)	17.1(2.3)	17.6 (2.5)
Act Inv	324(124)	334(74)	328(107)	341(160)	294(119)	333(154)	331(131)
CAMCOG	94.8(3.4)	95.6(4.3)	95(3.8)	94.3(4.4)	93.1(5.5)	94.1(4.6)	94.6 (4.2)
BMI	25.9(2.6)	26.5(3.9)	26.1(3.1)	25.8(5.8)	27.1(6.0)	26.0(5.8)	26.1(4.6)*
YWKcal	7688(4312)	8351(4674)	7946(4428)	7373(4711)	7743(4698)	7437(4670)	7694 (4537)
Yale Index	63.8(18.3)	58.8(21.7)	61.8(19.6)	54.8(24.2)	52.4(16.2)	54.4(22.9)	58.2(21.6)
VO_2**	34.9(8.1)	34.9(10.6)	34.9(8.9)	25.8(8.3)	28.8(6.2)	26.4(8.0)	30.8(9.4)

Abbreviations: Act Inv-Activity Inventory; YWkcal- Yale Weekly Kilocalories; VO_2 - Estimated VO_2 max *n=109 for BMI due to missing data **n for estimated VO_2 max -total dataset =77; Male subset total=40;-$\varepsilon4^-$=27; $\varepsilon4^+$=13; Female subset total = 37; $\varepsilon4^-$ = 30; $\varepsilon4^+$=7

Descriptive statistics for cognitive tests are listed in Table 2 for original and imputed datasets. While interference scores or reaction times on the five cognitive tasks analyzed were of main interest, performance on accuracy measures of the cognitive tasks or on subcategories that were used to calculate interference scores for some variables are important to note, particularly to compare performance by genotype. *t* test anlaysis revealed no significant differences in accuracy for Eriksen Flanker or Sternberg percent correct when comparing *APOE* ε4 carriers and non-carriers. For the Stroop conditions, there were significant differences by genotype for some conditions of the task, with *APOE*-ε4 carriers showing significantly better performance on Word (p = .03) and a tendency towards significance on Color-Word (p = .05).

Table 2

Means and Standard Deviations for Cognitive Performance- Original and Imputed Datasets

	Original Dataset*			**Imputed Dataset *n*=117**		
	ε4$^-$	**ε4$^+$**	**Total**	**ε4$^-$**	**ε4+**	**Total**
Stroop Word	111.7(14.2)	102.8(15.0)	110.3(15.9)	111.7(14.2)	102.8(15.0)	110.3(15.9)
Stroop Color	77.1(12.6)	74.2(11.4)	76.4(12.3)	77.1(12.70	74.2(7.9)	76.3 (12.3)
Stroop CW	49.3(8.2)	45.9(7.9)	48.4(8.21)	49.2(8.2)	45.9(7.9)	48.3(831)
Stroop INT	4.0(6.7)	3.0(8.2)	3.7(7.1)	4.0(6.7)	3.0(8.1)	3.7(7.1)
Fl Cong%	99.9(.35)	99.7(.60)	99.9(.44)	99.8(.36)	99.7(.61)	99.8(.45)
Fl Cong RT	5662(867)	5700(998)	5673(902)	5650(851)	5683(169)	5660(882)
Fl Incon%	99.2(1.1)	99.1(1.3)	99.2(1.02)	99.2(1.1)	99.1(.21)	99.2((1.2)
Fl Incon RT	6926(1365)	6963(1344)	6937(1353)	6928(1338)	6968(1301)	6939(1323)
Fl Interference	22.2(12.9)	22.1(9.7)	22.2(12.1)	22.5(12.8)	22.6(9.7)	22.59(12.0)
Stern 6 %cor	85.2(11.9)	83.7(13.2)	85.0(12.2)	84.7(12.8)	85.5(14.0)	85.0 (13.1)
Stern 6RT	12543(2584)	13112(3137)	12703(2747)	12542(2505)	13194(3090)	12971 (1991)
Stern 8 %Corr	72.5(14.8)	71.9(20.6)	72.3(16.5)	74.1(16.8)	73.2(20.8)	73.8(17.81)
Stern 8 RT	13310(3072)	13782(3818)	13442(3284)	13390(3284)	14010(4100)	15043(5767)
WCST	8.17(2.5)	8.18(2.5)	8.3(3.7)	8.06(2.6)	8.18(2.4)	8.5(3.7)

*Original dataset : Eriksen Flanker: *n* =111; Stroop: *n*=116; Sternberg 6: *n*=108; Sternberg 8: *n*=109; WCST: *n*=114
Abreviations: Fl Cong%-Eriksen Flanker Congruent-% Correct; Fl Incon %- Eriksen Flanker Incongruent % Correct; Fl Inter-Flanker Interference; Stroop CW – Stroop Color-Word, Stroop Int- Stroop Interference; Stern 6% Cor-Sternberg 6 % Correct; Stern 6RT-Sternberg 6 Match Correct Reaction Time; Stern 8 Cor-Sternberg 8 % Correct; Stern8 RT – Sternberg 8 Match Correct Reaction Time; WCST-Wisconsin Card Sort Test

Correlational Analysis Between Variables

As expected, **s**ignificant positive correlation was revealed between estimated VO_2 max and Yale Index ($p < .001$). Yale weekly kilocalorie expenditure was positively related to the Yale Index ($p < .001$), and similarly with estimated VO_2 max ($p = .031$). Both age and sex were negatively related to estimated VO_2 max ($p < .001$ for both, while education was positively related to estimated VO_2 max ($p = .02$). Female gender was negatively related to education ($p = .02$). Results are summarized in Table 3.

Table 3

Pearson Correlations-Imputed Dataset *n*=117

	1	2	3	4	5	6	7	8	9	10	11	12
1.Age												
2.Sex	-.236											
3.Ed	-.109	-.224										
4.*APOE*	-.189	**-.242**	.088									
5.Act Inv	-.069	.020	.046	-.040								
6.YWkcal	-.035	-.056	.059	.066	.172							
7.YIndex	-.060	-.173	.179	-.038	-.036	**.465**						
8.VO2	**-.362**	**-.436**	**.384**	.119	-.185	.235	**.496**					
9.Stroop	.159	-.136	.224	-.061	-.081	.005	**.111**	.076				
10.Flank	-.061	-.033	.112	.005	-.098	-.064	-.086	-.152	-.220			
11.Stern6	-.004	-.129	-.041	.110	-.090	-.066	.193	.015	-.051	-.023		
12.Stern8	-.024	-.105	.051	.080	-.059	-.190	-.031	.069	-.066	-.025	**.656**	
13.WCST	.144	-.142	-.046	.021	-.111	.098	.022	-.080	.098	.047	-.095	-.056

Bold: $p<.01$ Underlined: $p<.05$ Abbreviations: Ed-Education; Act Inv-Activity Inventory; YWkcal-Yale weekly kilocalories; VO_2 - Estimated VO_2 max ; Flank-Eriksen Flanker Interference Score; Stern 6 and 8-Sternberg 6 and 8 Match Correct Reaction Time; WCST-Wisconsin Card Sort Test % perseverative erors

Hierarchical Regression Analysis

Eriksen-Flanker task

There were no significant main effects in relation to the Eriksen-Flanker Interference score when considering 1) weekly kilocalorie, 2) Yale Index, or 3) estimated VO_2 max as predictor variables. There were no significant interactive associations with regard to *APOE* genotype and kilocalorie energy expenditure or cardiovascular fitness measures. Regression diagnostics revealed three discrepancy outliers for this task, but analysis showed no effect on significance, so they remained in the dataset. Results are summarized in Tables 4 and 5.

Stroop Color and Word Test

There were no significant main effects or interactions with genotype related to weekly kilocalorie expenditure, Yale Index, or estimated VO_2 max for this task. Results are summarized in Tables 4 and 5.

Wisconsin Card Sort Test

There were no significant main or interactive effects in relation to percentage of perseverative errors on the WCST for weekly kilocalorie expenditure or Yale Index. Results for estimated VO_2 max revealed no significant positive associations after one high-leverage sedentary ε4 homozygote was removed from the dataset. Results are summarized in Tables 4 and 5.

Sternberg 6 and 8 Match Correct Reaction Time tasks

Working memory performance on the Sternberg 6 Match Correct Reaction Time task was positively related to weekly kilocalorie expenditure in *APOE*-ε4 carriers, while no such relationship was observed for non-carriers. *APOE*-ε4 status x Yale Weekly Kilocalorie score was revealed as a negative slope for performance on the Sternberg 6 Match Correct Reaction Time task ($p < .001$, $\Delta R^2 = .111$, $\beta = -0.72$). Working memory performance was positively related to weekly kilocalorie expenditure for both ε4 carriers and non-carriers on the Sternberg 8 Match Correct Reaction Time task. Both Yale Weekly Kilocalorie score and *APOE* x Yale Weekly Kilocalorie score were revealed as negative slopes for cognitive performance ($p = .04$, $\Delta R^2 = .038$, $\beta = -0.20$ and $p = .04$, $\Delta R^2 = .035$, $\beta = -0.41$, respectively). (It is noted that as measure is for reaction time, a negative slope corresponds to a conceptually positive association.) Effect size f^2 for Sternberg 6 Match Correct Reaction Time *APOE* x Yale Weekly Kilocalorie score was

0.1, f3or Sternberg 8 Wkcal, f^2 was 0.04, and for Sternberg 8 x Yale Weekly Kilocalorie score interactive effect, f^2 was 0.04. Yale Index was revealed as a positive predictor for the Sternberg 6 Match Correct Reaction Time, with those participants with a higher Index displaying poorer reaction time for correct performance on this task (p = .04, R^2 = .036, β = 0.20). There were no significant effects related to estimated VO_2 max for this task. Hierarchical regression results for Sternberg task are summarized in Tables 4 and 5. Scatterplots showing slopes of interaction results are shown in Figures 1 and 2.

For further exploration, a median split stratified by Yale Weekly Kilocalorie measure was conducted. Results revealed working memory performance on the Sternberg 6 Match Correct Reaction Time task was positively related to weekly kilocalorie expenditure in *APOE*-ε4 carriers, while no such relationship was observed for non-carriers. Yale Weekly Kilocalorie score x *APOE* was revealed as a negative slope for performance on the Sternberg 6 Match Correct Reaction Time task for the upper median split (p=.01, ΔR^2= .138, β = -1.20), with high-active *APOE*-ε4 carriers performing better than equally active ε4 non-carriers. However, for the lower median split there was no such interactive association; results revealed working memory performance on the Sternberg 6 Match Correct Reaction Time task was negatively related to *APOE*-ε4 genotype such that those carrying the *APOE*-ε4 allele showed slower reaction times for correct Sternberg 6 performance compared to *APOE* -ε4 non-carriers. *APOE*-ε4 genotype was revealed as a positive slope (p = .02, ΔR^2 = .100, β = 0.33). There were no significant findings for a median split analysis of Sternberg 8 Match Correct Reaction Time task. Results are detailed in Table 6, and scatterplots are shown in Figure 3.

Figure 1

Sternberg 6 and 8 Match Correct Reaction Time –Yale Weekly Kilocalories

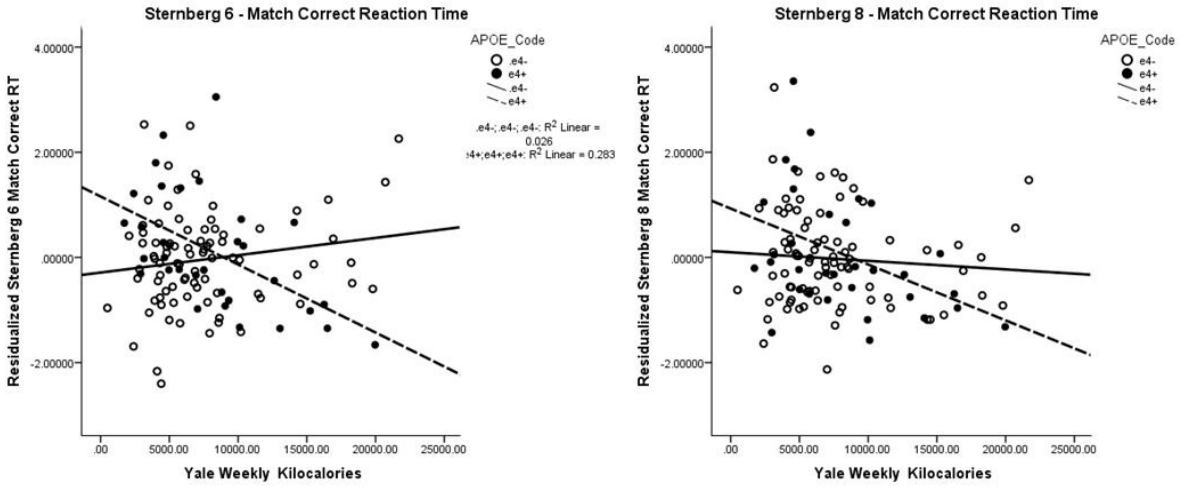

Figure 1. Genotype x physical activity interaction for Sternberg 6 and 8 Match Correct Reaction Time task. *APOE*-ε4 carriers show decreased reaction time with increasing time spent in physical activity participation (Yale Weekly Kilocalories) in the Sternberg 6 condition. In the Sternberg 8 condition; a decrease in reaction time is observed for both ε4 carriers and non-carriers. ε4 carriers are represented by dotted lines and solid circles.

Figure 2
Sternberg 6 Match Correct Reaction Time – Yale Index Score

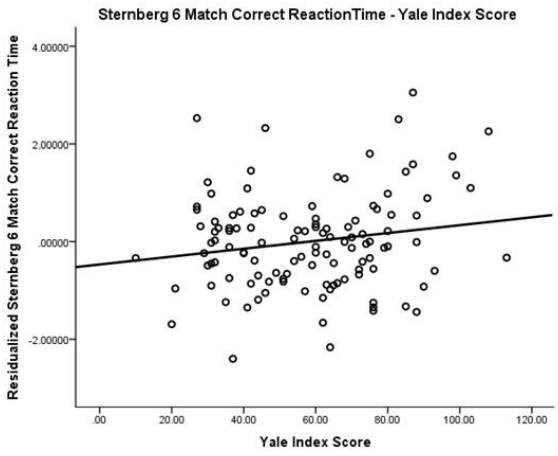

Figure 2. Increased reaction time on Sternberg 6 Match Correct task is observed as high-aerobic intensity physical activity increases (Yale Index score).

Figure 3

Sternberg 6 Match Correct Reaction Time Task – Median Split by Weekly Kilocalories

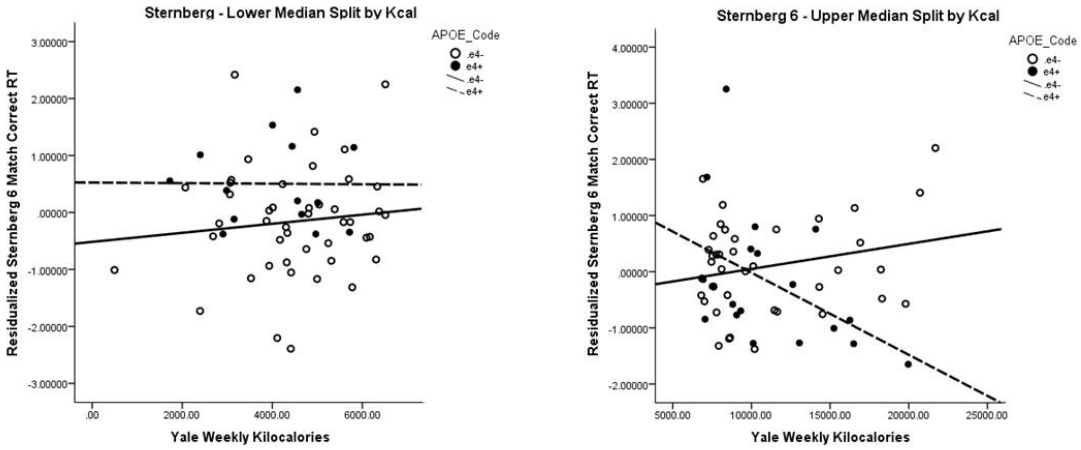

Figure 3. Genotype x physical activity interaction for Sternberg 6 Match Correct Reaction Time task by median split. *APOE*-ε4 carriers show decreased reaction time with increasing time spent in physical activity participation(Yale Weekly Kilocalories)in the upper median split, with no decrease seen for non-carriers. No genotype x physical activity interaction differences are noted in the lower median split. ε4 carriers are represented by dotted line and solid circles.

Table 4

Hierarchical Regression – Yale Weekly Kilocalorie /Yale Index *n* =117; ε4$^+$=33

	Flanker			Stroop			WCST			Stern 6			Stern 8		
Predictor	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**
Age	.52	.004	0.06	.09	.025	0.16	.12	.021	0.14	.97	.000	-.004	.79	.001	-0.02
Sex	.84	.000	-0.02	.27	.010	-0.11	.23	.012	-0.11	.15	.018	-0.14	.22	.013	-0.12
Educ	.21	.014	0.12	**.01**	**.050**	**0.23**	.51	.004	-0.06	.40	.006	-0.08	.83	.000	0.02
APOE	.89	.000	0.01	.49	.004	-0.07	.87	.000	0.02	.43	.005	0.08	.64	.002	0.05
Act Inv	.30	.010	-0.10	.36	.007	-0.08	.29	.010	0.12	.37	.007	-0.08	.53	.003	-0.06
Wkcal	.58	.030	-0.05	.90	.000	0.01	.21	.014	0.12	.51	.004	-0.06	**.04**	**.038**	**-0.20**
*APOE*x	.26	.012	0.23	.16	.016	-0.28	.29	.009	0.21	**.00**	**.111**	**-0.72**	**.04**	**.035**	**-0.41**
YIndex	.25	.012	-0.11	.49	.004	0.07	.88	.000	0.02	**.04**	**.036**	**0.20**	.544	.371	-0.03
*APOE*xYInd	.26	.011	0.32	.13	.019	-0.42	.10	.024	-0.46	.45	.005	-0.21	.958	.003	-0.16

Abbreviations: Ed-Education; Act Inv-Salthouse Activity Inventory; Wkcal-weekly kilocalories Flanker-Eriksen Flanker Interference Score; Stroop-Stroop Interference Score; Stern 6 and 8– Sternberg 6 and 8 Match Correct
Reaction Time Task; WCST-Wisconsin Card Sort Task % perseverative errors;YIndex-Yale Index; *APOE*xYInd-*APOE* x Yale Index

Table 5

Hierarchical Regression – Estimated VO_2 max *n* =77; ε4$^+$=20

	Flanker			Stroop			WCST*			Stern 6			Stern 8		
Predictor	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**
Age,Sex,Ed	.97	.003	0.01	**.001**	**.214**	**0.42**	.78	.014	0.12	.98	.002	0.02	.55	.029	0.10
APOE	.77	.001	0.04	.19	.019	-0.15	.53	.006	-0.08	.64	.003	-0.06	.83	.001	-0.03
Act Inv	.87	.000	0.04	.34	.010	-0.10	.11	.035	-0.19	.49	.007	-0.08	.59	.004	-0.06
VO_2	.26	.018	-0.20	.69	.002	0.06	.26	.017	-0.19	.30	.015	-0.18	.08	.042	-0.30
*APOE*xVO_2	.18	.026	0.61	.55	.004	-0.24	.61	.004	-0.27	.79	.001	0.12	.61	.004	0.23

Abbreviations: Ed-Education; Act Inv-Salthouse Activity Inventory;VO_2-Estimated VO_2 max; flanker-Eriksen Flanker Interference score; Stroop-stroop Interference score; WCST-Wisconsin Card Sort; Stern 6 and 8 -Sternberg 6 and 8 Match Correct Reaction Time Task; *One high leverage outlier was removed from dataset (an e4$^+$ homozygote); with outlier included- Est VO_2 max, p=.069, ΔR^2= .044, β=-0.306; *APOE* x Est VO_2 max, p =.039, ΔR^2=.054, β=-0.890

Table 6

Hierarchical Regression-Sternberg 6 and 8 -Median Split by Yale WKcal

	Sternberg 6 Match Correct RT						Sternberg 8 Match Correct RT					
	Lower Split *n* = 58, ε4$^+$=20			**Upper Split *n* = 59, ε4$^+$=14**			**Lower Split *n* = 58, ε4$^+$=20**			**Upper Split *n* = 59, ε4$^+$=14**		
Predictor	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**
Age,Sex,Education	.50	.042	-0.12.	.86	.014	-0.04	.78	.020	-0.01	.53	.040	0.03
APOE	**.02**	**.100**	**0.33**	.28	.022	-0.16	.10	.048	0.23	.36	.015	-0.13
Act Inv	.45	.009	-0.10	.78	.001	-0.04	.63	.004	-0.07	.82	.001	0.03
YWKcal	.42	.011	0.11	.90	.000	-0.02	.50	.008	0.10	.27	.023	-0.17
APOE x YWkcal	.61	.004	-0.23	**.01**	**.138**	**-1.20**	.34	.017	0.46	.17	.034	-0.59

Abbreviations: Act Inv-Salthouse Activity Inventory; YWkcal- Yale Weekly kilocalories

Discussion

This study investigated physical activity in relation to cognitive function in older adults ages 50-70, with additional regard for *APOE* genotype. To augment findings of past research, this study was designed to more clearly define specificity in the physical activity/cognition association, employing a cognitive task battery to clarify performance differences regarding inhibitory control, cognitive flexibility, and working memory aspects of executive function, incorporating multiple measures of physical activity to discern relative importance of high-intensity aerobic activity versus total energy expenditure. In addition, *APOE* genotype, known as a factor related to Alzheimer's disease and general cognitive decline, yet sensitive to physical activity involvement, was examined for its role in the relationship between physical activity, fitness, and cognition. As the participants in this study were generally healthy, well-educated, and highly intellectually engaged, with little variance remaining for association of physical activity with cognition, this study provided a conservative analysis of the relationship.

