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Regular Aerobic Exercise and Cognitive Function: The Roles of Vascular Function and Plasma Insulin

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Dedication

To my family, who have continuously nurtured my curiosity, challenged my philosophies, and encouraged me to follow my own destined path.

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Regular Aerobic Exercise and Cognitive Function: The Roles of Vascular Function and Plasma Insulin

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There is an increasing recognition that vascular disease risk is associated with a greater incidence of cognitive impairment and dementia. Such link is supported by the physiological observation that cerebral metabolism heavily relies on vascular supply of oxygen and energy substrates. Cerebral hypoperfusion which results from vascular dysfunction causes a mismatch between energy demand and supply and is associated with the pathological features of dementia, including the impairments of action potential generation and protein synthesis, glutamatergic excitotoxicity, and the deposition of cerebral amyloid-β proteins. In contrast, habitual aerobic exercise is an established strategy to ameliorate the risk factors for vascular disease and is increasingly recognized in improving cognitive function.

Accordingly, the primary purpose of this dissertation study was to investigate whether the exercise-related improvement in cognitive function was attributable to ameliorated vascular function and risk factors for vascular disease. In order to address this as comprehensively as we could, both cross-sectional and interventional studies were conducted. The primary findings from the present study were as follows. In the cross-

sectional study, a greater cognitive performance observed in endurance-trained adults was associated with higher levels of cerebral CO_2 reactivity and brachial endothelium-dependent vasodilation and lower levels of central arterial stiffness and plasma insulin. In the interventional study, a 3-month aerobic exercise training intervention did not improve cognitive function although central arterial stiffness and brachial endothelium-dependent vasodilation made favorable changes. However, we found that the improvement in memory performance after aerobic exercise training was associated with the reduction in central systolic blood pressure.

Taken together, a better cognitive performance observed in endurance-trained adults may not directly be attributable to greater vascular function because there were discrepant changes in cognitive and vascular functions after a 3-month aerobic exercise intervention. The correlation between the changes in memory performance and central systolic blood pressure is interesting but needs further investigation using a larger sample size. The discrepancy in the results between the cross-sectional and interventional studies could be explained by the duration of exercise training and/or the time it takes for the effect of improved vascular function to translate into cognitive function.

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Chapter 1: General Introduction

Cognition is one of the most important determinants for autonomy, quality of life, and functional ability in late life[1]. With advancing age, cognitive function progressively deteriorates in the multiple domains, especially memory and attention-executive function[2]. Due to the rapid population aging and a lack of the established treatment, the prevalence of dementia is exponentially rising and imposes a major healthcare problem to the Western societies[3]. The most frequent forms of dementia are Alzheimer's disease and vascular dementia, which have traditionally been believed to originate from the distinct pathologies. Yet an accumulating body of evidence suggests that vascular dysfunction and risk factors for vascular disease increase the risk of both types of dementia[4-7].

The brain weighs only 2% of body mass but consumes 20-25% of the body's oxygen. Due to the lack of energy reserve, the brain heavily relies on the perfusion through the vascular system to support its energy demand and receives ~15% of cardiac output at rest[8]. Therefore, the impairment in vascular function can elicit the disturbance in the stringently-regulated cerebral homeostasis and triggers a pathological cascade leading to cognitive decline. For example, stiffening of intra- and extra-cranial arteries leads to the failure of Windkessel function, which allows the transmission of pulsatile pressure energy into the delicate microcirculation and increases the risk of aneurysm and rupture[9-11]. At the level of cerebral arterioles, vascular risk factors cause the hypertrophy in the vessel wall, which results in the elevated vascular resistance and reduced cerebral blood flow and vasomotor capacity[12-15]. From the functional standpoint, endothelial

dysfunction leads to the development of atherosclerosis and disruption of blood-brain barrier. Vascular endothelium tonically releases vasoprotective substances such as nitric oxide (NO)[16]. Not only does NO exert potent vasodilatory effect on vascular smooth muscle, it also prevents the atherogenic process by inhibiting vascular oxidative stress and inflammation[17, 18]. Moreover, the capillary endothelium composes blood-brain barrier, which regulates the molecular trafficking between the brain and the blood[19]. The breakdown of blood-brain barrier has strongly been suggested to increase the accumulation of cerebral amyloid and the risk of neurodegeneration[20]. Furthermore, insulin resistance and hyperinsulinaemia may reduce cerebral metabolic rates and glucose utilization and may accelerate cognitive decline[21].

Lifestyle modifications such as regular physical activity and dietary modifications are the first line of defense against vascular disease and risk factors[22, 23]. In particular, habitual aerobic exercise has consistently been reported to ameliorate central arterial stiffness, vascular endothelial function, and insulin sensitivity[24-26]. However, these findings are mostly confined to the effects on cardiovascular system, and it remains elusive whether the similar adaptations take place in cerebrovascular system.

Accordingly, the overall goal of this dissertation was to investigate whether the exercise-related improvements in vascular function and plasma insulin level translate into a better cognitive function. Middle-aged men and women were specifically recruited because the pathological alterations in the brain are thought to precede years before the clinical onset of cognitive impairment and because the association between vascular disease risk and cognitive function could be negated by the effects of primary aging[27, 28].

In order to systematically address these aims, two different but complementary approaches were utilized: Study 1 (cross-sectional study) was designed to investigate the associations among cognitive function, cerebral and peripheral vascular functions, and plasma insulin level in sedentary and endurance-trained adults. Study 2 (interventional study) was designed to determine whether exercise-related improvement in cognitive function after a 3-month aerobic exercise training intervention is accompanied by the concomitant improvements in vascular function and plasma insulin level.

The specific aims of this project were to determine 1) the efficacy of regular aerobic exercise on cerebral and peripheral vascular functions and plasma insulin level; 2) whether exercise-related improvement in cognitive function is accompanied by the concomitant improvements in cerebral and peripheral vascular functions and plasma insulin level.

Hypotheses

Study 1:

1. The endurance-trained middle-aged adults would demonstrate:
    - Higher cognitive performance in memory and attention-executive function than the age-, sex-, education-matched sedentary subjects.
    - Greater cerebral and peripheral vascular functions (i.e., cerebral CO_2 reactivity, resting regional cerebral perfusion, central arterial stiffness, and brachial endothelium-dependent vasodilatation) than the age-, sex-, education-matched sedentary subjects.

- Lower plasma insulin concentrations than the age-, sex-, education-matched sedentary subjects.

2. A better cognitive function is associated with greater levels of cerebral and peripheral vascular functions and lower concentration of plasma insulin.

Study 2:

1. A 3-month aerobic exercise training will:
    - Increase cognitive performance in memory and attention-executive function.
    - Improve cerebral and peripheral vascular functions (i.e., cerebral CO_2 reactivity, resting regional cerebral perfusion, central arterial stiffness, and brachial endothelium-dependent vasodilatation).
    - Decrease plasma insulin concentrations.
2. The exercise-related improvement in cognitive function would be associated with the concomitant improvements in cerebral and peripheral vascular functions and plasma insulin level.

Chapter 2: Cardiopulmonary Fitness and Cognitive Function: Associations with Cerebral and Peripheral Vascular Reactivity

ABSTRACT

The brain is a metabolically demanding organ that heavily relies on the vascular supply of oxygen and nutrients. It has recently been hypothesized that cerebral hypoperfusion, which results from impaired vascular function, may trigger the pathological cascade leading to cognitive impairment. In contrast, regular aerobic exercise is recognized to improve vascular function, an effect which may further translate into better cognitive function. **Purpose**: To determine the associations among cardiopulmonary fitness, cognitive function, and cerebral and peripheral vascular reactivity in sedentary and endurance-trained subjects. **Methods**: Cardiopulmonary fitness, neuropsychological assessment, and cerebral (i.e., cerebral CO_2 reactivity) and peripheral (i.e., brachial flow-mediated dilation (FMD)) vascular reactivity were measured in 27 healthy sedentary and 32 endurance-trained adults aged 43-65 years. **Results**: There were no significant group differences in age, sex, education, fasting blood glucose concentration, and blood pressures. The endurance-trained group demonstrated significantly greater cardiopulmonary fitness, cognitive functions in total composite, memory, and attention-executive function, and brachial FMD. Cerebral CO_2 reactivity exhibited a trend which was greater in the endurance-trained than in sedentary group ($P=0.052$). Partial correlation analysis controlling for age, sex, and education demonstrated that maximal oxygen consumption was significantly correlated with total

cognitive composite score (r=0.36) and cerebral (r=0.28) and peripheral (r=0.33) vascular reactivity measures. Moreover, cerebral CO_2 reactivity showed a significant correlation with total cognitive composite score (r=0.32). **Conclusions**: Cardiopulmonary fitness is positively associated with cognitive performance and both cerebral and peripheral vascular reactivity measurements. Furthermore, cerebral CO_2 reactivity is positively correlated with cognitive performance, suggesting that the beneficial effects of habitual aerobic exercise on cognitive function may be potentially mediated by cerebrovascular function.