The findings did not support a general association of physical activity with cognition, as there was no single relationship trend shown for all cognitive challenges. Instead, results supported the concept of specificity with regard to all three factors of the investigation. No significant relation was found for either measure of physical activity with inhibitory or cognitive flexibility executive function tasks, but significant associations were revealed for both the general study population and for those at higher genetic risk of cognitive decline when working memory was challenged with a Sternberg task. An *APOE*-ε4 x weekly kilocalorie interaction was found to account for significant variance for the Sternberg 6 Match Correct Reaction Time task, with faster processing

speed of this working memory capacity challenge for highly active *APOE*-ε4 carriers, and with no such benefit shown for non-ε4 carriers. With the more challenging 8-letter condition of the task, both main and *APOE*-related interactive effects were revealed, with more highly active individuals demonstrating faster processing of correct responses, but with a higher magnitude of benefit for *APOE*-ε4 carriers. Additionally, the Yale Index score revealed as a positive predictor for performance on the Sternberg 6 Match Correct Reaction Time task, with those exhibiting higher levels of high-aerobic intensity activity performing worse on the task, suggesting yet another variation of specificity of physical activity in relation to a cognitive process.

As mentioned previously, there were no associations between the Eriksen-Flanker task and any measure of physical activity. Of note, neither Yale Index, a measure weighted toward high-aerobic physical activity, nor estimated VO_2 max, a measure of aerobic capacity, predicted performance on the Eriksen Flanker Interference condition. These findings are in contrast to the results of Colcombe et al. (2004), who found better Flanker performance post-intervention in an aerobically-trained group. It is possible that in this study population, the high cognitive reserve in the group based on the high education level may have left little variability for an association with aerobic activity or fitness level, and with the exception of three discrepancy outliers, the overall performances indicated a relatively narrow range in performance with low interference scores. It is noted that there were no differences in performance by genotype, with *APOE*-ε4 carriers performing equally with non-carriers. The Flanker task is a frontally mediated inhibitory task (Hazeltine, Poldrack, & Gabrieli, 2000) that would be sensitive to frontal aging, which as espoused by West (1996) is an aspect of normal, non-

pathogenic aging that occurs in the general population. While some studies have shown steeper cognitive decline in aging for *APOE*-ε4 carriers in executive function-related tasks, they generally have involved cognitive flexibility tasks rather than attention-related tasks such as the Flanker task (Wetter et al., 2005; Wisdom et al., 2009). As such, it would be reasonable that there would not be significant differences for ε4 carriers.

Stroop task performance was not related to weekly kilocalorie expenditure, Yale Index, or estimated VO_2 max. This task involves written words and as such is influenced by education and reading ability **(**Leverett, Lassiter, & Buchanan, 2002; Van der Elst, Van Boxtel, Van Breukelen, & Jolles, 2006). With a mean education level for this study population of 17.6 years, there may have been a ceiling effect, with little room for additional variance to be consumed by cardiovascular fitness or physical activity energy expenditure. Additionally, review of the literature revealed mixed findings with other investigations related to physical activity and Stroop interference performance in older adults. While several researchers have determined benefits from aerobic training (Bixby et al., 2007, Dustman, Ruhling, & Russell, 1984; Smiley-Oyen, Lowry, Francois, Kohut, & Ekkekakis, 2008) others have found no benefit for aerobic fitness (Blumenthal et al.,1989), or benefit only for an added switching condition (Coubard, Duretz, Lefebvre, Lapalus, & Ferrufino, 2011; Predovan, Fraser, Renaud, & Bherer, 2012). Interestingly, the two interventions that found a benefit only for an added switching condition of the Stroop, with no interference benefit, incorporated aerobic dance as the sole exercise condition. It may be that the type of aerobic activity in which an individual is engaged may alter the cognitive relationship with Stroop Interference. With the wide variety of physical activities reported in this study population, there may have been influences from

cognitive aspects of the pursuits that contributed to variability in the results with this task. As with the Flanker task, there were no significant differences regarding the *APOE* x VO_2 max interactive association with this task, but despite lack of significance, performance of two *APOE*-ε4 homozygotes - one with high estimated VO_2 max with one of the top Stroop Interference scores, and the other with low estimated VO_2 with one of the poorest Interference scores -highlight extreme differences for those most at risk of cognitive decline with regards to a cardiovascular fitness measure. These findings reflect results of Wetter et al. (2005) who in an *APOE* genetic-related study (with no physical activity component), noted no significant differences in ε4 carriers in the Interference condition of the Stroop task, but commented that performance by ε4 carriers was more highly variable when compared to non-carriers.

The lack of significance with any measure of physical activity in relation to the Wisconsin Card Sorting task is in accord with results of Etnier et al. (2007) with regard to *APOE* genotype, and Smiley-Oyen, Lowry, Francois, Kohut, & Ekkekakis, (2008) with regard to a general population of older adults. While this task is a measure of executive function, a breakdown of the cognitive processes involved in the task reveal a multitude of varied cognitive processes, including maintenance of information, concept formation, inhibition of previously learned responses, attention, and set shifting (Cinan & Tanör, 2002). In addition, there may be a reliance on verbal strategies for some individuals (e.g., memorizing "three red circles") that involves the phonological loop for storage of verbal information (Cinan & Tanör). Miyake, Friedman, Emerson, Witzki, & Howerter (2000), in a latent-variable analysis of several executive function tasks, commented on a "task impurity" problem of this and other executive function tasks, with cognitive processes

not directly relevant to target executive function potentially interfering with results. Taken together, these multiple processes operating during WCST testing may complicate the delineation of a straightforward association with physical activity.

In the Sternberg working memory task, we found no main physical activity or genetic/physical activity interactive effect regarding accuracy of performance, but did find differences in reaction time, with a main effect for the Sternberg 8 condition, and interactive effects on both Sternberg 6 and 8 conditions of the Match Correct reaction Time task for *APOE*- ε4 carriers. In the Sternberg 6 condition, the interactive effect showed better performance by ε4 carriers with higher energy expenditure, with no such benefit revealed for non-ε4 carriers. In the more challenging Sternberg 8 condition, more highly-active individuals from the general population exhibited better reaction time performance; however, more highly-active *APOE*-ε4 carriers demonstrated significantly better performance even after considering the variance accounted for of the general study population.

To explore the genetic/physical activity interactive relationship further, a median split analysis stratified by weekly kilocalorie expenditure was conducted. This examination revealed significantly different findings for *APOE*-ε4 carriers based on their position in upper or lower portions of the analysis. *APOE*-ε4 carriers in the lower half of the split showed poorer performance on the Sternberg 6 Match Correct Reaction Time task compared to non-carriers regardless of their weekly kilocalorie expenditure. This finding relates to deficits reported in the literature in cognitive ability for ε4 carriers in the area of processing speed during working memory tasks (Hofer et al., 2002; Reinvang et al., 2010). However, higher levels of weekly energy expenditure appeared to offer

significant benefit in association with speed-related memory performance of ε4 carriers in this study, allowing them surprisingly to surpass the performance of equivalent higher-active non-ε4 carriers on the same task condition.

The better performance of the higher-active *APOE*-ε4 carriers in this study on a specific working memory task supports the research showing cognitive benefit for highly-active individuals of this genotype compared to lower-active ε4 carriers (Niti et al., 2008; Rovio et al., 2005; Schuit et al., 2001), but the revelation of significantly better performance when compared to equivalently active non-carriers in the Sternberg 6 condition was unexpected. However, there is support for equal or better performance from general *APOE*/cognition literature. Alexander et al. (2007), evaluating cognitive function related to *APOE* genotype across six decades in an international investigation (ages 6-65), found no deficits in verbal working memory reaction time among ε4 carriers compared to non-carriers across the entire age span. Jochemsen, Muller, van de Graaf, and Geerlings (2012), in a median split analysis by age of a four-year prospective study of individuals in the Netherlands (mean age 57±10 years), found a significant positive association with immediate and delayed memory recall for ε4 carriers below the age of 57, with better performance than non-carriers. The concept of antagonistic pleiotropy, whereby a gene exhibits a benefit early in life but a disadvantage later in life, has been primarily associated with young adult ε4 carriers, but as results of the two above general studies and this *APOE* x physical activity/cognition study suggest, this benefit may extend even into early older adulthood. While acknowledging the limitations of this cross-sectional investigation and the findings that relate to reaction time on a single working memory task, when evaluating physical activity levels as a moderating variable,

it appears from this study that this positive association in early aging may be limited to those *APOE*-ε4 carriers with a physically active lifestyle.

As reported previously, we did not find that cardiovascular fitness was significantly associated with the Sternberg memory task, instead finding a positive association with general physical activity as measured by weekly kilocalorie expenditure. While there is ample evidence for a positive effect of cardiovascular fitness in relation to cognitive function in older adults, there is evidence for a benefit for total physical activity as well. Our research is in accordance with results of several general older adult population studies without regard for *APOE* genotype. Flöel et al. (2010), specifically examining both self-reported kilocalorie expenditure and VO_2 max and lactate threshold in a cross-sectional study, found a positive association with performance on the Auditory Verbal Learning Task only with physical activity as measured by self-reported weekly kilocalorie energy expenditure. Buchman et al.(2012), in a four-year prospective study of an older age population (mean baseline age 81.6, SD 7.12), offered more objective evidence for a benefit for total energy expenditure using baseline accelerometer data. These researchers found that total daily physical activity predicted both reduced cognitive decline and incident Alzheimer's disease and improved performance on a cognitive test battery of episodic, semantic, and working memory, perceptual speed, and visuospatial ability regardless of whether it involved high-intensity or moderate level physical activity. The findings remained significant after controlling for *APOE*-ε4 status.

Of course, questions remain as to possible reasons for this relationship. Much of the physical activity reported by older adults includes everyday chores and moderately active leisure pursuits. A review of physical activities reported by participants in this

study with high levels of energy expenditure included such endeavors as caring for small children or elderly parents, farm work, or hiking. These types of activities tend to involve both cognitive and physical challenges, which together may evoke changes in the brain that are particularly helpful in improving verbal memory performance for those most at risk of cognitive decline. These findings are similar to those of Niti et al. (2008), who investigated cognitive decline in relation to *APOE* genotype and participation in physical, social, or productive activities. While acknowledging limitations and overlap when categorizing activities, these researchers found for ε4 carriers, compared to non-carriers, that while all three types of activities were beneficial in preventing a drop in the Mini Mental Status Exam (MMSE) scores at one year, activities that were deemed primarily "productive" (i.e – preparing meals, gardening, shopping, volunteering) were more effective at preserving cognition than those deemed to be primarily physical or social activities. (No significant benefits were found for ε4 non-carriers.) The movement aspect of these activities may be particularly important, a point supported by the finding in this study that when cognitive activity was measured through intellectual pursuits without much of a physical component, as reported on the Salthouse Inventory, there were no findings of significance relating to improved performance on the Sternberg task.

There was a finding that the Yale Index negatively predicted performance on the Sternberg 6 Match Correct Reaction Time task. It was surprising to find a negative association with working memory with any measure of physical activity, but given that overall kilocalorie expenditure did significantly positively predict performance, it is speculated that the amount and type of physical activity associated with high-intensity exercise may be the key to understanding this discovery. When examining the type of

physical activity common for high-intensity pursuits, typical modalities for aerobic workouts include treadmill or cycle exercise, which may not provide the cognitive stimulation needed for preserving working memory capacity. In support of this possibility, review of a cycling/cognition study surprisingly supported a negative relationship for this activity with select cognitive functions. This investigation provided two conditions for a 3-month aerobic activity cycling intervention for older adults living in retirement communities - one involving stationary cycling alone, and another with an added virtual reality cognitive component in which participants viewed a 3D scenic tour and competed against an avatar. While a significant improvement was noted in executive function for virtual reality cycling, those in the stationary cycling condition revealed no benefit for cognitive function; in fact, revealing a non-significant decline in an executive function composite score (with primary deficiency on a digit span backwards score). Neither condition offered any benefit for performance on the Rey Auditory Verbal Learning (RAVLT) task (Anderson-Hanley et al., 2012). Voelker-Rehage, Goode, & Staudinger (2010) offer behavioral and neuroimaging evidence for differing effects of different types of physical activity, showing that motor fitness (comprised of balance, flexibility, coordination, and fine motor skill), common to many moderate level physical activities, was associated with better performance on an identical picture recognition task, whereas physical fitness (aerobic capacity and muscular strength) showed no such association. These researchers also found different brain activations relating to the two different measures, commenting that different types of exercise facilitate different brain processes, with impact on “competing cortical networks” whereby motor fitness could possibly attenuate effects of physical fitness.

While mode of physical activity may influence cognitive function, time invested in the activity may be an additional factor influencing cognition. High-aerobic exercise performed by participants in this study was frequently completed in short bouts, with some participants engaging in little other physical activity throughout the day, often sitting for hours at work or engaged in inactive leisure pursuits. Pate, O'Neill, & Lobele (2008) highlighted the issue of time spent in sedentary activity, commenting that an individual could meet recommendations for physical activity participation by engaging in a short session of high-intensity exercise, yet if inactive the rest of the day, could expend considerably less energy when compared to another who is actively moving for much of the day in light to moderate physical activities.

The lack of significance related to estimated VO_2 max may involve a lack of power in this smaller dataset. Inability to accurately report estimated VO_2 for several participants receiving Beta blocker medication resulted in loss of eight individuals from the dataset, including three *APOE*-ε4 carriers (one homozygote). However, similar findings for the larger dataset showing no significant associations for the Yale Index score and cognitive performance on any of the cognitive tasks provide support that the same factors that may have resulted in non-significance for these tasks when using estimated VO_2 as a measure. Alternatively, as discussed previously, it may be that for this older adult population, cognitive enhancement may be necessary to augment cardiovascular fitness to reveal a positive association with performance on some executive function-related tasks.

Limitations of this study include that it was cross-sectional, so only associational inferences may be made. There was also limited power to detect dose-response effects of

APOE-ε4 genotype. While this study contained a reasonable percentage of ε4 carriers, there were very few ε4 homozygotes. The extreme differences in cognitive performance seen in two ε4 homozygotes on the Stroop task in relation to estimated VO_2 max - with one high fit and one sedentary – suggest that effects of this genotype may be stronger than reported, yet lack of participants in this category did not allow for any conclusions to be made for dose-response relationships. Use of estimated VO_2 max and self-reported physical activity offered less precision in measurement than other methods available; however, analysis correlating high-intensity physical activity and estimated VO_2 max offered support for accuracy of the instruments. Medical considerations were also a concern for this age group, with medications potentially interfering with cognitive performance or complicating interpretation of aerobic fitness testing, and obesity issues extending interpretation of estimated VO_2 max calculation to ranges beyond weights validated by the test.

Advantages of this study included the employment of a battery of cognitive tests and multiple measures of physical activity or aerobic fitness to allow for comparisons. Many previous studies have been limited in scope regarding cognitive or physical activity measures, resulting in incomplete and confusing information in the literature. This study was able to more clearly define cognitive/physical activity associations in areas of inhibitory control, cognitive flexibility, and working memory because of the comprehensive study design. We were also able to recruit individuals with a wide range of physical fitness and activity levels, which augmented ability to determine differences in cognitive performance. Overall, this study highlighted the importance of physical activity for older adults, particularly for those who are at greater genetic risk for

dementia, revealing relationships with improved memory for those at higher genetic risk of Alzheimer's disease. Finally, this research provides convincing evidence to foster promotion of community physical activity programs for older adults and to aid in providing guidelines for program design, and contributes to a body of knowledge to assist in future interventional physical activity/cognitive research design incorporating *APOE* genetic analysis.

Chapter 4: Literature review of physical activity/cardiovascular fitness and cognition pertaining to younger adult population

Physical Activity/Cardiovascular Fitness and Cognition in Young Adults

While much of physical activity/cognition research has centered on the older adult population, questions remain about cognition throughout the lifespan. There is research available in younger adult populations under age 50, and as one would expect it is a smaller body when compared to studies dealing with older adults, but results have revealed some differences in cognitive function of younger adults as related to physical activity or cardiovascular fitness. These studies have primarily taken the forms of epidemiological studies or cross-sectional comparison studies relating differences between young and older adult high and low-fit populations. Intervention studies are less common but the ones that exist have provided interesting results. Some of these studies evaluated emotional benefits of physical activity as well as cognitive advantage.

It is important to note that the young adult population spans several decades, and that there are likely differences between the extremes of ages. Cognitive findings may be different for individuals in their twenties compared to those approaching middle age. Indeed, effects of cognitive decline may begin well before age 50; Singh-Manoux et al. (2012) found that reduction in cognitive function as measured by a battery of tests occurred as early as age 45. Salthouse (2009) discovered that loss of regional brain volume, diminished serotonin receptor binding and striatal dopamine binding, and other brain changes could begin as early as the late twenties or early thirties.

Review of Physical Activity/Cardiovascular Fitness Studies as Related to Cognitive Performance in Young Adults

In a large epidemiological study, Richards, Hardy, and Wadsworth (2003) looked at an older age group of young adults of both sexes. Using measures of physical activity

and spare-time leisure activity participation, these researchers found in a 1946 British birth cohort (n = 1919), that both physical activities and spare-time leisure activities at age 36 were associated with higher verbal memory scores at age 43. A 15-item word list learning task/recall was employed to determine verbal memory, with number of words correctly recalled over three trials serving as score. The Minnesota leisure time questionnaire was used to determine engagement in physical activities, with evaluation of number of different physical activities over the previous month used as a measure.

This study also investigated the effect of change in physical activity level between age 36 and 43 and its effect on memory at age 53, finding that reduction of physical activities after age 36 resulted in a greater decline in memory score at age 53, but sustained or increased physical activity participation at age 43 predicted higher verbal memory scores at age 53. In other words, improved physical activity participation at age 43, even without previous exercise habits, suggested a protective effect on memory, while those with high levels of physical activity earlier did not receive an extended benefit if they discontinued their exercise habits. The underlying message from these results implied that recent and continual physical activity appeared to be protective of cognition in midlife. Physical activity scores at age 36 and 43 were also additive in predicting reduced memory decline at age 53 (Richards et al., 2003).

In an old versus young cross-sectional comparison study (105 male participants, grouped by ages 18-28, 35-45, or 60-73), Shay and Roth (1992) used the Astrand-Rhyming submaximal bicycle ergometer test to evaluate cardiovascular fitness, and a battery of ten cognitive tests to evaluate visuospatial and verbal memory, attention and concentration. Participants were recruited from local athletic clubs and community

groups, and included several individuals who were long-time athletes, assuring a wide range of physical training. Results revealed a significant fitness relationship for both older and younger groups for the visuospatial component only. The oldest group displayed a more robust cognitive relationship with cardiovascular fitness.

While epidemiological studies or cross-sectional investigations can illustrate general associations of physical activity or cardiovascular fitness with cognitive benefits, intervention studies can more closely explore the specificity of aerobic exercise or physical activity in relation to cognitive performance. Examination of a battery of tasks can help clarify the cognitive functions by which physical activity or cardiovascular fitness shows the greatest effect.

One investigation assessed the effect of aerobic endurance exercise on memory and affect in young adults ages 17-29 (Stroth, Hille, Spitzer, & Reinhardt, 2009). A randomized intervention was conducted with a class of 26 medical assistant students, who either trained aerobically by running 30 minutes a day, five days a week for six weeks, or who were assigned to a social control group that was matched to intervention participants by sex and baseline physical activity habits. All participants were assessed cognitively for short-term visuospatial and verbal memory using the Visual and Verbal Memory test, and concentration using the d2 Test of Attention. Results revealed significantly higher visuospatial scores for participants in the aerobic training group compared to controls. No difference between groups was reported on concentration or verbal memory. In addition, the runners group demonstrated significant benefits in positive affect that was not shown in controls.

An additional investigation by Stroth et al. (2010) in 75 young adults ages17- 47 found that a 17 week quasi-experimental (non-randomized) aerobic training intervention improved select executive functions. No benefit was found in reaction times for 2 *N*-back task in runners versus controls, nor was there an effect of exercise training for incongruent trial reaction times for the Stroop task. However, in a more complex executive function task, the dots-mixed task, runners did show improvement in reaction times for correct responses compared to controls.

This study also examined the role of a polymorphism of the catecho-O-methyltransferase (COMT) gene in relation to cognitive function. The researchers hypothesized that COMT Val^{158M}et can be used as an index of dopamine availability in the prefrontal area, since the Met allele results in lower activity of the COMT enzyme and higher dopamine levels, and the Val allele indicates higher enzymatic activity and lower dopamine levels. The study found that individuals with Val/Val genotype showed improved cognitive performance in relation to aerobic training to a greater degree than those with a Met allele, suggesting a mediating influence of dopamine (Stroth et al., 2010).

Ismail and El-Naggar (1981) examined effect of a 4-month physical fitness program in 48 males ages 24-68 (mean age 42) with 35 participating in intervention and 13 age-matched individuals serving as non-participatory controls. The participants were primarily engineering professors. The program was comprehensive, consisting of calisthenics, jogging, and recreational sports activities performed for three 90-minute sessions per week. All participants were assessed with a battery of ten cognitive tests (Verbal Reasoning, Successive Numbers, Digit Span, Trail-making A and B, Space

Relations, Series, Classification, Matrices, and Conditions). Successive numbers was the only task that revealed significant improvement in *t*-test comparisons of the experimental physical training and control group. Discriminant function analysis revealed that performance in temporal order tasks were more successful in discriminating between pre and post results than for visuospatial tasks.

Another young adult study examined differentiated effects of acute versus chronic exercise on cognition and affect (Hopkins, Davis, Vantieghem, Whalen, & Bucci, 2012). A four-week intervention of 54 adults ages 18-36 was conducted with four groups – two with an aerobic treadmill intervention (4 times per week for 30 minutes), with one of the trained groups performing an additional acute exercise bout post-intervention two hours before post-intervention cognitive testing of recognition memory. Participants in a third group maintained their regular physical activity level throughout the study, participating only in one acute bout of exercise two hours before cognitive post-test, and a fourth group served as a no-exercise control. Results showed that participants in the aerobic group with a post-intervention acute exercise bout and the acute bout-only group performed significantly better in object recognition memory, compared to the control group and aerobic intervention group without acute bout. These results in essence showed no effect of long-term aerobic training on an object recognition memory task. However, affect results revealed both aerobically trained groups showed improved positive mood. This study also contained a genetic component, examining effects of BDNF val^{66}met polymorphism, and researchers found that the improvement in recognition memory found after an acute exercise bout was observed exclusively in val/val homozygotes, with no

cognitive benefit for those with a Met allele, suggesting that lower release of BDNF in met allele carriers may attenuate benefits to cognition in an acute exercise condition.

Pereira et al. (2007) investigated the concept of exercise-induced neurogenesis and angiogenesis, looking at MRI measurements of cerebral blood volume (CBV) in the dentate gyrus before and after a 3-month, four times a week aerobic fitness intervention in 11 participants (ages 21- 45, mean age 33, 2 males, 9 females) with baseline low aerobic fitness levels. The aerobic fitness intervention allowed free choice among cycle ergometer, treadmill, Stair Master, or elliptical trainer. The Rey Auditory Verbal Learning Task (AVLT) was used to assess cognitive function pre-and post-intervention. Participants testing at post-intervention demonstrated improved CBV in the dentate gyrus area that correlated with higher VO_2 max and improved performance on trial 1 of the AVLT, with trending improvement on all-trial learning and delayed recall. No effect was found for recognition memory or source memory. No control group or alternative intervention existed for this study.

A study by Hansen, Johnsen, Sollers, Stenvik, & Thayer (2004) evaluated the effects of aerobic physical training and detraining in 37 male sailors ages 18-22. The intervention was conducted immediately after basic training was completed. An 8-week cardiovascular fitness intervention was employed for all participants. At the end of the intervention, executive and non-executive cognitive tests were administered. Tests consisted of simple reaction time, choice reaction time, serial pattern matching, and working memory (2-*N* back). After the cognitive tests were completed, participants were divided into two groups – one whose members continued shore-based duties and who maintained their aerobic training for an additional four weeks, and another group that was

assigned to ship duty and whose members received no aerobic physical training. The ship-based group members, however, were exposed to heavy manual work during the second period, so while they may have been detrained aerobically (and results showed decrease inVO_2 max for this group) they were still physically active. Cognitive tests were administered again after four weeks. Results showed that the group that continued aerobic physical training improved performance on executive tasks, while the detrained group performing manual work improved performance on the non-executive tasks.

To clarify some of the uncertainty posed by differing findings in previous studies, Hötting, et al. (2012) conducted an intervention in 68 sedentary middle-aged adults (40-55, mean age 48) examining cognitive change post-intervention in both cardiovascular fitness and stretching and toning groups, along with a no-activity control group. Cognitive tasks examined attention with the d2 test of Attention, episodic memory with the AVLT, perceptual speed with a task similar to Trail Making A, executive function with Stroop task, and spatial reasoning with a subtest of a German intelligence test.

Fitness results of 6-month physical training revealed increases of about 15% in VO_2 max in an aerobic cycling group, with no significant changes in stretching and toning and control groups. The cycling group also showed higher total energy expenditure compared to the two other groups, but there was no difference in amount of time spent in physical activity in the cycling and stretching groups. These researchers noted wide individual variation in changes in VO_2 max in both groups, and in light of this finding, additionally compared across-group variance in VO_2 peak improvement according to a median split.

Cognitive change results revealed that the cycling group showed greater improvement in episodic memory recognition score than either the stretching or control groups. Improvement in attention was significantly greater in the stretching group compared to the cycling group, but not significantly different from the control group. Both trained groups showed greater improvement in episodic memory learning compared to the control group. When examined across groups in relation to VO_2 peak, it was confirmed that improvement of episodic memory learning score was related to this fitness variable regardless of group intervention. No other cognitive variables showed significance based on cross-group VO_2 peak. There were no differences among groups in executive function, perceptual speed, and spatial reasoning, indicating a possible ceiling effect in this young adult group, according to the researchers. Of note, a follow-up of this study one year post-intervention of 25 participants (Hötting, Schauenburg, & Röder, 2012) found that while episodic memory scores decreased overall during this time period, those participants who maintained cardiovascular fitness did not show a reduction in episodic recognition memory. (Both cycling and stretching groups had high-fit members.)

In summary, it can be said that among the young adult age group, while relationships of physical activity or cardiovascular fitness with cognitive function were clearly revealed, it is difficult to determine a pattern of specific effects based on available literature. Differences in age and education level of groups tested, limitations of cognitive tasks chosen for investigation, complications of aerobic fitness interventions with modalities that could incorporate more motor learning (i.e.-sports play, work onboard a ship, free choice of several pieces of gym equipment), and small study sizes all

complicate ability to make a concise statement as to specificity of exercise with regard to cognitive processes in this age group.

Relationship of Physical Activity/Cardiovascular Fitness and Cognitive Training

Human physical activity or cardiovascular fitness studies that specifically examine the effect of cognitive practice are scarce. Most intervention studies, as described above, involve an exercise protocol with pre-and post-cognitive challenges, rather than a cognitive challenge with role of physical activity or cardiovascular fitness explored as a mediating variable. Therefore, evidence for improvement in complex cognitive processes in relation to physical activity or cardiovascular fitness comes mainly from an epidemiological study, from interpretations of animal studies, and from comparisons of structural and functional brain adaptations induced by cognitive training in activity/cognitive interventions in older adults.

Review of Cognitive Improvements From Cognitive Training

In order to understand the role that physical activity or cardiovascular fitness plays in learning, it is helpful to review the theories supporting cognitive training. Training studies capitalize on the concept of plasticity of the human brain, employing intense practice in specific cognitive tasks, with the belief that training can result not only in improvement in the trained task, but can also transfer to benefit more general cognitive functions in areas sharing the same neural circuitry. In particular, working memory and attentional/interference control tasks have been utilized in training studies since they are known to be important in higher order cognitive functioning (Buschkuehl & Jaeggi, 2010; Shipstead, Redick, & Engle, 2010) and can predict success in tasks ranging from reading comprehension (de Jong & de Jong, 1996) to foreign language vocabulary

acquisition (Service, 1992) to reasoning and problem-solving (Chein & Morrison, 2010; Buschkuehl & Jaeggi, 2010; Morrison & Chein, 2011). A study by Jaeggi, Buschkuehl, Jonides,and Perrig (2008) revealed that working memory training resulted in transfer to improvement in measures of fluid intelligence. These researchers commented on components of their training that may have aided in transfer – that training was adaptive to continually challenge all participants, that it engaged multiple executive processes such as inhibition control, monitoring, updating, and dual-task management, and that their choice of training task, a dual *N*-back incorporating both visual and auditory processes, resisted the use of simple strategies to improve performance, all of which may have facilitated attentional control necessary for improved success on a fluid intelligence task.

Despite success by Jaeggi et al.(2008) and others (Chein & Morrison, 2010; Jaeggi et al., 2010; Jaušovec & Jaušovec, 2012; Stephenson, 2010) in obtaining transfer to far-reaching cognitive function, these results have not been universal and several other studies have failed to find transfer (Li et al., 2008; Redick, Shipstead et al., 2012; Salminen, Strobach, & Schubert, 2012), leading to questions as to other factors that may be affecting whether transfer is shown from cognitive training. Morrison & Chein (2011) commented that it is important to explore differences in individuals that may be driving transfer, and Jaeggi et al. (2011) explored this issue in a discussion of a training study with children. These researchers found that greater improvement in the training task related to increase in performance on transfer to fluid intelligence tasks. Jaeggi et al. theorized that providing optimal challenge was crucial in this relationship, or alternatively, that a ceiling effect for some participants may have affected transfer. However, it should also be considered that physical activity or cardiovascular fitness

status of participants in this and other cognitive training studies has not been explored, and as reviewed above in relation to cognitive performance, physical fitness may play a critical role in cognitive learning, with higher fitness level contributing additional resources for improving cognition and potentially serving as a mediator in determining success of training and transfer. Evidence for this relationship is discussed below.

Physical Activity/Cardiovascular Fitness and Intelligence

Regarding the question of cognitive benefit from cardiovascular fitness relating to a global benefit in intelligence, one large epidemiological study investigating physical condition in young adult males in Sweden observed such an association. Aberg et al. (2009) followed over 1.2 million Swedish men enlisted in the military from 1950 -1976 and found that cardiovascular fitness scores at age 18 (determined upon enlistment in the military) were associated with better performance on the Wechsler Adult intelligence Scale. Additionally, when physical fitness scores at age 18 were compared to school physical education grades at age 15, those who had shown improved cardiovascular fitness over three years demonstrated significantly higher global intelligence scores than those with decreased fitness levels over the same time period. This significant association continued in both the cross-sectional and longitudinal investigation with subgroups of logical, verbal, visuospatial, and technical scores. Muscular strength did not correlate with higher scores in this population. These researchers examined heritability in a subgroup in this study, comparing results for 268,496 siblings, 3147 dizygotic twin pairs, and 1432 monozygotic twin pairs. It was found that cardiovascular fitness showed a heritability of 56% and heritability of cognitive measures ranged from 52-56%. When a cross-twin, cross-trait bivariate analysis was performed, however, genetic factors

explained less than 15% of the correlation between cardiovascular fitness and the five cognitive measures.

Neurobiological Similarities of Cognitive Training and Physical Training

As discussed above, working memory improvement has been shown to relate to success in several cognitive training studies, a cognitive process also known to show improvement through physical activity or cardiovascular fitness training in young adults, as described in a previous section of this paper (Hopkins et al, 2012; Ismail & El-Naggar, 1981; Pereira et al., (2007); Richards et al., 2003; Stroth et al, 2009; Hötting et al., 2012). Neuronal effects of working memory training have been studied to help more clearly elucidate mechanisms that may influence success of cognitive training (Buschkuehl, Jaeggi, & Jonides, 2012), and it is helpful to evaluate physical activity neurobiological mechanisms that may also relate.

Change in activation of select brain regions is one area where effects are seen with cognitive training. Olesen, Westerberg, and Klingbeg (2004) conducted a cognitive training intervention in young adults and found increased brain activity in the prefrontal and parietal regions along with decreased activation in the anterior cingulate cortex (ACC) after five weeks of visuospatial working memory training. Interestingly, an aerobic exercise intervention conducted with 29 older adults (mean age 65), and involving six months of physical training, also resulted in increased prefrontal and parietal activity and decreased activation in the ACC, along with improvement on a Flanker task (Colcombe et al., 2004). It is noted that these findings in an older population may not transfer to a younger adult population and that the regional changes shown may

relate to many different processes, but the similarities to cognitive training are interesting and warrant discussion.

Other neuronal effects of cognitive learning interventions reported by Buschkuehl et al. (2012), such as improved functional connectivity or larger gray matter density or stronger white matter integrity, have also been shown in physical activity interventions in older adults. Colcombe et al. (2006) conducted a 6-month randomized clinical trial aerobic exercise intervention for 59 individuals ages 60-79 years and found increased gray and white matter in prefrontal and temporal cortices in the aerobic training group, and Rovio et al. (2010) found in a longitudinal study that midlife physical activity in 75 older adults corresponded with greater total brain volume and increased gray matter in the frontal region area 21 years later. Voss, Prakash, et al. (2010) found improved connectivity in the DMN in 65 sedentary older adults ages 55-80 after a one-year intervention in both aerobic walking and balance and flexibility training groups.

Dopamine in particular may be related to working memory capacity, and has been found to be affected by both cognitive and physical training. McNab et al. (2009), using positron emission tomography (PET) and fMRI, found decreased dopamine binding potential for the D1 receptor in prefrontal and parietal cortices, which was associated with gains in working memory with cognitive training in young adult males. Landau, Lal, O'Neil, Baker, and Jagust (2009), using similar imaging methods but working with older adults, reported a role for dopamine in working memory by showing that striatal dopamine correlated positively with higher working memory performance on a Sternberg task and with brain activation of the prefrontal cortex during maintenance of working memory. As reported previously, dopamine release has been shown in animal studies to

be increased with physical exercise in several brain regions in younger populations (Goekint et al., 2012; Meeusen et al., 1997), and Stroth et al. (2010) suggested a mediating influence of dopamine availability in the prefrontal region in young adults based on results showing differing effects on cognition in val or met allele carriers of the COMT val^{158}met polymorphism. These findings could translate to a role for physical training providing support for improving working memory through cognitive training.

Is Physical Training Additive to Cognitive Training?

The similar neuronal and behavioral consequences with cognitive training and physical activity interventions leads to the question whether a combination of both types of training could be additive in improving cognitive function in young adults. There are studies that lend credence to this possibility. Fabel et al. (2009) found that providing wheel running opportunities for 10 days followed by 35 days of enriched environment promoting learning resulted in an increase of 30% in neurogenesis in mice compared to each condition individually. No cognitive task was presented in this study, but other animal studies have correlated increased neurogenesis with improved performance on a water maze task (van Praag, Christie, et al., 1999) or shown that reduced neurogenesis impairs spatial and object recognition memory (Jessberger et al., 2009). A note by Fabel et al. illustrates an important point of the differences between neurogenic effects of aerobic exercise (running wheel) and enriched environment (toys) - that running stimulates proliferation of precursor cells in the dentate gyrus, while enriched environment contributes to survival of new neurons.

Combined Physical and Cognitive Training Interventions in Humans

Two human studies of older adults also provide evidence that physical activity interventions are additive to cognitive training. In addition, these investigations provide insight into the puzzle as to whether some modalities of physical activity enhance cognition primarily because of cognitive learning, or instead because the movement involved with humans playing a sport or performing active household work is of major significance in the cognitive learning process.

Oswald, Rupprecht, Gunzelmann, & Tritt (1996) conducted a nine-month intervention study of 309 independent-living older adults ages 75-94, examining cognitive memory training or psycho-education alone or in combination with physical activity training (targeting primarily balance, coordination, and flexibility, using dancing, games, yoga and skilled activities such as family tennis or table tennis). Physical activity alone and a control group rounded out five groups. All participants participated in cognitive training, physical activity, or education in a once a week session of approximately two-three hours, with additional cognitive work provided to train at home.

Results from the cognitive training revealed significant effects in cognitive ability specific to trained tasks of processing speed, attention, memory, and reasoning when compared to the non-treatment control group. No cognitive benefits were found for the physical activity group. The combined physical activity training and cognitive training group experienced similar cognitive benefits as the memory training group, and in addition this group displayed transfer benefits in the area of reduction of symptoms of cognitive impairment. This combined group was also the only group to show reduced symptoms of dementia at one and two-year follow-ups (Oswald et al., 1996). At a five

year follow-up, the combined group continued to demonstrate stability of benefits for cognitive function and resistance to symptoms of cognitive impairment. Attrition did occur as with any longitudinal study, with older and less healthy individuals more likely to drop-out. However, there were no differences between groups in rate of attrition. The researchers commented on limitations of the study in that motivation or group dynamics, as well as 45 more minutes per week spent in group activity, may have played a factor in increased benefits for the combined group. It was also noted that after completing the training program many of the individuals involved continued with activities presented in the interventions, making it difficult to ascertain long-term effects of the initial training period alone (Oswald, Gunzelmann, Rupprexcht, & Hagen, 2006)

One other combined cognitive and physical training intervention explored the effects of aerobic exercise alone or in combination with cognitive training (Fabre, Chamari, Mucci, Massé-Biron, & Préfaut, 2002). Thirty-two participants (ages 60-76) were assigned to either a cognitive training group, an aerobic walking/jogging group, a combined training and exercise group, or control group for two months. The cognitive training centered on eight themes – "perceptive activities, attention, intellectual strucuration, association and imagination, language, spatial marks, temporal marks, and associated recruiting". Logical memory and paired associates learning scores from the Wechsler memory scale improved in all trained groups, but the achievement was significantly higher in the combined group on all cognitive tasks when compared to the other trained groups. All participants were sedentary at beginning of study. In this study, all groups, including the control group meeting for leisure time activities (i.e-painting, choral singing) spent the same amount of time in group sessions.

Genetic Influence of *APOE* Genotype on Cognition and the Role of Physical Activity in This Relationship

Although the study of the relationship of genetic factors and cognition is in its infancy, there is evidence that some genetic variations may influence cognitive function, with additional support for the role of physical activity in the genetic/cognition relationship. One gene of interest is Apolipoprotein E (*APOE*), a transporter of cholesterol and other lipids, and notable as a risk factor in development of late-onset Alzheimer's disease. *APOE* is characterized by three variants or alleles - ε2, ε3, and ε4, with the ε4 allele present in about 15% of the population (Bu, 2009). The ε4 allele has a different orientation, with amino acid substitutions that contribute to a more unstable protein with higher likelihood of fragmentation (Harris et al., 2003). As a result of these differences from ε2 or ε3, the ε4 genotype is associated with differences in structure and function of areas of the brain throughout life (Chen et al., 2007; Reiman et al., 2004; Scarmeas et al., 2005) and has been shown to negatively affect cognitive function in older healthy adults (Wisdom, Callahan, & Hawkins, 2009).

Characteristic Brain Differences in *APOE*-ε4 Carriers.

The characteristics of brain activity in *APOE* ε4 carriers throughout life show some distinct differences from non-carriers. Hypometabolism at rest has been shown repeatedly to be a distinct aspect of ε4 carriers' brain function, even in healthy young adulthood, with reduced metabolism and lower oxygen delivery to the frontal, parietal, medial temporal lobes (Reiman et al., 2004). In cognitively healthy older ε4 carriers, Reiman et al. (2005) showed that gene dose of ε4 negatively corresponded to cerebral metabolic rate for glucose in posterior cingulate, parietotemporal and frontal cortex.

Differences in brain structure in *APOE* ε4 carriers compared to non-carriers also exist. For example, older adult ε4 carriers frequently show more brain atrophy compared to non-carriers even in cognitively healthy individuals (Chen et al., 2007) and this difference is pronounced in the hippocampal area (Lind et al., 2006). Shaw et al. (2007) found that cortical thickness of the left entorhinal cortex was significantly thinner even in children and adolescents carrying the ε4 allele when compared to ε3's and ε2's. However, contrary to other structural comparisons, Richter-Schmidinger et al. (2011) found no differences in hippocampal volume in young adult ε4 carriers (mean age 24).