INTRODUCTION

The prevalence of dementia is exponentially increasing due to the rapid population aging and a lack of an established treatment, and is expected to impose a major health problem in the Western societies[29]. While age and genetic predisposition are currently regarded as the established risk factors for dementia, mounting evidence indicates that vascular dysfunction and risk factors for vascular disease are associated with accelerated cognitive decline[6, 30]. Because neurons in the brain are metabolically demanding without energy storage or reserve, they heavily rely on a continuous supply of blood and impairments in vascular function can result in cerebral hypoperfusion that may trigger a pathological cascade leading to cognitive decline and impairment[31, 32].

Cerebral blood flow increases in response to neuronal activation. This response, termed functional hyperemia, is accomplished by the coordinated vasodilatory responses that take place in both the local neurovascular unit inside the brain parenchyma and remote feeding arterioles surrounding the brain surface[33, 34]. Mechanistically, the vasodilation of downstream capillaries that is locally mediated by neuronal activation increases shear stress in the upstream feeding arterioles and elicits endothelium-dependent flow-mediated vasodilation[35-38].

Habitual aerobic exercise is an established strategy to improve conduit artery function, and is increasingly recognized in its benefits on cognitive function [24, 39]. However, it remains unknown whether habitual aerobic exercise is associated with greater cerebral vascular function and whether the exercise-associated enhancement in cerebral and/or peripheral vascular function is associated with greater cognitive function.

Accordingly, the primary purpose of the present study was to determine the associations among cardiopulmonary fitness, cognitive function, and cerebral and peripheral vascular reactivity measures. In order to isolate the effects of primary aging on cognitive and vascular functions, middle-aged adults who were free of overt vascular and neurological disease were specifically recruited. We hypothesized that cerebral and peripheral vascular reactivity would be greater in endurance-trained middle-aged adults and associated with greater cognitive function.

METHODS

Subjects

Fifty-nine community-dwelling adults aged 43-65 years were recruited through flyers and newspaper advertisements posted in Austin, Texas. All subjects were nonsmoking, normotensive (<140/90 mm Hg), non-diabetic (fasting blood glucose<126 mg/dL), and free of overt cardiovascular, cerebrovascular, or neurological disease as assessed by medical health questionnaire, blood chemistry, and hematological evaluations. None of the subjects were taking cardiovascular-acting medications including hormone replacement therapy. Physical activity status was verified by a modified physical activity questionnaire[40] and maximal oxygen consumption. Endurance-trained subjects reported running, cycling, and/or swimming at a moderate to vigorous exercise intensity for 7.6±0.6 hours/week. Sedentary subjects reported engaging in exercise less than once per week for the past year. Neuropsychological assessment and vascular function measurements were conducted on separate days within a one-month

period. During the period of data collection, participants reported no major changes in their physical activity or dietary habits. Vascular function measurements were conducted during the early follicular phase of the menstrual cycle in premenopausal women. Subjects fasted for at least 4 hours and abstained from alcohol, caffeine, and exercise for at least 24 hours before all of the measurements. The Human Research Committee reviewed and approved all procedures, and written informed consent was obtained from all subjects.

Measurements

Cardiopulmonary fitness and body composition. Maximal oxygen consumption (VO_2max) was measured during a modified Bruce protocol. After a 5-minute warm-up, subjects walked or ran while the treadmill slope was gradually increased 2% every 2 minutes until volitional exhaustion. Because VO_2max is influenced by age and sex and potentially complicates the association with other pertinent variables, we additionally reported the fitness percentile calculated based on age- and sex-adjusted regression established by the American College of Sports Medicine[41]. Body composition was measured by dual-energy X-ray absorptiometry (Lunar DPX, General Electric Medical Systems, Fairfield, CT).