With regards to regional brain activity and connectivity, cognitively healthy older adult *APOE*-ε4 carriers show altered brain activation under memory challenge tasks (Sperling, 2007; Bookheimer et al., 2000). Sperling reported that older ε4 carriers displayed increased hippocampal activation that converted to hypoactivation as memory became more impaired, possibly forecasting a path toward dementia. Filippini et al. (2009) found in an fMRI investigation that young adult ε4 carriers (ages 20-35) also displayed greater hippocampal activation during memory encoding compared to non-ε4 carriers, as well as increased coactivation bilaterally in the medial temporal region and medial-prefrontal cortex within the DMN during rest. Filbey, Slack, Sunderland, & Cohen (2006), using fMRI and magnetoencephalography (MEG), found increased neural activity in frontal and medial areas and cingulate gyri in young adults during a working memory task, (mean age 26), with regional BOLD responses correlating to increased theta power. Scarmeas et al. (2005), using positron emission tomography (PET), showed that young ε4 carriers exhibited higher activation in some temporal areas (left middle

temporal gyrus and right transverse temporal gyrus) and lower in others (right superior temporal gyrus and left inferior temporal-fusiform gyrus) during encoding of a memory task. Dennis et al. (2010) found greater bilateral activity in the medial temporal lobe and the posterior cingulate during encoding, but reduction overall in connectivity across anterior and posterior cortices. Performance of cognitive tasks in the above studies generally showed no differences between young adult ε4 carriers and non-carriers, despite differences in brain activation as shown in neuroimaging. One study of 340 young adults showed different findings of brain activation, however, with decreased brain activity over three learning trials in ε4 carriers, as opposed to ε2 and ε3 carriers, who increased activity. This lower activation corresponded to better episodic memory retrieval of words in a delayed free recall task in carriers of the ε4 allele (Mondadori et al., 2007).

Some additional findings in younger adults have shed some new light on possible mechanisms for effects of the ε4 allele. One study that examined postmortem gene transcripts from brain cortical samples of young adults who were either ε3 or ε4 revealed differences relating to mitochondrial function with ε4 carriers that was occurring before evidence of plaque or tangle formation (Valla et al., 2010). Another study revealed mitochondrial damage postmortem in the posterior cingulate cortex in young adults, again found without plaque (Conejero-Goldberg et al., 2010). Both of these studies offer support for a theory of mitochondrial dysfunction mediating, at least in part, the role the *APOE*-ε4 allele, and may offer an explanation behind the hypometabolism seen in ε4 carriers.

Behavioral Investigations Regarding *APOE* Genotype in Young Adults.

Cognitive behavioral studies of *APOE* genotype in young adults have revealed some conflicting findings. Similar to Mondadori et al's (2007) findings of better performance of *APOE* ε4 carriers in episodic memory, Alexander et al. (2007), looking at cognition in 415 participants ages 6 – 65, also found an advantage for ε4 carriers in a specific cognitive function - verbal fluency. This was observed in all age groups studied. However, performance on a spatial maze task was lower in ε4 carriers in the 16-30 age group (with no differences for non-carriers in other age groups). There were also no differences from non-carriers on an attentional task. Contrary to findings of enhanced functioning in select cognitive tasks in ε4 carriers found by Mondadori et al. and Alexander et al, Liu et al. (2010), in a study of 2208 adults (mean age 49) found no differences in performance on the AVLT in ε4 carriers under age 50, but diminished performance for the task in individuals over 50. These researchers commented that cognitive effects were seen as early as age 40 for ε4 homozygotes. Bunce, Anstey, Burns, Christensen, & Easteal (2011) found no evidence of higher cognitive function in ε4 carriers in any task administered to adults in a large study (n = 6560) stratified by age (20-24, 40-44, and 60-64). This study examined simple and choice reaction times, as well as a cognitive battery to assess processing speed, working memory, immediate and delayed recall, and lexical decision making. A meta-analysis by Ihle, Bunce, & Kliegal (2012) examined 20 studies and also found no evidence of antagonistic pleiotropy as found by Mondadori et al. and Alexander et al.

Physical Activity and Cognition in Relation to *APOE* Genotype.

With all of the findings showing differences in brain structure and function between *APOE* genotypes, one may wonder about the benefit of physical activity for ε4 carriers in comparison to the general population. Few such studies are currently available, but findings from them suggest there may be particular benefit of either moderate physical activity or high-intensity aerobic physical activity for *APOE*-ε4 carriers.

Only one *APOE* genetic study evaluating cardiovascular fitness in relation to cognitive function in young adults is known to this student. In a cross-sectional study, Woo et al. (unpublished abstract, 2008), determined event related potentials in a group of 59 young males between the ages18-23 (29 ε4 carriers) during both a go/nogo executive function task and a non-executive function oddball task. A Stroop task was administered additionally as a behavioral task. Sedentary carriers of the ε4 allele displayed smaller P300 amplitude bilaterally at frontal and central regions compared to higher fit ε4 carriers, who were undifferentiated from both high and low-fit non-carriers. Participants with higher aerobic fitness levels from both ε4 and non-ε4 categories exhibited shorter P300 latency at all sites measured when compared to lower-fit individuals of any genotype. There were no differences between genotypes on the Stroop interference scores.

Physical activity studies in older adults have revealed differences in cognitive performance by those carrying an ε4 allele. Schuit et al. (2001) conducted a prospective study of the relationship of physical activity participation and cognitive decline over a three-year period in 347 older men ages 65 - 84. Incidence of cognitive decline was indicated by a drop of three or more points on the Mini Mental Status Exam over the

three-year period as related to physical activity level and genotype. These researchers observed that the risk of cognitive decline was 13.7 times higher in sedentary ε4 carriers relative to physically active non-carriers, or four times higher compared to active ε4 carriers. Deeny et al. (2008) investigated the effect of exercise on working memory in adults 50-70 years of age with regard to *APOE* genotype. Using both behavioral and MEG brain imaging evidence, these researchers examined cortical dynamics and cognitive performance on a behavioral Sternberg memory task, comparing carriers and non-carriers of the *APOE*-ε4 allele. Highly active ε4 carriers showed faster reaction time in correct responses on the memory task compared to sedentary carriers. Sedentary ε4 carriers also showed a lower level of activation in the right medial temporal lobe compared to highly active ε4 carriers, who were undifferentiated from non-carriers in activation. Etnier et al. (2007) examined working and episodic memory in relation to aerobic capacity (VO_2 max) in older women who were classified according to *APOE*-ε4 carrier or non-carrier groups. They reported that the cognitive behavioral effects of ε4 showed evidence of a dose-response for sedentary ε4 carriers, with ε4 homozygotes of lower aerobic fitness exhibiting poorer response to a delayed memory task of the AVLT compared to ε4 heterozygotes or non-carriers.

In terms of the neurobiological mechanisms provided through physical activity for *APOE*-ε4 carriers, one animal study shed light on possible factors by examining exercise and cognition in 10-12 month old *APOE*-ε3 and *APOE*-ε4 transgenic mice. Nichol, Deeny, Seif, Camaclang, & Cotman (2009) engaged one-half of both genotypes in training on a running wheel while the other half served as sedentary controls. Cognitive testing was conducted at baseline and after six weeks of physical training with an object

recognition task, a place recognition task, and a radial-arm water maze task. No effects of exercise were observed for the object recognition task in either genotype. *APOE* ε4 mice were impaired at baseline for the place recognition task compared to ε3 mice, but improved performance with exercise training, as did the ε3 mice. The ε4 mice improved performance on the radial arm maze after exercise to the point that their error rate was indistinguishable from that of the ε4 mice. These researchers determined that sedentary ε4 mice were not deficient in BDNF at baseline when compared to ε3 mice, but did exhibit 50% less of tyrosine kinase B (Trk B), the high affinity receptor for BDNF. Exercise training increased BDNF levels in ε4 mice and brought levels of Trk B to equal that of ε3 mice. Running also dramatically increased levels of synaptophysin, suggesting improved synaptic function.

Chapter 5: Variability in cognitive performance and learning in younger adults explained by cardiovascular fitness, physical activity, and *APOE* genotype.

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Abstract

Both cognitive training and physical activity have been shown to be positively related to cognitive function, and there are questions about genetic influences on cognition as well, but there has been no research investigating possible interactions of these factors in this relationship. To explore these multiple issues, a cognitive training intervention was conducted in 106 adults ages 22-50, additionally examining the roles of physical activity participation, cardiovascular fitness, and *APOE*-ε4 genotype. Hierarchical regression analyses were performed to explain variance accounted for by these variables in relation to performance on a battery of cognitive tests presented pre-and post-intervention and with regard to transfer observed on three novel post-intervention learning challenges. Cognitive training revealed benefit only for tasks specifically trained, with no transfer to post-intervention learning tasks. However, cardiovascular fitness was positively related to performance on a post-intervention proactive interference learning challenge, and a trend was observed for performance on an additional transfer challenge - a foreign vocabulary divided-attention task. No significant findings were observed related to *APOE* genotype on post-intervention tasks or learning challenges. This study has shown that cardiovascular fitness is associated with improved cognitive performance and learning even in young adulthood, revealing a positive relationship with higher performance on learning challenges that were not affected by a computer-based cognitive training program.

Introduction

Interest in those factors affecting cognition is high among young and middle-aged adults as they work to stay mentally sharp and delay age-related cognitive decline. As an indicator of this interest, popularity of computer-based "brain games" is increasing, with many playing these games in the hope that practicing specific mental tasks will transfer to benefit more complex cognitive processes and improve thinking and reasoning ability. Some studies examining these cognitive training programs have shown them to be effective in improving memory and other executive functions in adults (Buschkuehl & Jaeggi, 2010; Jaušovec & Jaušovec, 2012), with some interventional studies supporting benefit transfer to other areas of cognition, including improvement in reasoning, problem-solving, and fluid intelligence (Chein & Morrison, 2010; Jaeggi, Buschkuehl, Jonides, & Perrig, 2008; Morrison & Chein, 2011). However, these findings are equivocal, with other studies finding no or limited effects of cognitive training (Li et al., 2008; Redick, Shipstead et al., 2012; Salminen, Strobach, & Schubert, 2012). The equivocal findings support the need to investigate other variables that account for the differences in findings across studies.

The importance of physical activity in maintaining cognitive function has received attention as well, but much of the research in this area has concentrated on older adult populations, with less known about benefits to cognition in younger adults. Studies of young adults have revealed benefits in the performance of tasks of verbal memory (Hötting, Schauenburg, & Röder et al., 2012), visuospatial memory (Stroth, Hille, Spitzer, & Reinhardt, 2009), and cognitive flexibility (Stroth et al., 2010), but investigations examining more complex learning processes through cognitive practice are

scarce in this age group. As such, questions remain about the ability of physical activity to augment the effects of cognitive training.

The role of genetic factors in cognition is also of interest, with some studies of older adults revealing cognitive benefit from physical activity for carriers of the *APOE* - ε4 genotype, characterized by attenuation of cognitive decline or improved performance on memory tasks for higher-active ε4 carriers when compared to lower-active ε4 carriers (Deeny et al., 2008; Schuitt et al, 2001). The ε4 genotype, however, has been shown to affect brain structure and function throughout life, with even young adults consistently revealing hypometabolism at rest in several areas of the brain (Reiman et al., 2004), and exhibiting differences in brain structure (Shaw et al., 2007) and activation during challenge (Filippini et al., 2009; Scarmeas et al., 2005). Some behavioral investigations have shown young *APOE*-ε4 carriers to display superior performance compared to non-carriers on some executive or memory tasks (Alexander et al., 2007; Marchant et al., 2010; Mondadori et al., 2007), while other studies revealed no difference in cognitive performance between carriers and non-carriers (Bunce et al., 2011; Ihle, Bunce, & Kliegal, 2012; Jorm et al., 2007). One study revealed poorer visuospatial memory performance in children and adolescent ε4 carriers with family history of dementia (Bloss, Delis, Salmon, & Bondi, 2008). Overall there is a lack of consensus about the role that *APOE* plays in cognition in young populations, and given the positive association shown for exercise in older adults and the discordance in findings of the effects of this gene in younger adult cognitive function, consideration of physical activity in relation to this genotype as a moderating variable warrants investigation in this population.

In light of the questions that remain regarding the association of physical activity with cognition in a younger adult population, this study aimed to investigate the relationships of both general physical activity participation and cardiovascular fitness with cognitive performance as well as the effects of cognitive training in adults ages 22-50. The influence of the *APOE* genotype was also considered. This study separately evaluated the contribution of cardiovascular fitness and physical activity to explain variance in performance on a battery of cognitive tests prior to and following 5 weeks of cognitive training, as well as examination of any transfer to learning tasks. It was hypothesized that both weekly energy expenditure and estimated VO_2 max would be positively related to cognitive performance, and that a greater magnitude of benefit would be revealed for carriers of the *APOE*-ε4 allele compared to non-carriers.

Methods

Participants

Participants were recruited from list-serves at the University of Maryland and through local community online newsletters, through advertising on Craig's List, and through posters and flyers asking about interest in improving memory through playing "brain games". A goal of 200 individuals in the age range of 22-50 was set for study population size, with stratification by age to include approximately equal numbers of individuals 22-30, 30-40, and 40-50.

Procedures

After expressing interest, volunteers were screened by a coordinator, who explained the study and inquired about age, education, handedness, and vision. A brief medical history was obtained and those with neurological or psychiatric conditions or on

psychotropic medication were eliminated from consideration. Those participants who continued to express interest in the study after the screening interview were appointed for an initial visit at a University of Maryland psychology lab, and were provided with an informed consent form to sign. Participants were also informed of payment of $100.00 for participation in study.

At the initial lab visit, informed consent was obtained and participants were provided with four surveys to complete – Need for Cognition, Beliefs about Malleability of Intelligence, the Paffenbarger Physical Activity Questionnaire, and the Physical Activity Readiness Questionnaire (PAR-Q).

Upon completion of questionnaires, cognitive testing commenced, with administration of seven different tasks – Attentional Networks, *N*-back 2, 4, and 6, Ravens Matrices, Nelson-Denny Reading Comprehension, Reading Span, Task Switching, and Shapebuilder. These tests were provided in three blocks randomized as to order of presentation. Block A consisted of Task Switching, Reading Span, and Attentional Network tasks; block B consisted of Ravens Matrices, Nelson-Denny, and Swahili dual-task (post-intervention only); Block C consisted of Shapebuilder, *N*-back tasks, and Proactive Interference and Artificial Grammar parts 1 and 2 (post-intervention only). Table 1 summarizes the cognitive tasks and times tested. Participants were provided with breaks during testing and a DNA mouthwash sample was collected during one of these breaks. Upon completion of the cognitive tasks, participants were allowed a practice session to gain understanding of the at-home online intervention.

For the final procedure of the first visit, anthropometric measurements of height, weight, and waist circumference of participants were obtained, and cardiovascular fitness

was assessed with the YMCA submaximal cycle ergometer test. If participants were unable to complete the cycle test at the first visit it was rescheduled for the post-intervention visit. Participants were paid $30.00 for completion of first testing session.

The intervention was administered online and was completed by participants at home or at other location of choice. Participants were randomized to four separate groups. One group practiced online games that emphasized interference control (Floop and *N*-back); another group trained with visuospatial tasks (Memnosyne and Shapebuilder); a third group performed both the interference control and visuospatial tasks; and a fourth group served as control, executing less challenging tasks (Remember Me, Follow Me). All participants were required to complete 26 minutes of cognitive training each day for five weeks, for a total of 35 training sessions.

After completion of online cognitive training, participants returned to the lab and completed the initial battery of cognitive tasks again. Three additional tasks were added to post-intervention testing – Artificial Grammar, Proactive Interference and Swahili vocabulary divided attention task. A summary of cognitive testing is presented in Table 1. Upon completion of these tasks, participants were paid the remaining $70.00 for participation. Participants were also asked to report any changes to their physical activity that would affect Paffenbarger Index score during the intervention period.

Table 1

Cognitive Tasks, Training Tasks, and Post-Intervention Learning Transfer Tasks

Cognitive Tasks Pre-Intervention	Cognitive Training Tasks and Conditions	Cognitive Tasks/Transfer Learning Challenges Post-Intervention
Block A	**Interference Control**	**Block A**
Task Switching	*N*-back	Task Switching
Reading Span	Floop*	Reading Span
Attentional Network		Attentional Network
	Working Memory Condition	
Block B	Shapebuilder	**Block B**
Ravens Matrices	Memnosyne*	Ravens Matrices
Nelson-Denny		Nelson-Denny
	Combined Condition	Swahili Divided-Attention**
Block C	*N*-back	
Shapebuilder	Floop*	**Block C**
N-back	Shapebuilder	Shapebuilder
	Memnosyne*	*N*-back
		Proactive Interference**
	Active Control Condition	Artificial Grammar**
	Follow Me*	
	Remember Me*	

*Task used for cognitive training only; not tested pre-or post-intervention ** Transfer task-tested post-intervention only

Questionnaires.

Need for cognition.

This scale identifies an individual's "tendency to engage in and enjoy thinking" (Cacioppo & Petty, 1982) and was used in this study to account for differences in attitude about cognitive challenge. Participants are asked to evaluate 18 sentences using a Likert-type scale to represent their feelings about the statements. Some examples of items rated include whether an individual enjoys challenges to find solutions to problems, whether he or she strives to find understanding of why things happen, or whether he or she prefers short or more comprehensive projects. This scale was developed and validated by Cacioppo and Petty, with additional validation contributed by Osberg (1987).

Implicit Theories of Intelligence Scale.

This scale is a measure of a belief that intelligence is dynamic – that it is not fixed and can adapt with learning. It is theorized that if an individual holds the belief that intelligence can be changed it may affect motivation during cognitive training, resulting in improved outcomes when compared to someone who does not hold this belief. This scale contains eight statements related to intelligence. Some examples of statements in this scale include "The effort you exert improves your intelligence." or "You are born with a fixed amount of intelligence." This instrument was developed by Abd-El-Fattach and Yates (2006) in accordance with the implicit theory of intelligence as espoused by Dweck (Mangels, Butterfield, Lamb, Good, & Dweck, 2006).

Physical Activity Readiness Questionnaire (PAR-Q).

This survey was designed to evaluate possible medical reasons contraindicating participation in physical activity or exercise or to determine if medical supervision or approval is necessary before performing a cardiovascular fitness assessment. This brief survey asks seven questions concerning known cardiac medical conditions, physician recommendations against physical activity, chest pain during exercise or at rest, dizziness or loss of consciousness with exercise, bone or joint problems, medications prescribed for heart conditions or hypertension, or any other reason contraindicating physical activity. This questionnaire was developed by the Canadian Society for Exercise Physiology (2002) and has been recommended for use by the American College of Sports Medicine as a basic screening to determine risk factors for exercise (ACSM, 2004, 2009).

Cognitive tasks-pre and post intervention tests.

Attentional Networks Executive Task.

This task examines three networks of attention - alerting, orienting, and executive attention. In this test, participants are instructed to focus on a middle arrow in a row of five by pressing a button on a computer keyboard to show the direction in which it is pointed. The rows are presented either aligned congruently with all arrows pointing the same direction (< < < < <), in a neutral condition with the middle arrow pointing with lines surrounding it (- - < - -), or in an incongruent condition with the middle arrow pointing in a different direction than the others in the row (> > < > >). The difference in reaction times between congruent and neutral or incongruent conditions assesses executive control of attention in a task involving cognitive conflict. In addition to the arrow pointing conditions, cue variations are provided prior to appearance of arrows in either a no-cue, center cued double-cue, or spatial cue to assess either alerting or orienting effects. The reaction time of the double-cued conditions minus no-cue conditions measure alerting effect, and the reaction time of the spatial cued minus center-cued condition examine orienting. Participants completed three test blocks of 96 trials each. The task is scored by the number correct and the reaction times for the different conditions. This task was developed by Fan, McCandliss, Sommer, Raz, & Posner (2002) and is a variation of the Eriksen Flanker task (Eriksen & Eriksen, 1974) with added cued reaction time as developed by Posner & Peterson (1990). This task was evaluated for psychometric properties in a review by MacLeod et al. (2010).

Raven's Advanced Matrices.

This non-verbal instrument measures problem solving ability and is used as a fluid intelligence test. With this test, participants view 3 x 3 matrices of detailed geometrical shapes, with the right item in the bottom row missing from the set. Eight choices are presented and participants are asked to select the one that is an appropriate match to complete the matrix. A total of 18 problems are presented in this 10-minute test and the dependent variable is the number of correctly solved problems. Two versions of this task were presented for pre-and post-testing, with randomization of version presented. This test has been in use since its development in 1932 and validity and reliability have been demonstrated over time in numerous population groups (Raven, 2000). The Advanced Matrices instrument is an adapted version of the test designed to provide discrimination of performance at upper levels of intelligence (Raven, Raven & Court, 1998)

Reading Span.

This is a complex span task that measures storage and processing functions of working memory capacity (Daneman and Carpenter, 1980). During this task, participants view sentences followed by letters. Participants are asked to respond to the sentences presented on the computer screen, pressing a computer key representing "true" or "false" indicating if the sentence makes sense. After responding to the question about the sentence, a letter is presented for 800ms and participants are instructed to commit each letter to memory in the order it was presented. After viewing three to seven sentence/letter sequences, participants must reproduce the letters viewed in the order that they were presented. The dependent variables for this task are the total score – the total

number of correctly recalled letters, and an absolute score - the number correct when the complete trial is recalled correctly. This test has been correlated with performance on reading comprehension measures (Daneman and Carpenter, 1980) and strong association with fluid intelligence has been observed by Redick, Unsworth, Kelly, & Engle (2012). Redick, Broadway, et al. (2012) determined high reliability and validity of the instrument.

N-back, levels 2, 4, and 6.

The *N*-back test is a test of verbal memory capacity where participants must recall information in a temporal order, but this task also measures interference control as an individual taking the test must inhibit a response to similar letters in different serial positions than the *N-back* position (Szmalec, Verbruggen, Vandierendonck, & Kemps, 2011). With this task, participants view letters presented one at a time for 500ms on the computer screen and are asked to respond if the letter presented is the same one viewed *N* items previously. This task tests at different levels for 2, 4, and 6 items back. If the letter on the screen is presented at the appropriate level back, participants respond with a "yes" response on the keyboard, otherwise a key representing "no" is pressed. Lures are added in this test, showing identical letters to the *N*-back letter, but in a different position. Fifty trials are presented at each level. Each sequence has 9-11 targets, 16-17 lures, and 32-34 distracters. The dependent variables are the percent correct for each item type, the reaction time for each item type, and *d*-prime and bias for target and lure trials. This test was developed by Kirchner in 1958. Miller, Price, Jaeggi, Buschkuehl, Perrig, & Meier (2010) evaluated this task for concurrent validity as a working memory measure.

Nelson-Denny reading comprehension.

The Nelson-Denny test is a measure of reading rate, vocabulary, and comprehension. Participants read passages and are presented with questions about the readings afterwards without having access to passages. This instrument contains 38 questions with five answer choices. The reading time to complete reading each passage serves as a dependent variable for this task, as does the number of correctly answered questions. This instrument was developed by Nelson and Denny in 1929. Validation and scoring reference information is provided in a test manual (Brown, Fishco, & Hanna, 1993).

Task switching.

This task is a measure of cognitive flexibility, an ability to quickly adapt to a different rule. In the task switching test, participants are instructed to press a computer key in response to a number displayed after one of four cue words are presented. Cue words "Magnitude" and "Low-High" prompt participants to select one key if the number presented is greater than the number five, and a different key if the number is less than five (the number five is never displayed). Cue words "Parity" or "Odd-Even" signal participants to select an appropriate key based on if the number presented is odd or even. The task switching model is based on work by Rogers & Monsell (1995). The 2:1 cue-task mapping used in this task was evaluated by Schneider & Logan, (2011), who found that this cueing resulted in only minor differences in switch cost with 2:1 mapping compared to 1:1 mapping.

Shapebuilder.

This task tests visuospatial memory by challenging participants to remember the color, order and spatial position of a series of geometric shapes presented. Participants view between 2 and *N* shapes on a 4 x 4 grid of connected squares and after the final shape in a series is shown, they are instructed to recreate the sequence by clicking on the correct colored shape and dragging it to the appropriate spatial position. The score for this "brain game" is based on points earned for correct spatial location, color, and shape, with partial credit given if location or location and shape are recalled. This task was developed by atkins et al.(submitted).

Cognitive tasks for working memory and interference control conditions.

Working memory group training tasks.

Shapebuilder.

This task was described above as a task tested pre and post-intervention

Memnosyne.

In addition to Shapebuilder, previously described, an additional visuospatial task is employed in the visuospatial working memory training and in combination working memory/interference control training intervention. In Memnosyne, participants view blue blocks appearing in various sections of a 4 x 4 grid. Participants are instructed to commit the location and order of appearance of the blocks, and reproduce the sequence. The number of blocks in the sequence begins at two and increases in level as needed to adapt to participant ability as training improvement occurs. Test scores are shown for each training period score along with participant's "high score" for training- to-date.

Interference control group training tasks.

N-back.

This task was described above as a task tested pre and post-intervention

Floop.

This game is presented as one of two tasks in the interference control training intervention, along with an *N*-back task previously described, and as one of four tasks in the combined working memory/interference control training intervention. This task trains to improve ability to resist distractions. It is a combination of Stroop and Flanker tasks. With this game, participants view five letters in a row, and hear a letter spoken at the same time. Participants are instructed to press a computer key representing "yes" if the spoken letter corresponds to the middle letter in the string, and "no" if it is not. Letters are presented in congruent (i.e.- F F G F F –and spoken letter is "G") or incongruent (i.e.- G G F G G, and spoken letter is "G") grouping, or in other mixed strings (G L X V J or L X G Y J). On the screen, score is shown along with "high score" for training-to-date.

Cognitive training control group tasks.

Follow Me.

This task instructs participants to click on a square in a 4 x 4 grid when a block with a flower design on it appears instead of a plain block. This task provides an active control testing situation, using a vigilance task instead of a working memory challenge. Scores, high score, and reaction times are shown for the participant.

Remember Me.

With this task, nature photographs are presented and participants are instructed to click a key corresponding to "yes" when a picture is presented that they have seen before, and "no" when the picture is new. This active control task tests recognition memory but doesn't engage working memory. Total score, reaction time, and high scores are posted for participant. This task has been validated by Sprenger et al. (in preparation).

Cognitive tasks-post-intervention learning challenges.

Artificial Grammar task.

The Artificial Grammar task is presented to participants during post-intervention cognitive testing to evaluate transfer of cognitive training to proficiency in this process. This task evaluates participants' ability to discern rules of grammar from one session where strings of letters are shown that adhere to a set of rules, to application of these rules to new sequences of letters that may or may not follow the rules presented in the first session. The grammar rules presented in the first session are complex enough that participants are unlikely to be aware of any strategy, and must instead rely on a "hunch" in determining if the rule applies with the novel letter strings in the second session. This task was originally developed by Reber (1967) with additional research contributed by Knowlton & Squire (1996).

Proactive Interference task.

Proactive interference is defined as a reduction in memory performance in learning new information due to intrusion from previously learned related information (Jacoby, Wahlheim, Rhodes, Daniels, & Rogers, 2010). This task presents 40 word pair associations (i.e.- ale-brew, sugar -candy) in a session with each pair viewed three times

for learning purposes, followed by a challenge with the first word in the pair presented, with participants asked to recall the second. A second session presents a viewing of a facilitated condition of 20 word pairs that matched pairs presented in the first session list exactly, then a proactive interference condition where 20 word pairs are changed from the previously presented associations to new ones (i.e.- ale-beer, sugar-honey), and finally 20 new word pairs presented for control purposes. During a recall session after the 60 items are presented, participants view one word from a pairing and are required to present the other word in the pair, inhibiting their memory of the first pairing by typing the second word from the association presented in the second session. This proactive interference task is based on work by Jacoby et al., who researched whether experience through practice with proactive interference can diminish its effects. It is being used in this study as a post-intervention test to examine transfer of learning from cognitive training.

Swahili divided-attention task.

This task, presented post-intervention, examines ability to transfer cognitive training to the task of foreign vocabulary learning, and also explores the ability to remember these words while performing a secondary task that divides attention to learning. During this task, 20 Swahili words are presented in pairs along with their corresponding English word. Participants view these pairs for seven seconds and remember these pairings while simultaneously performing a finger tapping task, presented in two difficulty levels. Participants hear auditory tones of different pitch during encoding portion of task, and they must respond to the tones by finger tapping the appropriate computer key corresponding to the tone presented. At the end of each viewing session, participants are presented with a testing session where they are

instructed to type the English translation for the Swahili word shown on the screen. Three trials of both "easy" and "hard" conditions comprise the testing. The dependent variables for this test are mean percent correct recall and mean percent correct for tapping for each of the three easy and hard trials. This test was developed by Nelson and Dunlosky (1994) as a measure to determine normative values for recall of foreign language vocabulary, using paired associates of a language using the English alphabet that has limited exposure among test-takers. The finger-tapping task used for dual-task interference was developed by Moscovitch (1994).

Physical activity or cardiovascular fitness instruments.

Paffenbarger Physical Activity Questionnaire.

The Paffenbarger Physical Activity Questionnaire, also known as the Harvard Alumni Activity Survey or College Alumnus Activity Survey, (Paffenbarger, Wing, & Hyde, 1978; Paffenbarger, Blair, Lee, & Hyde, 1993) was employed to assess aspects of physical activity. This measure, comprised of eight questions, was selected to meet the needs of the study for a concise instrument validated for use by self-report. The primary measure of the questionnaire is a Physical Activity Index that provides an estimate of weekly caloric expenditure through calculations of metabolic equivalent units of task (METs) for sport and recreational activities over the past year, as well as estimated daily walking and stair climbing reported by participants. Duration of participation in each recreational activity is also detailed. The Index can be broken down by METs to categorize light, moderate, or high intensity physical activity subtotals. In addition to the Index measure, this survey questions about intensity of exercise, requesting the number of times per week that a participant engages in activity that causes sweating, heavy

breathing, or heart thumping. A measure of total daily activity in this survey asks participants to categorize two 24-hour periods (one weekday, one weekend) into five physical activity levels- vigorous, moderate, or light activity, sitting, and sleeping or reclining. Two questions concerning a participant's self-reflection of exercise habits and exertion complete the instrument. Details of this instrument including validation information are contained in Appendix C.

YMCA cycle ergometer test.

The YMCA cycle ergometer was used for submaximal VO_2 testing. This protocol, based on descriptions from *The Y's Way to Physical Fitness* (Golding, Joseph, & Campagna, 1989) tests at multiple stages at different resistance levels and is designed to elicit heart rate response to increased workloads on the cycle ergometer. The protocol for the test involves initial weighing of participant, fitting bike seat appropriately so that left leg has only a slight bend at the knee, and fitting with heart rate monitor and blood pressure cuff. Resting heart rate and blood pressure are obtained and participant is instructed to maintain steady 50rpm intensity throughout the procedure. Stages are timed for three minutes, with heart rates recorded at second and third minutes of each session. If two heart rate measures in the same session have more than a five beat discrepancy, an additional minute is allowed to allow heart rate to plateau, with additional minutes added if necessary to ensure that beats are within five beats from previous reading. Blood pressure is checked during each session, and at the end of each session, participants are asked to report their perceived exertion level (Borg, 1982).

The test consists of two-four different sessions, with the first rate set at 150 kgm/minute, followed by additional sessions with increased resistance that are based on

each participant's heart rate at end of previous session. Two sessions with heart rate over 110 beats per minute are required to provide adequate data for valid scoring. Testing is stopped when an individual reaches 85% of his or her estimated age-predicted heart rate maximum or if participant experiences adverse symptoms (ACSM, 2009). The test is scored by an equation that reflects the slopes of power output for each workload using the ACSM metabolic equation for cycle ergometry. Details of this instrument including validation information can be found in Appendix D.

DNA collection procedure.

A mouthwash procedure was used in this study to obtain a DNA sample to analyze for *APOE* genotypes (Lum & Marchand, 1998). With this procedure, participants are asked to rinse with 10 ml of mouthwash for 45 seconds and then expectorate into a 30 ml test tube. This procedure is conducted after abstinence from food or drink for one hour as dictated by procedure protocols, and was completed during pre-intervention cognitive testing by research assistants. After collection, samples were stored at -20^{o} C for one to five months before DNA isolation and genotyping was conducted. This method has been shown to be a reliable collection method for obtaining buccal cell DNA. All researchers were blind to genotype during the study. Details regarding DNA isolation and genotyping can be found in Appendix B.

Statistical Analysis

Descriptive statistics were determined for study participants and correlation matrices were generated for cognitive and physical activity/fitness variables. Change in cognitive performance was determined by using post-intervention cognitive test scores as dependent variables while controlling for pre-intervention performance. Independent *t*

test analysis was used to assess differences between *APOE* genotypes and differences between those who completed the cognitive intervention and those who did not. Regression diagnostics were conducted to ascertain outlying points of leverage or discrepancy or global or local influence.

Hierarchical regression was conducted for this study, examining the relation of general physical activity participation, as measured by total weekly kilocalorie expenditure self- reported by participants on the Paffenbarger Physical Activity Questionnaire, or cardiovascular fitness, as measured by the YMCA Cycle Ergometer Test of estimated VO_2 max, with cognitive performance separately across the battery of tasks tested pre-intervention. Each of the cognitive measures of interest was regressed in a hierarchical manner in two models. The first model included 1) Age, 2) Sex, 3) Education, 4) *APOE* genotype, 5) Need for Cognition, 6) Estimated VO_2 max, and 7) *APOE* genotype x Estimated VO_2 max interaction. A separate regression was conducted for the second model, including 1) Age, 2) Sex, 3) Education, 4) *APOE* genotype, 5) Need for Cognition, 6) Weekly Kilocalorie Expenditure, and 7)*APOE* genotype x Weekly Kilocalorie Expenditure. Simple regressions were conducted separately when any interaction term achieved significance to determine which group exhibited a significant relationship between physical activity or cardiovascular fitness measure and cognitive performance.

Hierarchical regression was also conducted using the same measures of physical activity and cardiovascular fitness described above to examine the relationship between them and cognitive improvements after completing cognitive training, as measured by post-cognitive intervention scores for cognitive tasks tested pre-intervention, and

measures on transfer cognitive tasks of Proactive Interference, Artificial Grammar, and Swahili Language Divided-Attention Task. For these regressions, the following were entered in to the regression equation: 1) Time 1 task performance, 2)Age, Sex, and Education, 3) *APOE* genotype, 4) Need for Cognition, 5) Cognitive Training Group Condition, 6) Estimated VO_2 max (in Model 1) or Weekly Kilocalorie Expenditure (in Model 2), and 7) *APOE* genotype x Estimated VO_2 max interaction (Model 1) or *APOE* genotype x Weekly Kilocalorie Expendiute interaction (Model 2). Simple regressions were conducted separately when any interaction term achieved significance to determine which group exhibited a significant relationship between physical activity or cardiovascular fitness measure and cognitive performance.

An *a priori* power analysis was conducted with G Power 3 (Heinrich Heine Universität Düsseldorf, 2012) evaluating a final study size of 165 for pre-intervention analysis (with estimated VO_2 max and DNA analysis) and 106 for completed cognitive training analysis, and seven predictors, respectively. Assuming a need for 80% power and an alpha of .05, results for effect size needed to achieve adequate power were as follows: for pre-intervention (n = 165), an effect size of f^2 = 0.06 is required, which is equal to a small positive effect needed to achieve adequate power. For analysis post-intervention (n = 106), an effect size of f^2 = 0.09 is required, which is equal to a small positive effect to achieve adequate power.

Results

Descriptive Statistics

A total of 267 individuals responded to recruitment efforts and scheduled appointments for an initial visit. Of those tested, nine either did not complete the pre-intervention test battery or had missing data. Of the participants who did complete the first battery of cognitive testing, 223 had DNA and weekly kilocalorie data available (31 opted not to provide DNA sample, four provided sample but DNA could not be isolated). Of those with DNA, kilocalorie, and cognitive data, 165 participants additionally consented to participate in estimated VO_2max testing and 106 of those completed the cognitive training intervention.

Descriptive statistics are presented in Table 2 for the pre-intervention group of participants who completed both a DNA analysis and estimated VO_2max test (n=165). There was a higher percentage of female participants in the study (65% female; 35% male), and the study population was highly educated, with 81% of participants possessing at least a college degree, and 34% holding advanced degrees. The study population tended towards lower physical fitness levels, with 33% classified as overweight and 19% as obese, and with 46% reporting less than 200 kilocalories expended per week in high-aerobic intensity physical activity (measured as six METS or greater). Estimated VO_2 max ranged from 18.2 to 49.8 ml/kg/minute, and weekly energy expenditure from self-reported physical activity pursuits, walking, and stair-climbing ranged from 56 to12655 kilocalories. Data regarding age stratification revealed a higher percentage of young adults ages 20-29, comprising 42% of the study population compared to 29% in the 30-39 age group, and 29% in the 40-51 age group.

Table 2

Descriptive Statistics-Means and Standard Deviations of Participants

	Male			Female			Total
	ε4-(n=40)	ε4+(n=18	Total(n=58	ε4-(n=72)	ε4+(n=35)	Total(n=107	*n* = 165
Age	31.2(8.3)	32.39(8.4)	31.5(8.3)	33.9(26.3)	36.49(9.8)	34.7(9.3)	33.6(9.1)
NFC score	28.7(19.8)	29.4(12.9)	28.9(17.8)	26.3(18.8)	25.2(17.1)	26.0(18.2)	27.0(18.1)
Education*	4/6/14/3/13	2/4/8/1/3	6/10/22/4/16	3/10/26/8/25	0/2/11/7/15	3/12/37/15/40	9/22/59/19/56
Wkcal	2686(2266)	3406(2997)	2910(2511)	2303(1711)	2267(1390)	2278(1607)	2500(1988)
Est VO2	36 .0(7.1)	36.4(6.4)	36.2(6.9)	30.4(7.1)	30.9(6.5)	30.6(6.9)	32.5(7.4)
> 6 METS	1021(1372)	1190(1677)	1074(1460)	665(1111)	525(762)	619(1008)	778(1202)
< 6 METS	1664(1632)	2216(1988)	1835(1752)	1638(1235)	1702(909)	1659(1135)	1720 (1381)
BMI	25.7(4.2)	25.6(3.2)	25.7 (3.9)	25.8(5.1)	24.9(4.3)	25.5(4.9)	25.6(4.5)
Waist Cir	34.2(4.8)	34.3(5.1)	34.3(4.9)	31.4(5.1)	30.6(4.3)	31.1(4.9)	32.2(5.1)
**R span ** **	37.8(17.3)	35.9(18.7)	37.2(17.6)	38.7(17.5)	39.6(18.2)	39.0(17.6)	38.4(17.6)
**N-back ** **	.11(1.3)	-.14(1.8)	.03(1.5)	-.01(0.8)	.07(1.0)	.02(0.9)	.02(1.1)
Executive	86.9(37.4)	72.3(33.6)	82.3(36.6)	101.6(38.8)	86.4(39.6)	96.6(39.6)	91.6(39.0)
**Shape ** **	1554(519)	1596(555)	1567(526)	1291(482)	1285(516)	1290(490)	1389(519)
Ravens	10.4(2.9)	10.5(3.0)	10.4(2.9)	9.3(3.5)	9.3(3.3)	9.3(3.4)	9.7(3.3)
TaskSwtch	249(284)	286(350)	261(303)	274(262)	219(242)	255(256)	258(272)
Nelson	23.5(7.5)	22.2(8.7)	23.1(7.8)	23.7(6.8)	23.1(7.3)	23.5(6.9)	23.4(7.2)

NFC-need for cognition; *Education- high school graduate/some college/college grad/some grad school/grad degree; ** Missing data or incompletion of task resulted in a different *n* for these cognitive tasks R span=n=164;n back n=155; Shapebuilder-n=156

Participant completion of cognitive training

For participants who completed both estimated VO_2 max testing and the cognitive training intervention (n = 106), t test comparisons revealed a higher mean age for completers (34.9 compared to 31.3 for non-completers, p = .013). There were no significant differences in pre-intervention cognitive task scores. There was no significant difference in estimated VO_2 max or number of times per week spent in vigorous physical activity between completers and non-completers, but there was a significant difference in energy expenditure between the two groups, with a lower mean weekly kilocalorie expenditure (2223 compared to 2999 for non-completers, p = .04) .

APOE genotype

There was a surprisingly large percentage of participants with the *APOE*- ε4 allele, with 32% of the study population as ε4 carriers, compared to a general population percentage of approximately 15%. Interestingly, *APOE*-ε4 carriers were more likely to remain in the study for the duration of the intervention, with individuals possessing the *APOE*-ε4 allele comprising 37% of those who completed five weeks of cognitive training (with a tendency towards significance of $p = .076$). Chi square and *t* test comparisons revealed no other significant differences between genotypes. Due to the low numbers of *APOE*-ε4 homozygotes (five in original dataset; three with estimated VO_2max data, one in VO_2 completion group) the division of genotype was based on carriers versus non-carriers of the ε4 allele. While there are questions about whether individuals with the *APOE*- ε 2/4 genotype should be considered cognitively at risk due to the protective nature of the ε2 allele (Corder et al., 1993), Farrer et al. (1997) revealed that the possession of this genotype is associated with a higher risk of cognitive decline in older adults with an odds-ratio of 2.6 relative to ε 3/3 genotype in Caucasians. (In the same study, OR for ε 3/4 was 3.2, ε 4/4 was 14.9, ε 2/2 and 2/3 were 0.6.) With these findings in consideration, analysis was conducted with and without this genotype included, with no significant differences between analyses, so these cases remained with other ε4 carriers in this analysis. There were five participants exhibiting this genotype in the original dataset, with three who completed estimated VO_2max testing, and two in the VO_2 completion dataset. A summary of *APOE* status by age group along with details on each of the six *APOE* genotypes is contained in Table 3.

Table 3

***APOE* Carriers and Non-Carriers and Allele Frequencies by Age Group**

	***APOE* Carriers and Non-Carriers**			***APOE* Allele Frequencies**					
Age	**ε4⁻**	**ε4⁺**	**Total**	**2/2***	**2/3**	**3/3**	**2/4****	**3/4**	**4/4*****
Time									
22-29	49(70%)	21(30%)	70	0	9(13%)	40(57%)	2(3%)	19(27%)	0
30-39	33(70%)	14(30%)	47	0	6(13%)	27(57%)	1(2%)	12(26%)	1(2%)
40-51	30(62%)	18(38%)	48	0	4(8.3%)	26(54.2%)	0	16(33.3%)	2(4.2%)
Total	112 (68%)	53(32%)	165	0	19(12%)	93(56%)	3(2%)	47(28%)	3(2%)
Time 2									
22-29	26(65%)	14(35%)	40	0	4(10%)	22(55%)	1(2%)	13(33%)	
30-39	18(64%)	10(36%)	28	0	2(7%)	16(57%)	0	9(32%)	1(4%)
40-51	23(61%)	15(39%)	38	0	3(8%)	20(53%)	1(2.6%)	13(34%)	1(2.6%)
Total	67(63%)	39(37%)	106	0	9(8%)	58(55%)	2(2%)	35(33%)	2(2%)

*one participant exhibited ε2/2 genotype but did not complete estimated VO_2 testing; **two additional participants exhibited ε2/4 genotype but did not complete estimated VO_2 testing ***one additional participant exhibited ε4/4 genotype but did not complete estimated VO_2 testing

Correlations

Pearson correlations were produced for demographic and physical activity variables and for cognitive test variables. Significant correlations provided support for validity of estimated VO_2 max, which correlated positively with reported weekly kilocalories (p = .003), energy expenditure at levels equal to or greater than 6 METS (p= .001), and reported vigorous physical activity - number of times per week for participation that causes one to "get out of breath" or "work up a sweat" (p < .001). Both BMI and waist circumference were negatively associated with estimated VO_2 max (p < .001), as were age and sex (p < .001). Need for cognition scores were positively correlated with estimated VO2max (p =.009), but education did not show a significant correlation (p = .750). Participation in physical activity at levels less than 6 METS did not correlate significantly to estimated VO_2 max (p = .123). Table 4 summarizes physical activity correlations. A correlation analysis of participants post-intervention revealed the

same findings as for pre-intervention dataset, except that Need for Cognition scores were no longer significantly correlated with estimated VO_2.

Table 4

Pearson Correlations - Demographic Variables/ Physical Activity-Related Variables

	1	2	3	4	5	6	7	8	9	10	11
1.Age											
2.Sex	.168										
3.Educ	**.289**	.168									
4.NFC	.072	-.079	**.282**								
5.VO_2	**-.306**	**-.361**	-.025	**.202**							
6.Vig/wk	.047	-.015	.123	.072	**.287**						
7.Wkcal	.001	-.152	-.019	.051	**.232**	**.408**					
8.>6METS	-.106	**-.181**	-.034	-.053	**.245**	**.235**	**.730**				
9.<6METS	.094	-.061	.002	.120	.120	**.383**	**.804**	.180			
10.BMI	.199	-.014	-.139	-.129	**-.386**	-.092	-.135	-.147	-.067		
11.WaistCir	**.237**	**-.299**	-.138	-.057	**-.283**	-.118	-.096	-.124	-.031	**.858**	
12.*APOE*	**.114**	**.017**	**.045**	**-.013**	**.023**	**.068**	**.044**	**-.016**	**.078**	**-.066**	**-.050**

Bold $p < .01$; Underlined: $p < .05$ Abbreviations: Educ-Education; NFC-Need for Cognition; VO_2-Estimated Vo_2 max; Vig/wk-Vigorous exercise/number of time per week; Wkcal-Weekly kilocalories; METS-Metabolic equivalent Units; Waist Circ-Waist Circumference

Strong correlations among cognitive tasks were also revealed. Of note, Ravens Matrices, a spatial test of fluid intelligence, was positively correlated at $p < .001$ to several other tasks – Nelson reading comprehension, Shapebuilder, *N*-back, Reading Span, and Proactive Interference. The post-intervention Proactive Interference learning task showed strong correlations to *N*-back and Shapebuilder in addition to Ravens and Nelson. The Proactive Interference task also positively correlated to the other learning transfer tasks of Swahili vocabulary percent correct ($p < .001$) and Artificial Grammar ($p = .035$). Summary of cognitive test correlation can be found in Table 5.

Table 5

Pearson Correlations – Cognitive Tests									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1.R Span									
2.N back d'	**.333**								
3.Executive	-.052	-.154							
4.Shape	**.370**	**.450**	**-.280**						
5.Ravens	**.301**	**.523**	-.164	**.577**					
6.TskSwtch	**-.235**	-.108	.150	-.138	-.105				
7.Nelson	**.270**	**.280**	-.102	**.236**	**.302**	**-.262**			
8.Pro Int	.215	**.340**	-.061	**.358**	**.459**	-.173	.244		
9.Swahili	.202	.173	.093	.167	.189	-.109	.158	**.388**	
10.ArtGram	.064	.074	.034	.013	.107	**-.279**	.189	.206	.081

Bold: p <.02; Underlined: p <.05 Abbreviations: R span-reading span; Shape-Shapebuilder task; Raven-Ravens Matrices; Tsk Swtch-Task Switch ; Nelson-Nelson reading Comprehension test; Swahili-Swahili divided attention vocabulary learning task; Art Gram-Artificial grammar

Hierarchical Regression Analyses

Pre-intervention cognitive performance

Hierarchical regression analysis of pre-intervention cognitive task scores revealed significant associations of cognitive task performance with education and Need for Cognition score (NFC) for a majority of tasks, and additional significant associations with age and sex for fewer tasks**.** A statistically significant negative association between performance for *APOE* genotype on the Attentional Network Executive task was revealed (p = .012, ΔR^2 = .037, β = -0.194), such that *APOE* ε4 carriers performed significantly better on this task. Effect size f^2 for this result was 0.04. (For this task, it should be noted that a lower score of congruent minus incongruent is considered better performance.) Three discrepancy outliers were identified and analysis was conducted with them included and removed; results did not alter conclusions and they were retained. (With removal of the outliers, n =162, $\varepsilon 4^+$=52, p = .003, ΔR^2 =.053, β = -0.231). Summaries of regression analyses for pre-intervention cognitive task scores are presented in Table 6. **(**Due to missing data, some l tasks have a lower n; noted in table summaries).

For further evaluation, the Attentional Network Executive task was examined by median split stratified by estimated VO_2 max. Cases were stratified to reflect percentile norms according to age and sex. Results revealed *APOE* x estimated VO_2 max as a statistically significant negative predictor for this task for the top half of the median split (n=82; $\varepsilon4^+$ =28, p = .009, ΔR^2 = .071, β=-2.128). Effect size f^2 for this result was 0.08 (As noted previously, a negative association for this reaction time task relates to better performance.) The additional regression analysis also revealed a tendency towards significance for *APOE* genotype alone as a significant negative predictor (p = .064, ΔR^2 = 0.039, β=-0.199). The median split bottom half revealed no significant findings for either *APOE* genotype or *APOE* x estimated VO_2 max interaction. Analysis of subsets by *t* test and chi square revealed no significant differences between *APOE*-ε4 carriers and non-carriers, and percentages of *APOE*-ε4 carriers in each subset remained similar to the total. Results are summarized in Table 7 and in Figure 1.

Table 6

Hierarchical Regression-Pre-Intervention

Time 1	Reading Span			*N*-back			Flanker Exec			Shape Score			Ravens Matrix			Task Switching			Nelson Correct		
	n=164 $\varepsilon4^+$=53			n=155 $\varepsilon4^+$=49			n=165 $\varepsilon4^+$=53			n=156 $\varepsilon4^+$=49			n=165 $\varepsilon4^+$=53			n=165 $\varepsilon4^+$=53			n=165 $\varepsilon4^+$=53		
	p	ΔR^2	B	p	ΔR^2	B	p	ΔR^2	B	p	ΔR^2	B	p	ΔR^2	B	p	ΔR^2	B	p	ΔR^2	B
Age	.50	.00	-0.05	.30	.01	0.08	.07	.02	0.14	**.00**	**.09**	**-0.30**	.28	.01	-0.09	.47	.00	-0.06	.07	.02	0.14
Sex	.47	.00	0.06	.94	.00	0.01	**.05**	**.02**	**0.16**	**.01**	**.05**	**-0.22**	**.05**	**.02**	**-0.15**	.99	.00	0.00	.97	.00	0.00
Educ	**.02**	**.04**	**0.20**	**.00**	**.10**	**0.33**	.27	.01	-0.09	**.00**	**.06**	**0.26**	**.00**	**.13**	**0.39**	.35	.01	-0.08	**.01**	**.04**	**0.21**
ApoE	.99	.00	0.00	.99	.00	0.00	**.01**	**.04**	**-0.19**	.61	.00	0.04	.98	.00	-0.00	.69	.00	-0.03	.36	.01	-0.07
NFC	**.00**	**.07**	**0.29**	**.00**	**.05**	**0.23**	**.03**	**.03**	**-0.17**	**.01**	**.03**	**0.19**	**.00**	**.08**	**0.29**	**.00**	**.07**	**-0.27**	**.00**	**.06**	**0.26**
VO_2	.30	.01	-0.09	.94	.00	0.01	.73	.00	-0.03	.63	.00	0.04	.15	.01	0.12	.91	.00	-0.01	.71	.00	0.03
ApoExVO_2	.32	.01	-0.42	.90	.00	-0.06	.16	.01	-0.59	.87	.00	-0.07	.65	.00	0.18	.15	.01	0.62	.97	.00	-0.02
Wkcal	.94	.00	0.01	.35	.01	0.07	.60	.00	-0.04	.35	.00	-0.07	.36	.00	-0.07	.76	.00	0.02	.61	.00	-0.04
ApoE xWkcal	.08	.02	-0.44	.76	.00	0.08	.92	.00	-0.02	.99	.00	-0.00	.74	.00	-0.08	.30	.01	-0.27	.81	.00	-0.06

Table 7

Hierarchical Regression -Attentional Networks Executive Task – Median Split by Estimated VO_2 max

	Median Split Bottom n=83 $\varepsilon4^+$=24			Median Split Top n=82 $\varepsilon4^+$=28		
	p	ΔR^2	β	p	ΔR^2	β
Age	.996	.000	-0.001	.014	.072	0.269
Sex	.207	.020	0.143	.179	.021	0.147
Education	.491	.006	-0.080	.311	.012	-0.120
APOE	.126	.029	-0.174	.064	.039	-0.199
NFC	.161	.024	-0.172	.280	.013	-0.122
VO_2	.099	.033	0.224	.074	.035	-0.212
***APOE* xVO_2**	.760	.001	-0.313	**.009**	**.071**	**-2.128**

Abbreviations: NFC-Need for Cognition; VO_2-estimated VO_2 max

Figure 1

Attentional Networks Executive Task – Lower / Upper Median Splits – Estimated VO2 max

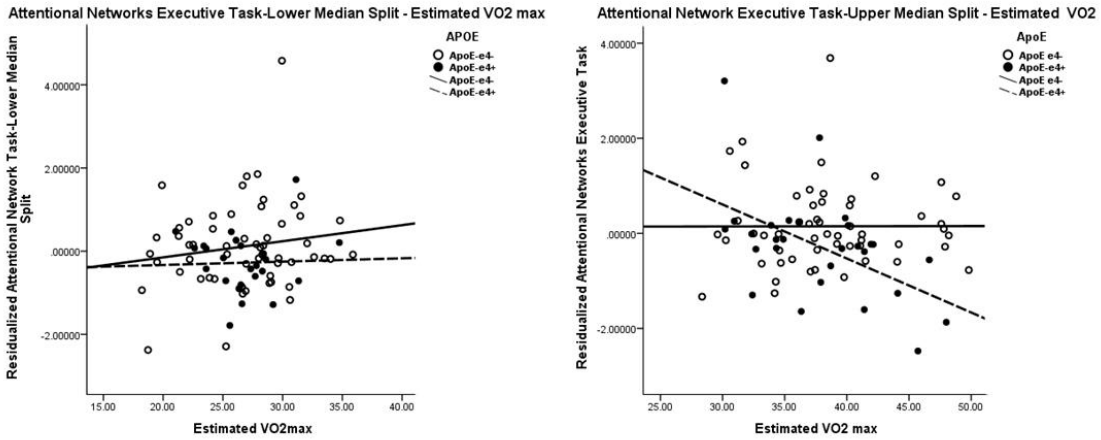


Figure 1. Genotype x cardiovascular fitness interaction for Attentional Networks Executive task - median split by estimated VO_2 max. *APOE*-ε4 carriers show decreased reaction time with increasing cardiovascular fitness (estimated VO_2 max) in the upper median split, with no decrease seen for non-carriers. No genotype x cardiovascular fitness interaction differences are noted in the lower median split. ε4 carriers are represented by dotted line and solid circles.

Cognitive performance after cognitive training intervention

Hierarchical regression analysis of cognitive task performance change scores post-intervention revealed fewer significant associations for age, sex, and education

(combined into one regression step for post-intervention) than at pre-intervention**,** with significance remaining only for Nelson number correct (p = .037, ΔR^2 = .038, β = 0.105). Condition of cognitive training was a significant positive predictor for change scores for Shapebuilder (p < .001, ΔR^2 = .215, β = 0.477), and for *N*-back (p = .022, ΔR^2 = .039, β = 0.202). Post hoc analysis revealed that significance for Shapebuilder was associated with the working memory and combined training conditions compared to control and interference control groups (p < .001) and significance for *N*-back was associated with the combined group compared to control (p =.015) and memory (p = .014) conditions. No significant associations were found for *APOE* genotype, estimated VO_2 max main effect or interactive effect with *APOE*. No associations were found for weekly kilocalorie expenditure, but a weekly kilocalorie x *APOE* interactive association was revealed as a negative predictor for Shapebuilder post-intervention score (p = .030, R^2=.018, β= -0.520). This task had a lower *n* than other cognitive tasks due to missing data, which may have led to a bias of results. An additional hierarchical regression analysis was conducted for this task, using weekly kilocalorie expenditure from the larger post-intervention dataset of those participants who completed the intervention but did not complete estimated VO_2 assessment,and this analysis showed no significant findings (n=114, p = .905, R^2=.000, β=-0.027). Post-intervention regression analyses summaries can be found in Table 8.

Table 8

Hierarchical Regression-Cognitive Task Scores After Intervention

Time 2	Reading Span n=106 $\varepsilon4^+$=39			N-Back n=97 $\varepsilon4^+$=35			Flanker Exec n=106 $\varepsilon4^+$=39			Shape Score n=95 $\varepsilon4^+$=34			Ravens Matrix n=106 $\varepsilon4^+$=39			Task Switching n=105 $\varepsilon4^+$=39			Nelson Correct n=106 $\varepsilon4^+$=39		
	p	ΔR^2	β	p	ΔR^2	β	p	ΔR^2	β	p	ΔR^2	β	p	ΔR^2	β	p	ΔR^2	β	p	ΔR^2	β
Time 1	**.00**	**.51**	**0.72**	**.00**	**.25**	**0.50**	**.00**	**.45**	**0.67**	**.00**	**.41**	**0.64**	**.00**	**.46**	**0.68**	**.00**	**.16**	**0.39**	**.00**	**.53**	**0.72**
Age/sex/ed	.85	.00	-0.01	.06	.06	0.25	.36	.02	0.05	.22	.03	0.16	.76	.01	0.09	.46	.02	-0.07	**.04**	**.04**	**0.11**
ApoE	.90	.00	-0.01	.32	.00	-0.09	.35	.00	0.07	.91	.00	-0.01	.09	.02	0.13	.85	.00	-0.02	.53	.00	-0.04
NFC	.84	.00	-0.02	.86	.00	0.02	.66	.00	0.03	.18	.01	0.12	.54	.00	0.05	.27	.01	-0.11	.25	.00	0.08
Cond	.75	.00	-0.02	**.02**	**.04**	**0.20**	.78	.00	0.02	**.00**	**.22**	**0.48**	.69	.00	0.03	.80	.00	-0.02	.90	.00	-0.01
VO_2	.67	.00	0.04	.87	.00	0.03	.63	.00	0.04	.56	.00	-0.04	.33	.01	-0.09	.87	.00	0.02	.20	.01	0.10
ApoExVO_2	.66	.00	0.17	.11	.02	-0.77	.80	.00	0.11	.67	.00	-0.15	.76	.00	-0.12	.42	.01	0.41	.08	.01	-0.64
Wkcal	.82	.00	-0.02	.78	.00	0.03	.50	.00	0.05	.74	.00	0.02	.91	.00	0.01	.65	.00	0.05	.57	.00	-0.04
ApoEx Wkcal	.12	.01	0.42	.23	.01	-0.39	.21	.00	0.36	**.03**	**.02**	**-0.52**	.68	.00	-0.12	.85	.00	0.07	.16	.01	-0.36

Post-intervention learning transfer tasks

Hierarchical regression analyses of three post-intervention learning transfer tasks– Proactive Interference, Swahili divided-attention task vocabulary leaning, Artificial Grammar -revealed significant positive associations with estimated VO_2 max for percent correct in both the Interference and Facilitated testing conditions of the Proactive Interference (PI) task (p = .028, ΔR^2 = .043, β= 0.241 and p = .027, ΔR^2 =.044, β= -0.245 respectively), and a tendency toward a positive significant association with estimated VO_2 max with Trial 1 of the hard condition in the Swahili vocabulary dual task (p = .059, ΔR^2 = .031, β = 0.212). Effect sizes f^2 for the Proactive Interference task Interference and Facilitated conditions were both 0.05. The tapping aspect of this task was controlled for in regression analysis to emphasize the association with vocabulary learning since an association with this fine motor task could conceivably be driving this relationship for participants with higher levels of cardiovascular fitness. There were no

significant associations with cardiovascular fitness for the easy condition of this task. There were no significant associations of cardiovascular fitness with any condition of the Artificial Grammar task. Summaries of regression analyses can be found in Tables 9, 10, and 11 and Figures 2 and 3.

Analysis of weekly kilocalorie expenditure revealed a significant negative finding for *APOE* x Wkcal interaction, (p = .024, ΔR^2 = .046, β=-0.819), such that a higher level of energy expenditure was associated with poorer performance on the PI Interference condition for carriers of the *APOE*-ε4 allele, while non-ε4 carriers showed a benefit. Removal of one high leverage outlier resulted in loss of significance (p= .072, ΔR^2 = .03, β=-0.631). There were no other significant findings for weekly kilocalorie expenditure or for interactions of Wkcal x *APOE* for the other learning task dependent variables.

Table 9

Hierarchical Regression Analysis -Proactive Interference

***n* =106 ε4$^+$=39**	**PC L2pi**			**PC L2pf**		
	p	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**
Age,Sex, Ed	.119	.055	0.235	.069	.067	0.247
APOE	.135	.021	0.145	.268	.011	0.107
NFC	.050	.035	0.197	.166	.018	0.140
Condition	.803	.001	-0.024	.882	.000	-0.014
VO_2	**.028**	**.043**	**0.241**	**.027**	**.044**	**0.245**
***APOE* x VO_2**	.892	.000	0.006	.393	.006	0.004
Wkcal	.562	.003	0.059	.180	.017	0.136
***APOE* x Wkcal**	.072*	.030	-0.631	.380	.007	-0.322

*One high-leverage outlier was removed from dataset, with inclusion, p=.024, R^2=.046, β=-0.819)
Abbreviations: Ed-education; NFC-Need for Cognition; VO_2-Estimated VO_2 max; Wkcal-Weekly kilocalories

Figure 2

Proactive Interference - Interference and Facilitated Conditions –Estimated VO_2 max

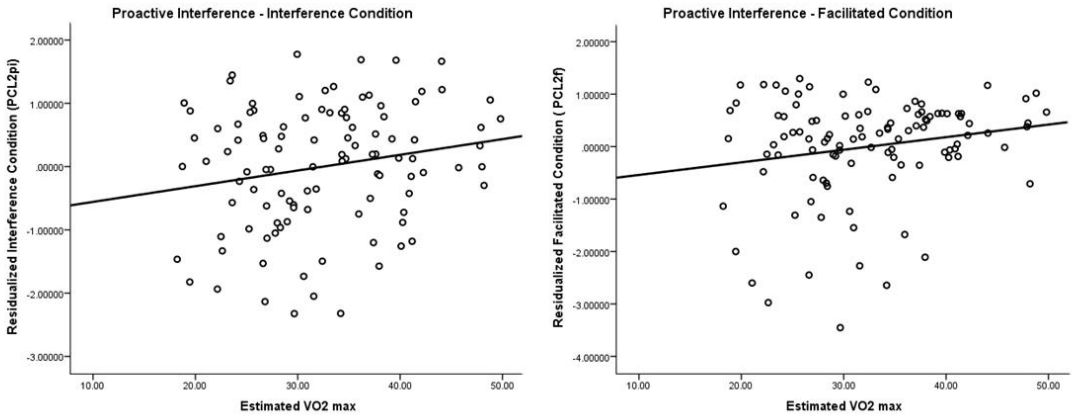


Figure 2. Percent correct on Proactive Interference Task Interference and Facilitated conditions. Percent correct increases with increasing cardiovascular fitness (Estimated VO_2 max)

Table 10

Hierarchical Regression-Swahili Divided Attention Task

***n* =106 ε4⁺=39**	**PC Hard1**			**PC Hard2**			**PC Hard3**		
	p	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**	***p***	**ΔR^2**	**β**
Tap Hard	.873	.000	0.016	**.022**	**.050**	**0.223**	**.008**	**.065**	**0.255**
Age,Sex, Ed	**.032**	**.083**	**0.277**	.342	.031	0.160	.451	.024	0.137
APOE	.131	.021	-0.145	.194	.015	-0.125	.175	.017	-0.130
NFC	.141	.020	0.149	.093	.026	0.172	.102	.024	0.165
Condition	.355	.008	0.088	.220	.013	0.118	.089	.025	0.161
VO_2	**.059**	**.031**	**0.212**	.236	.013	0.133	.368	.007	0.099
***APOE* x VO_2**	.486	.004	0.359	.870	.000	0.085	.999	.000	0.001
Wkcal	.512	.004	0.066	.693	.001	0.040	.965	.000	-0.004
***APOE* x Wkcal**	.424	.006	-0.291	.218	.014	-0.447	.173	.016	-0.490

Abbreviations: Ed-education; NFC-Need for Cognition; VO_2-Estimated VO_2 max; Wkcal-Weekly kilocalories; PC-percent correct

Figure 3

Swahili Divided Attention Task – Estimated VO2 max

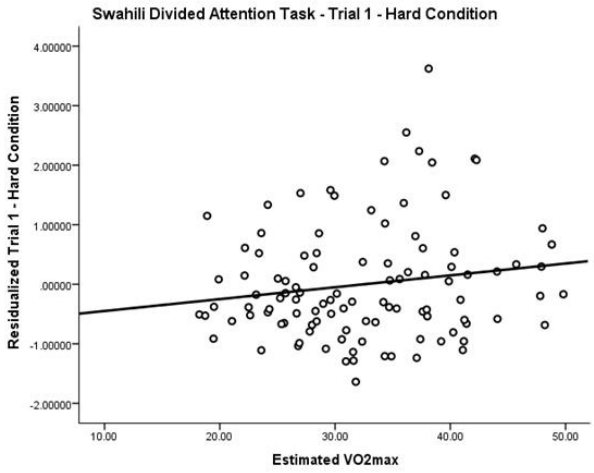


Figure 3. Percent correct on Swahili Divided-Attention Task- Trial 1 of Hard condition. Percent correct increases with increasing cardiovascular fitness (Estimated VO_2 max)

Table 11

Hierarchical Regression – Artificial Grammar

***n*=105; ε4$^+$=38**	**CHgY**			**CLgY**			**CHgN**			**CLgN**		
	p	**R^2**	**β**	***p***	**R^2**	**β**	***p***	**R^2**	**β**	***p***	**R^2**	**β**
Age,Sex,Ed	.12	.06	-0.02	.33	.03	0.02	.22	.04	0.19	.68	.02	0.07
APOE	.12	.02	-0.15	.61	.00	0.05	.87	.00	-0.02	.65	.00	-0.05
NFC	.07	.03	0.19	.14	.02	0.15	.82	.00	0.02	.12	.02	0.16
Condition	.24	.01	-0.11	.75	.00	-0.03	.94	.00	0.01	.98	.00	0.01
VO_2	.57	.00	0.06	.33	.01	0.11	.37	.01	0.10	.23	.01	0.14
APOE -VO_2	.08	.03	-0.91	.84	.00	-0.11	.63	.00	0.27	.32	.01	0.54
Wkcal	.40	.01	-0.09	.84	.01	-0.02	.48	.00	-0.08	.81	.00	0.03
APOE -WK	.77	.00	0.11	.76	.00	-0.12	.32	.01	-0.39	.40	.01	0.33

Abbreviations: Ed-education; NFC-Need for Cognition; VO_2-Estimated VO_2 max; Wkcal-Weekly kilocalories; APOE-WK-APOE x Weekly kilocalories; ChgY-High chunk strength-grammatical; ClgY-Low chunk strength-grammatical; CHgN-High chunk strength-grammatical; CLgN-low chunk strength-non-grammatical

Discussion

An aim of this study was to investigate the role that physical activity plays in relation to cognitive learning in an adult population, and to gain understanding about what types of physical activity may mediate this relationship and what specific cognitive processes may preferentially benefit. Additionally, a genetic/physical activity interaction with cognition was explored with regard to the *APOE* genotype, known as a factor related to cognitive decline in older adults, but with unclear associations with cognition in a younger population. The study examined cognitive processes across a battery of tasks before and after a cognitive training intervention and explored association with learning through post-intervention challenges, incorporating two measures of physical activity to discern relative importance of high-intensity aerobic activity versus total physical activity energy expenditure. It was hypothesized that both measures of physical activity would be positively associated with cognitive performance and that the magnitude of benefit would be greater for higher-active or more cardiovascularly fit *APOE*-ε4 carriers when compared to lower-active or less fit ε4 carriers.

The findings of this study did not support a broad positive association of physical activity with cognition, instead revealing support for the concept of specificity for population, cognitive task, and physical activity measure. Significant or near-significant relationships were limited to positive associations with two post-intervention learning challenges for the main population, and one pre-intervention executive task in relation to a genetic/physical activity interaction. All significant cognition/physical activity relationships were associated with estimated cardiovascular fitness, with no significant associations detected between cognitive performance and total energy expenditure.

Pre-Intervention and Post-Intervention Cognitive Task Scores

Results from this study revealed no relationship between physical activity and performance on the seven cognitive tasks tested before and after the intervention. A genetic effect was noted for pre-intervention performance on the Attentional Networks executive control Flanker task, with a significant positive association for carriers of the *APOE*-ε4 allele compared to non-carriers, and additional findings of a significant positive interactive effect for the top half of a median split by estimated VO_2 max, with no association for bottom half low-fit participants. There was also a significant negative relationship with a small variance accounted for (ΔR^2 =.018) for weekly kilocalorie expenditure for post-intervention Shapebuilder task. This result may have been related to a pre-intervention score used for a control that showed virtually no relationship. There were also 11 missing cases for this variable, so analysis was conducted with a smaller dataset compared to other cognitive tasks. An additional analysis was conducted with the larger dataset that had post-intervention weekly kilocalorie scores available, adding 19 additional cases, with 6 six more ε4 carriers, and this analysis revealed no significant findings.

While this study's main concentration was with physical activity-related issues, it is important to note other significant factors in the regression analysis. Education level and a Need for Cognition score were entered into regression analysis as potential confounders, and both revealed significant relationships with cognitive performance at pre-intervention. However, their relation post-intervention was limited, with no associations revealed for Need for Cognition, and an education association only for the Nelson reading comprehension test. Age and sex accounted for additional significant

variance pre-intervention, with age being associated negatively with Attentional Networks Executive and Shapebuilder scores, and sex showing a more positive association for males in the visuospatial tasks of Shapebuilder and Ravens Matrices and for the Attentional Network Executive task. Association with these variables showed no significant associations with post-intervention change scores for cognitive performance.

Conditions of cognitive training significantly predicted post-intervention change performance on *N*-back and Shapebuilder scores, and post hoc analysis revealed that the conditions responsible for significance were ones in which the two tasks were specifically trained during the cognitive intervention. However, no other tasks revealed a significant benefit from cognitive training, nor did the post-intervention learning tasks. This discovery contradicts findings from some previous studies (Chein & Morrison, 2010; Buschkuehl & Jaeggi, 2010; Morrison & Chein, 2011; Jaeggi, Buschkuehl, Jonides, & Perrig, 2008) and lends doubt to the notion that "brain training" can result in wide-ranging cognitive benefits.

Post-Intervention Learning Challenges

As discussed previously, with regards to main effects of either weekly kilocalorie expenditure or cardiovascular fitness in relation to cognitive performance, we did not find that either measure predicted an association with performance for the seven cognitive tasks presented at pre-and post-intervention. However, when three post-intervention learning experiments were evaluated, we found that estimated VO_2max was a significant positive predictor for performance on both the Facilitated and Interference conditions of the Proactive Interference task, and estimated VO_2max also showed a tendency toward significance for Time 1 of the hard condition of the Swahili divided-attention vocabulary

learning evaluation. These cognitive tests involved greater complexity than the seven tasks tested pre-and post intervention, relying on longer-term memory retention for word pairings and great interference control or resistance to distraction while learning. Given that this study population consisted primarily of highly educated healthy young adults at the peak of physical and brain performance, it is not surprising that significant associations would be limited to these demanding learning tasks.

It was particularly interesting to find that performance on both the Proactive Interference and the Swahili vocabulary acquisition tests was positively associated with cardiovascular fitness when the cognitive intervention conditions (training in memory and/or interference control) were unable to show transfer benefits to these learning tasks. Given the continual quest for optimal techniques to improve memory and learning, it could be interpreted that these findings suggest that an attainment of higher aerobic capacity (which in this study was significantly correlated with amount of time spent in high-aerobic physical activity) may offer a better approach towards achieving cognitive improvement when compared to practice through cognitive "brain games". However, another possibility may be that the cognitive training is only effective in those individuals who possess higher cardiovascular fitness – an interactive benefit augmented by the combination of physical and cognitive conditioning. Future research allowing for aerobic exercise and combined exercise/cognitive training conditions as well as cognitive training alone is warranted to clarify the relationships found in this study.

The literature provides broad support for why cardiovascular fitness would provide a benefit for learning these challenging tasks. Both the Swahili and the Proactive Interference tasks involve a combination of memory with interference control, and these

cognitive processes are supported in several physical activity/cognition studies. Höting et al. (2012) and Peirera et al. (2007) reported improved scores on word recall memory tasks for aerobically-trained young or middle-aged adults in aerobic exercise interventional studies, and Masley, Roetzheim, and Gualtieri (2009) and Stroth et al. (2010) found benefit for inhibition-related executive function tasks.

These two tasks are believed to engage both the prefrontal region and hippocampus (Caplan, McIntosh, & De Rosa, 2007; Jonides & Nee, 2006; Moscovitch, 1994; Schlegel, Rudelson, & Tse, 2012). Findings for benefit of cardiovascular fitness are strong for frontally-mediated executive function tasks (Colcombe et al., 2004) and for hippocampally-related memory tasks (Erikson et al., 2011; Peirera et al., 2007), and human neuroimaging studies have provided structural evidence of increased gray matter in both the prefrontal region and cingulate cortex (Floe et al., 2010; Ruscheweyh et al., 2009; Weinstein et al., 2012) and increase in hippocampal volume (Erikson et al.) in association with cardiovascular fitness, with corresponding high cognitive performance on select tasks. In addition, animal research provides neurobiological evidence for improved learning ability after aerobic training that relate to both regional and whole-brain benefits. Classic work by van Praag, Christie, Sejnowski, & Gage (1999) demonstrated that running wheel exercise in mice resulted in increases in long-term potentiation (LTP) and increased neurogenesis in the dentate gyrus, and Fabel et al. (2009) showed additionally that when running wheel exercise preceded a five-week exposure to a cognitively challenging enriched environment, neurogenesis was increased by 30% compared to either condition alone. Other animal research has shown that aerobic exercise increases brain vasculature (van de Borght et al., 2009), improves synaptic

density (Redila & Christie, 2006) and results in elevation of cognition-related neurotransmitters (Meeusen et al., 1997) and neurotrophins (Neeper, Gomez-Pinilla, Choi, & Cotman, 1995), all of which likely provide for more efficient brain processing of novel information.

While the Proactive Interference task involves the need to resist interference from old learning in favor of new information presented, the Swahili vocabulary acquisition task, administered concurrently with a secondary finger-tapping task, engages a different aspect of inhibitory control, an ability to learn while under divided attention. Again, this is a pre-frontally mediated task that has been shown to be particularly affected by aerobic exercise training (Harada, Okagawa, & Kubota, 2004; Hawkins, Kramer, & Caoaldi, 1992). Interestingly, a tendency towards significance for superior cardiovascular fitness-related performance on this task was concentrated on trial 1 of the hard condition. Trials 2 and 3 showed an evening out of performance, with less advantage for higher cardiovascular fitness. (There were no significant differences in the three trials of the easy condition based on aerobic fitness levels.) This could be interpreted to illustrate a more efficient response for higher-aerobically fit individuals, with greater conflict control to resist distraction from a secondary task. The motor aspect of the tapping task may have shown particular benefit for individuals with high cardiovascular fitness (Issacs et al., 1992), and indeed there were significant positive relationships with the tapping task at Trials 1 and 2 of the hard condition. However, positive association with vocabulary acquisition was maintained at near significance levels even after controlling for tapping performance.

There were no significant findings for any condition of the Artificial Grammar task. This task evaluates ability to discern rules of grammar without any conscious strategy, and while this task was significantly correlated with the Proactive Interference task, it does not involve an interference control component, an aspect of inhibition-related executive function that is most likely to benefit from high-aerobic intensity physical activity during learning (Barenberg et al., 2011). Several studies have reported that increased aerobic capacity due to exercise training is specific rather than general in relation to cognitive improvement (Barenberg et al., 2011, Colcombe et al., 2003, Kramer et al., 1999), and it may be that processes invoked during Artificial Grammar task are not conducive to an association with cardiovascular fitness.

The lack of significant positive findings related to weekly kilocalorie expenditure was contrary to what was hypothesized, but there are several reasons why this may have been so. A majority of the cognitive tasks tested involved some sort of inhibitory-related executive component, and high performance on this cognitive process in particular has been associated with high cardiovascular fitness (Barenberg et al., 2011; Kramer et al., 1999). Since the weekly kilocalorie measure gives weight to low and moderate physical activity that is not as likely to be contributing to improved aerobic capacity as much as high-intensity exercise, it would not be likely to show a positive association. An alternative explanation may be that this self-report instrument may not have been sensitive enough to adequately capture participants' total physical activity energy expenditure, since questions related mainly to sport or leisure activities, with physical activities of daily living being estimated only through self-reported daily walking and stair climbing. However, a validation study by Jacobs, Ainsworth, Hartman, & Leon

(1993) found this survey to be more highly correlated to accelerometer data than others with more extensive questioning that may lead to overestimations, so this may not be a likely reason for lack of significance. An additional alternative explanation for lack of significance for this measure on post-intervention cognitive tasks may relate to significant differences regarding kilocalorie expenditure for those who completed the intervention, with physically active individuals more likely to withdraw from the cognitive training, which would have limited the range for comparison for post-intervention testing.

There was a tendency towards significance showing a negative association for *APOE*-ε4 carriers with the Interference condition of the Proactive Interference task (significance was present prior to removal of a high-leverage outlier, a 50-year old *APOE*-ε4 homozygote). With loss of several participants with higher kilocalorie expenditure due to non-completion of the cognitive intervention, this result may be biased to a less-active group. Nevertheless, a post hoc exploration of the two subsets of the kilocalorie expenditure measure – moderate physical activities less than six METS and higher intensity activities greater than six METS –revealed that this association was limited to low and moderate intensity physical activities, with non-ε4 carriers showing a positive association for performance on the Interference condition but no benefits for ε4 carriers. While it appears illogical that higher kilocalorie expenditure, rather than lower, would drive the negative relationship, there is behavioral and neuroimaging evidence that motor fitness factors such as balance, coordination, or flexibility that are common in moderate physical activities, are differentially associated with cognitive processes, and through "competing cortical networks" may attenuate effect of aerobic-related physical

activities (Voelcker-Rehage, Godde, & Staudinger, 2010). With this result relating only to *APOE*-ε4 carriers, it is speculated that this finding may be foreshadowing cognitive aging and a stronger need for aerobic exercise to maintain certain cognitive function for this genotypes.

APOE-ε4 Genotype and Genotype x Estimated VO_2 Max Interactions

The discovery that the *APOE*-ε4 genotype was associated with better performance on an Attentional Networks Executive task at pre-intervention, both alone in the total dataset, and in interaction with cardiovascular fitness in the upper half of a median split analysis by estimated VO_2 max, was surprising, but a review of the literature finds neurobiological evidence why this may be so. Research by Marchant, King, Tabet, & Rusted (2010), offer some clues to cognitively protective mechanisms of the ε4 genotype. These researchers found that young adult ε4 carriers displayed better cognitive benefit at baseline in a frontally-mediated executive challenge, and additionally showed greater executive performance improvement after cholinergic stimulation (through a nicotine spray) when compared to non-carriers. (No performance difference was noted for ε4 carriers on a working memory task also tested.) It is of interest that in this investigation it was shown that the ε4 carriers' performance was not compensatory with achievement gained from low initial performance - it was instead a cumulative benefit, with high initial performance and greater ability to profit from an intervention. It should be noted that animal studies have revealed that the cholinergic system benefits from aerobic exercise, with reports of increase in acetylcholine synthesis or attenuation of age-associated decline of acetlcholine receptor (Fordyce et al., 1991; Park et al, 1992), and also of importance, the cholinergic system has been shown as an important factor in the

attentional network, with neuroimaging evidence for particular benefit for filtering out distracters (Sarter, Givens, & Bruno, 2001). Cardiovascular fitness was not a significant predictor of Executive performance post-intervention, most likely due to specific cognitive training in inhibitory control in approximately 50% of who completed the study.

While it is of interest that *APOE*-ε4 carriers showed better performance on an executive function task compared to non-carriers, the question remains how the *APOE* -ε4 genotype may confer benefits in select cognitive functions at a young age yet be associated with impaired cognitive function and Alzheimer's type dementia at an older age. The concept of antagonistic pleiotropy, a theory whereby natural selection allows for a benefit early in life that evolves into a disadvantage later in life (Williams, 1957) may be expressed with the *APOE*-ε4 genotype, with advantages in the brain expressing in early life, progressing to mild cognitive changes as early neurodegenerative processes occur in individuals with this genotype, and eventually advancing toward risk for dementia as compensatory abilities in brain regions deteriorate (Han & Bondi, 2008). There is an hypothesis that the ε4 allele, believed to be the ancestral allele for *APOE*, is a beneficial allele in areas where food scarcity exists, possibly through mechanisms of providing greater amounts of needed cholesterol to areas including the brain, and through efficient metabolism of brain glucose, thus providing an advantage for survival, yet conveys a disadvantage, especially with aging, in Western cultures with high-fat diets and low activity levels that may promote degenerative changes in this genotype (Corbo & Scacchi, 1999). Research by Puttonen, Elovainia, Kivimäki, Lehtimäki, & Keltikangas-Järvinen (2003) provides support for this hypothesis. Examining cognitive function of

Finnish young adult ε4 carriers and non-carriers in relation to low-density lipoprotein (LDL) levels, they found that ε4 carriers performed a mental arithmetic task significantly better if they had low LDL levels, whereas non-carriers performed better at higher levels of LDL.

Conclusion

This study has limitations in that it was cross-sectional in nature, so only associations can be shown. Use of self-report physical activity questionnaires and estimated VO_2 max testing offered less precision in measurement than other methods. However, correlation analysis revealed significant associations of estimated VO_2 to both high-intensity physical activity levels and frequency of vigorous activity, while showing no significant relation to low-intensity energy expenditure, which would provide support for accuracy of the measures. There were few participants in the higher levels of cardiovascular fitness, which limited findings to a less physically-fit group, but this could also be construed as more realistically reflecting the fitness levels of the general population, and significant findings from this research could be interpreted that positive cardiovascular fitness associations with cognitive benefit are not limited to elite athlete fitness standards. Indeed, Kramer et al. (1999) found in an older adult interventional study that only a 5% increase in VO_2 max was needed to achieve significant improvement on several executive function tasks. In addition to possible physical fitness limitations, a potential bias towards participants with higher intelligence or educational level, especially for those who completed the cognitive training, may have limited generalizability of these findings. Future research endeavors should emphasize community outreach to incorporate participants from a wider range of educational

achievement. Strengths of the study included carefully-planned intervention testing of a wide range of cognitive processes with appropriate controls in place, and proper standardization of both cognitive and exercise testing conditions. Physical activity questionnaires revealed stability of physical activity patterns of at least one year, with many participants reporting several years duration of their exercise habits and physically active leisure pursuits.

In conclusion, this study has shown that cardiovascular fitness is associated with improved cognitive performance and learning even in younger adulthood, revealing a positive relationship with higher performance on learning challenges that were not affected by a computer-based cognitive training program. In addition, a particular association with cognitive benefit for *APOE*-ε4 carriers was shown for executive inhibitory function, and this appeared to be stronger in relation to higher cardiovascular fitness. Taken together, these findings offer insight into the role that physical activity plays throughout the lifespan and suggests that a positive association of physical activity with cognition is not limited to older age groups but instead may promote a benefit for the general adult population and for a specific genotype in young adults even when physical and cognitive health are at their peak.

Appendix A

The YALE PHYSICAL ACTIVITY
SURVEY
FOR OLDER ADULTS

INTERVIEWER: PLEASE MARK TIME: HR__MIN__ SEC__

INTERVIEWER: (Please hand the subject the list of activities while reading this statement.) Here is a list of common types of physical activities. Please tell me which of them you did during a typical week in the last month. Our interest is learning about the types of physical activities that are a part of your regular work and leisure routines.

For each activity you do, please tell me how much time (hours) you spent doing this activity during a typical week. (Hand subject card #l.)

Work	Time(hrs/wk)	Code
Intensity (Kcal/min)		
Shopping (e.g., grocery, clothes)	_____	3.5
Stair climbing while carrying a load	_____	8.5
Laundry (time loading, unloading, hanging, folding only)	_____	3.0
Light housework: tidying, dusting, sweeping, collecting trash in home, polishing, indoor gardening, ironing	_____	3.0
Heavy housework: vacuuming, mopping, scrubbing floors and walls, moving furniture, boxes, or garbage cans	_____	4.5
Food preparation (10+ minutes in duration): chopping, stirring, moving about to get food items, pans	_____	2.5
Food service (10+ minutes in duration): setting table, carrying food, serving food	_____	2.5
Dish washing (10+ minutes in duration): clearing table, washing/drying dishes, putting dishes away	_____	2.5
Light home repair: small appliance repair, light home maintenance/repair	_____	3.0
Heavy home repair: painting, carpentry, washing/polishing car	_____	5.5

Other: _____ _____ #

*Taylor et al. (1978) or McCardle et al. (1981) # determined by the specific activity

Intensity	Time(hrs/wk)	Code
Yardwork (Kcal/min)		
Gardening: planting, weeding, digging, hoeing	_____	4.5
Lawn mowing (walking only)	_____	4.5
Clearing walks/driveway: sweeping, shoveling, raking	_____	5.0
Other: _____	_____	___#
Caretaking		
Older or disabled person (lifting, pushing wheelchair)	____	5.5
Childcare (lifting, carrying, pushing stroller)	_____	4.0
Exercise		
Brisk walking (10+ minutes in duration)	_____	6.0
Pool exercises, stretching, yoga	_____	3.0
Vigorous calisthenics, aerobics	_____	6.0
Cycling, Exercycle	_____	6.0
Swimming (laps only)	_____	6.0
Other: _____	_____	___#
Recreational Activities		
Leisurely walking (10+ minutes in duration)	_____	3.5
Needlework: knitting, sewing, needlepoint, etc.	_____	1.5
Dancing (mod/fast): line, ballroom, tap, square, etc.	_____	5.5
Bowling, bocci	_____	3.0
Golf (walking to each hole only)	_____	5.0

Racquet sports: tennis, racquet ball	_____	7.0
Billiards	_____	2.5
Other: _____	_____	___#

Intensity	Time(hrs/wk)	Code
Yardwork (Kcal/min)		
Gardening: planting, weeding, digging, hoeing	_____	4.5
Lawn mowing (walking only)	_____	4.5
Clearing walks/driveway: sweeping, shoveling, raking	_____	5.0
Other: _____	_____	___#
Caretaking		
Older or disabled person (lifting, pushing wheelchair)	____	5.5
Childcare (lifting, carrying, pushing stroller)	_____	4.0
Exercise		
Brisk walking (10+ minutes in duration)	_____	6.0
Pool exercises, stretching, yoga	_____	3.0
Vigorous calisthenics, aerobics	_____	6.0
Cycling, Exercycle	_____	6.0
Swimming (laps only)	_____	6.0
Other: _____	_____	___#
Recreational Activities		
Leisurely walking (10+ minutes in duration)	_____	3.5
Needlework: knitting, sewing, needlepoint, etc.	_____	1.5
Dancing (mod/fast): line, ballroom, tap, square, etc.	_____	5.5

Bowling, bocci	_____	3.0
Golf (walking to each hole only)	_____	5.0
Racquet sports: tennis, racquet ball	_____	7.0
Billiards	_____	2.5
Other: _____	_____	___#

INTERVIEWER: (Please read to subject.) I would now like to ask you about certain types of activities that you have done during the past month. I will ask you about how much vigorous activity, leisurely walking, sitting, standing, and some other things that you usually do.

1. About how many times during the month did you participate in vigorous activities that lasted at least 10 minutes and cause large increases in breathing, heart rate, or leg fatigue or caused you to perspire? (Hand subject card #2)

   Score: 0 = Not at all (go to Q3)
   1 = 1-3 times per month
   2 = 1-2 times per week
   3 = 3-4 times per week
   4 = 5+ times per week
   7 = refused
   8 = don't know Frequency score
   = _____

2. About how long do you do this vigorous activity(ies) each time? (Hand card #3)

   Score: 0 =Not applicable
   1 = 10-30 minutes
   2 = 31-60 minutes
   3 = 60+ minutes
   7 = refused
   8 = don't know Duration score = _____
   weight= 5

VIGOROUS ACTIVITY INDEX SCORE:
FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____
(Responses of 7 or 8 are scored as missing.)

3. Think about the walks you have taken during the past month. About how many times per month did you walk for at least 10 minutes or more without stopping which was not strenuous enough to cause large increases in breathing, heart rate, or leg fatigue or cause you to perspire'? (Hand subject card #2)

Score:
0 = Not at all (go to Q5)
1 = 1-3 times per month
2 = 1-2 times per week
3 = 3-4 times per week
4 = 5+ times per week
7 = refused
8 = don't know

Frequency score = _____

4. When you did this walking, for how many minutes did you do it? (Hand #3)

Score:
0 = Not applicable
1 = 10-30 minutes
2 = 31-60 minutes
3 = 60+ minutes
7 = refused
8 = don't know

Duration score = _____

weight = 4

LEISURELY WALKING INDEX SCORE:
FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____

(Responses of 7 or 8 are scored as missing.)

5. About how many hours a day do you spend moving around on your feet while doing things? Please report only the time that you are <u>actually moving.</u> (Hand card #4)

Score:
0 = Not at all
I = less than I hr per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = refused
8 = don't know

Moving score = _____

weight = 3

MOVING INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____

(Responses of 7 or 8 are scored as missing.)

6. Think about how much time you spend standing or moving around on your feet on an average day during the past month. About how many hours per day do you stand? (Hand card #4)

Score:
0 = Not at all
1 = less than 1 hr per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = refused
8 = don't know

Standing score = _____

weight = 2

STANDING INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ =

_____

(Responses of 7 or 8 are scored as missing.)

INTERVIEWER: (Please read to subject.) I would now like to ask you about certain types of activities that you have done during the past month. I will ask you about how much vigorous activity, leisurely walking, sitting, standing, and some other things that you usually do.

1. About how many times during the month did you participate in vigorous activities that lasted at least 10 minutes and cause large increases in breathing, heart rate, or leg fatigue or caused you to perspire? (Hand card #2)

Score:
0 = Not at all (go to Q3)
1 = 1-3 times per month
2 = 1-2 times per week
3 = 3-4 times per week
4 = 5+ times per week
7 = refused
8 = don't know

Frequency score = _____

2.About how long do you do this vigorous activity(ies) each time? (Hand card#)

Score: 0 =Not applicable

1 = 10-30 minutes
2 = 31-60 minutes
3 = 60+ minutes
7 = refused
8 = don't know Duration score = ___weight= 5

VIGOROUS ACTIVITY INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____

(Responses of 7 or 8 are scored as missing.)

3. Think about the walks you have taken during the past month. About how many times per month did you walk for at least 10 minutes or more without stopping which was not strenuous enough to cause large increases in breathing, heart rate, or leg fatigue or cause you to perspire'? (Hand subject card #2)

Score: 0 = Not at all (go to Q5)
1 = 1-3 times per month
2 = 1-2 times per week
3 = 3-4 times per week
4 = 5+ times per week
7 = refused
8 = don't know Frequency score = _____

4. When you did this walking, for how many minutes did you do it? (Hand card #3)

Score: 0 = Not applicable
1 = 10-30 minutes
2 = 31-60 minutes
3 = 60+ minutes
7 = refused
8 = don't know Duration score = _____weight = 4

LEISURELY WALKING INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ =

_____

(Responses of 7 or 8 are scored as missing.)

5. About how many hours a day do you spend moving around on your feet while doing things? Please report only the time that you are actually moving. (Hand subject card #4)

Score: 0 = Not at all
I = less than I hr per day

2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = refused
8 = don't know Moving score = _____weight = 3

MOVING INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ = _____

(Responses of 7 or 8 are scored as missing.)

6. Think about how much time you spend standing or moving around on your feet on an average day during the past month. About how many hours per day do you stand? (Hand subject card #4)

Score: 0 = Not at all
1 = less than 1 hr per day
2 = 1 to less than 3 hrs per day
3 = 3 to less than 5 hrs per day
4 = 5 to less than 7 hrs per day
5 = 7+ hrs per day
7 = refused
8 = don't know Standing score = _____
weight = 2

STANDING INDEX SCORE:

FREQ SCORE _____ x DUR SCORE _____ x WEIGHT _____ =
_____

(Responses of 7 or 8 are scored as missing.)

Appendix B

DNA Isolation and Genotyping

DNA Isolation Procedures

DNA was isolated through methods prescribed by Puregene EP DNA Purification Kit (Gentra). Isolation began with addition of 1 ml of cell lysis solution to DNA samples that had been centrifuged for 10 minutes with supernatant poured off afterward. Samples were vortexed for 20 seconds after solution was added and then incubated for 15 minutes at room temperature. 10μL of proteinase K solution (20mg/mL) was added to cells and samples were vortexed for 20 seconds. Samples were then incubated for 10 minutes at room temperature. 340 μL of protein precipitation solution was added to cells and samples were again vortexed for 20 seconds. Samples were then incubated at 4^{o} C for 10 minutes.

After incubation, supernatant was poured into previously prepared 15-mL tubes containing 1 mL of 100% isoproponal, and samples were inverted 50 times and then centrifuged for 10 minutes. Supernatant was poured off and 1 mL of 70% ethanol was added and tubes were inverted 5 times, followed by a 1 minute centrifuge. Ethanol was poured off and samples were centrifuged again for 1 minute. A pipette was used to remove residual ethanol from tube and samples were then placed in 37^{o} C incubator for 10 minutes to dry. After drying, 400μL of DNA hydration solution was added and samples were incubated at 37^{o} C for 48 hours.

A second isolation procedure was performed 48 hours after first isolation. Cell lysis was performed as before, adding 2 mL of cell lysis solution. Samples were then incubated in a 65^{o} C water bath for one hour. After incubation, 800 μL of protein

precipitation solution was added and samples were vortexed for 20 seconds and then centrifuged for 15 minutes.

After incubation, supernatant was poured into previously prepared 15-mL tubes containing 2.4 mL of 100% isoproponol. Tubes were inverted gently for 50 times and centrifuged for five minutes. Supernatant was poured off and 2.4 mL of 70% ethanol was added to samples. Tubes were inverted five times and after a three minute centrifuge ethanol were poured off. Samples were centrifuged again for three minutes and a pipette was used to remove residual ethanol from tube. Samples were incubated at 37^{o} C for 10 minutes to dry, and 400 μL of DNA hydration solution was added to tube. Samples were incubated for 48 hours at 37^{o} C. Upon completion of incubation, samples were centrifuged for one minute and transferred to 1.5 mL Eppendorf tubes for storage at 4^{o} C. It has been shown that storage of unprocessed samples for up to one week at room temperature of 37^{o} C and up to six months of -20^{o} storage did not affect DNA yield of samples and ability to PCR amplify.

DNA Genotyping

Genotyping of *APOE* ε2, ε3, and ε4 alleles was performed using the following PCR primers: F-5' ACT GAC CCC GGT GGC GGA GGA GAC- 3'; R-5' TGT TCC ACC AGG GGC CCC AGG CGC TC- 3'. The thermal PCR cycle profile consisted of incubation at 95^{o} C for 5 minutes followed by 45 cycles of 95^{o} C for 30 seconds, 63^{o} C for 30 seconds, and 72^{o} C for 30 seconds and then incubation at 72^{o} C for 5 minutes. Two restriction digests containing 15 μL each of PCR product and either 0.2 μl of HaeII OR 0.75 μl of AflIII were incubated at 37^{o} C overnight and analyzed separately on a 3% agarose gel for two hours. Genotypes were determined for each sample according to

fragment sizes observed from each of the digests. Genotype accuracy was determined by the use of DNA sequence-verified controls with each genotyping reaction.

Appendix C

Paffenbarger Physical Activity Form: Scoring and Validation

Scoring of the Physical Activity Index allows for 8 kcal for each city block participant reports walking, or 96 kcal for each mile estimated. Stair climbing is estimated at 4 kcal for each flight of ten steps. Sport and recreational activities are scored using MET units from scoring guide for the instrument, augmented for activities not in use at time of publication of original instrument with MET values from the 2011 Compendium of Physical Activities (Ainsworth et al., 2011). All kilocalorie determinations are converted to reflect weekly totals.

This instrument has been validated for use in the age group of this study population. The total Physical Activity Index was found to have a Pearson correlation of .29 to Caltrect accelerometer measure of METs/day by Ainsworth, Leon, Richardson, Jacobs, & Paffenbarger (1993) in men and women between 21 and 59, and Jacobs, Ainsworth, Hartman, & Leon (1993), examining results among different questionnaires in men and women ages 20-59, found a correlation of the total index to accelerometer METs/day of .30, compared to correlation coefficients of .18 and .19 for the longer Minnesota Leisure Time and Baecke questionnaires, respectively. Albanes, Conway, Taylor, Moe, and Judd (1990) found Spearman correlations of .49 with energy intake level in kilocalories in 21 men ages 28-55. Albanes et al. also found that this questionnaire compared well to other exercise questionnaires, with Spearman correlation of .54 with Minnesota Leisure Time Survey, and .56 with Baecke questionnaire. Rauh, Hovell, Hofstetter, Sallis, & Gleghorn (1992) found a correlation of .34 to accelerometer use for Latinos ages 18-55.

As is the case with many physical activity questionnaires, comparison against validity criteria was stronger for vigorous activities. When calculated separately, high-intensity recreational activities displayed a correlation to accelerometer use of .69 (Ainsworth et al.,1993), and a .49 correlation for high-intensity physical activity(over 6.0 METs) was found by Strath, Bassett, & Swartz (2004) when compared to a heart-rate motion sensor in men and women ages 20-56. Strong validity was shown between the total Index and VO_2 max by Ainsworth et al. and Jacobs et al. (1993) , with correlations of .60 and .52, respectively.

Interestingly, the Paffenbarger questionnaire correlated to physical activity diary records at .30, compared to .74 of the highly detailed Minnesota questionnaire in one validation study(Jacobs et al., 1993), yet it correlated higher to accelerometer use than the Minnesota survey (.30 and .18, respectively). Jacobs et al. commented that length or detail did not necessarily transfer to better performance against validation measures, and it may be that querying about numerous physical activities may contribute to an overestimation of energy expenditure whereas estimated daily walking and stair climbing may surprisingly represent a more realistic assessment of general daily physical activity.

Ainsworth et al. (1993) found reliability of the Paffenbarger questionnaire was high for retest at a one month interval with a correlation coefficient of .72, with less reliability over a longer period of time of eight to nine months, with reliability decreasing to .34 and .43 respectively. Jacobs et al. (1993) found a correlation of .50 for total Index weekly kilocalories with a retest at nine months, with an additional correlation of .63 for sport and recreational activities. Rauh et al. (1992) found a reliability correlation of sport and recreational activity of .67, and total kilocalories of .34.

Paffenbarger Physical Activity Questionnaire

1. How many city blocks or their equivalent do you normally walk each day?

   ☐ Blocks/day (*Let 12 blocks = 1 mile*)

2. What is your usual pace of walking? *(Please check one)*
   - ☐ casual or strolling (less than 2 mph)
   - ☐ average or normal (2 to 3 mph)
   - ☐ fairly brisk (3 to 4 mph)
   - ☐ brisk or striding (4 mph or faster)

3. How many flights of stairs do you climb up each day?

   ☐ Flights/day (*Let 1 flight = 10 steps*)

4. List any sports or recreation you have actively participated in during the past year? (*Please remember seasonal sports or events*)

Sport, recreation, or other physical activity	Number of times/year	Hours	Minutes	Years Participation

5. Which of these statements best expresses your view? (*Please check one*)
   - ☐ I take enough exercise to keep healthy.
   - ☐ I ought to take more exercise.
   - ☐ Don't know.

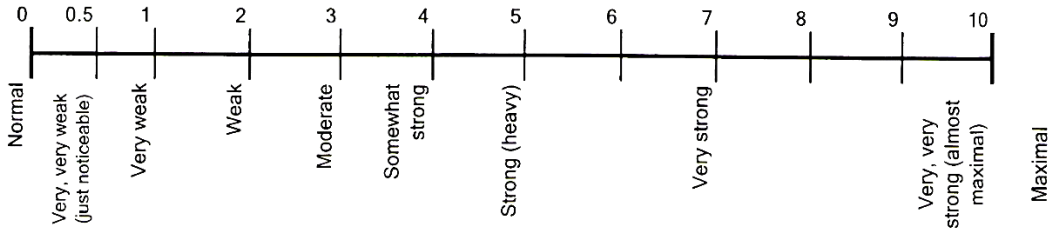
6. At least once a week, do you engage in regular activity akin to brisk walking, jogging, bicycling, swimming, etc. long enough to work up a sweat, get your heart thumping, or get out of breath?
   - ☐ Yes
     How many times per week? _____
     Activity: _____
   - ☐ No
     Why not? _____

(turn over)

Subject Code: Date: / /

Paffenbarger Physical Activity Questionnaire

7. When you are exercising in your usual fashion, how would you rate your level of exertion (degree of effort)? *(Please circle one number)*



8. On a usual weekday and a weekend day, how much time do you spend on the following activities? (*Total for each day should add to 24 hours*)

	Usual Weekday Hours/Day	Usual Weekend Day Hours/Day
A. Vigorous activity (digging in the garden, strenuous sports, jogging, aerobic dancing, sustained swimming, brisk walking, heavy carpentry, bicycling on hills,etc.)		
B. Moderate activity (housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing, light carpentry,ballroom dancing, bicycling on level ground, etc.)		
C. Light activity (office work, driving car, strolling, personal care, standing with little motion, etc.)		
D. Sitting activity (eating, reading, desk work, watching TV, listening to radio, etc.)		
E. Sleeping or reclining		
COLUMN TOTAL:		
MUST EQUAL:	24	24

Subject Code: Date: / /

Appendix D

YMCA Cycle Ergometer Scoring and Validation

This test is practical in that testing can be performed in most cases without medical supervision. Use of a cycle for submaximal testing is also appealing because it is familiar to most individuals, is more portable for field use than other testing equipment, enables ease of monitoring blood pressure since the upper body is immobile during testing, and can more easily be used by individuals with orthopedic issues that may prohibit them from using other equipment such as a treadmill.

VO_2 max, estimated with this test, is a standard measure of cardio-respiratory fitness, reflecting the capacity of the heart, lungs, and blood to transport oxygen to muscle, and the ability of muscle to utilize oxygen during exercise (Heyward, 2006, p. 55). VO_2 max captures the increased aerobic capacity of physically trained individuals. The submaximal test utilizes the principle that the relation of heart rate to VO_2 is linear with increasing work rate (Garatachea, Cavalcanti, García;-López, González-Gallego 2007) and can be extrapolated beyond the range tested to predict VO_2 max.

This protocol was cross-validated by Beekley et al. (2004) comparing to maximal tests using both treadmill and cycle ergometer. These researchers found that the YMCA submaximal test showed a significant correlation both to treadmill VO_2 max (r=.77, p <.05) and to cycle ergometry (r =.79, p< .05). Validated by sex, Beekley et al. found that the YMCA correlated well to maximal testing in both sexes, with a higher correlation to maximal treadmill performance in women (r = .90, p <.05) compared to men (r =.63, p<.05), with the YMCA test slightly overpredicting actual VO_2 max in men. Garatachea et al., (2007) also validated this test and found significant correlations between this

submaximal test and a maximal test performed on a cycle ergometer, (men, $r = .677$, $p < .01$; *women* $r = ,726$, $p<.010$).

Limitations of the YMCA method of obtaining a submaximal estimate of VO_2 max include assumptions that heart rate will maintain a steady-state for each work rate, that estimated maximal heart rates for a given age are similar, and that mechanical efficiency is constant for each individual taking the test. Participants who have limited experience with cycling or who have poor leg strength may encounter premature leg fatigue with the test, or may have difficulty maintaining a steady cadence. Beekley et al. (2004) commented that these issues can contribute up to 6% variation in accuracy. Additionally, scores for older individuals with significant deviations from average maximal age-predicted heart rates will reflect a less accurate estimation of VO_2 max. Garatachea et al. (2007) found that variations in maximal heart rate are a major limitation to accuracy of this submaximal test.

Standardization of conditions for testing helps limit variation, and a strength of testing for this study is that all participants were tested after two-three hours of quiet seated work so baseline heart rates were uniformly low. In addition, both temperature of room and eating and drinking patterns were controlled before testing. Use of trained research assistants also contributed to standardization of procedure, which assures appropriate handling of equipment and consistency in administration and use of verbal encouragement techniques.

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