Brachial flow-mediated dilatation (FMD). After at least 15 minutes of rest in the supine position, endothelium-dependent vasodilation was assessed by FMD using a noninvasive, standardized procedure[42]. The left (non-dominant) arm was extended and placed in a customized arm support system to prevent movement of the arm and to standardize the position of an ultrasound transducer. Brachial artery diameter and blood flow velocity

were measured from images derived from a Doppler ultrasound machine equipped with a high-resolution linear array transducer (Philips iE33 Ultrasound System, Bothel, WA). Once the subject was resting in a comfortable position, the pneumatic arm cuff was placed on the forearm, 3-5 cm distal to the antecubital fossa and connected to a rapid cuff inflator (E20 Rapid Cuff Inflator, D.E. Hokanson; Bellevue, WA Hokanson). Once a longitudinal image of the brachial artery, 5-10 cm proximal to the antecubital fossa was obtained, the transducer was stabilized in a secure position. Baseline brachial artery diameters and blood flow velocities were recorded prior to cuff inflation. The arm cuff was then inflated to 100 mmHg above resting systolic blood pressure (measured prior to baseline image capture) for 5 minutes. Blood flow velocity was recorded 10 seconds prior to cuff deflation and continued until 20 seconds after cuff deflation. Then, the ultrasound was switched to 2D mode to optimize the image for brachial artery diameter measurements for the next 160 seconds. The image files were transferred to an offline computer and stored for later data analysis. Brachial arterial diameter during end-diastole, as determined from the ECG trace, was measured by a single investigator using automated image analysis software (Brachial Analyzer, Medical Imaging Applications; Coralville, IA). FMD was calculated using the following equation: [(peak diameter - baseline diameter)/baseline diameter] × 100. The baseline end-diastolic diameter was calculated by taking the average of at least 20 cardiac cycles before cuff inflation. Peak end-diastolic diameter was taken from the average of 3 consecutive cardiac cycles demonstrating the largest brachial artery dilation after cuff deflation.

Cerebral CO_2 reactivity. Blood flow velocity (BFV) of the middle cerebral artery (MCA) was measured by transcranial color-coded duplex ultrasonography (iE 33 Ultrasound System, Philips, Bothell, WA) during normocapnic, hypocapnic, and hypercapnic steady states. MCA was insonated from the left posterior temporal window using a 1.6 MHz transcranial Doppler probe which was mounted on a custom-made probe fixation device attached to commercially available headgear (Dia Mon, DWL Compumedics, Charlotte, NC)[43]. Subjects wore nose clips and breathed only through a mouthpiece with a Y-way valve (Hans-Rudolph, Shawnee, KS), one end connected to a 5-liter air reservoir containing a mixture of 5 % CO_2 and 21 % O_2 balanced with nitrogen and another end open to room air. End-tidal CO_2, an estimate of arterial CO_2 level, was measured from expired air and analyzed by a capnograph (Capnocheck Plus, Smiths Medical, Waukesha, WI). Non-invasive beat-by-beat blood pressure was measured by Portapres (Finapres Medical, Amsterdam, Netherlands).

After at least15 minutes of rest in the supine position, 3 minutes of baseline recordings were taken during spontaneous breathing of room air. Next, subjects underwent 1 minute of maximal voluntary hyperventilation with a duty cycle of 1 second. This short period of hyperventilation was intended to reduce end-tidal CO_2 level to ~25 mmHg without causing respiratory muscle fatigue or central hypoxia possibly associated with a prolonged hyperventilation. The MCA-BFV was recorded during the last 20 seconds of hyperventilation. From the pilot study conducted prior to data collection, 30-40 seconds of maximal hyperventilation effectively decreased end-tidal CO_2 to near minimal levels (~25 mmHg). After the MCA-BFV returned to the baseline following

hyperventilation, a respiratory valve was switched to an air reservoir containing 5% CO_2 and 21% O_2 and the subjects were asked to breathe spontaneously for 3 minutes. The air reservoir was continuously filled from a cylinder whose air pressure was manually adjusted to subject's respiratory volume. The MCA-BFV was recorded during the last minute of hypercapnia.

The MCA-BFV waveform was manually traced by a single investigator who was blinded to subject characteristics and study design. Time-averaged peak velocity (i.e., area under curve of BFV waveform) was recorded from at least 10 cardiac cycles in normocapnic, hypocapnic, and hypercapnic steady states. Because transcranial Doppler does not measure blood flow per se, cerebral CO_2 reactivity index (CVRi) was calculated as a percent change in MCA-BFV over an absolute change in end-tidal CO_2[43]. The percent change in MCA-BFV has been reported to have a strong correlation with an absolute change in cerebral blood flow measured by intravenous Xenon dilution technique[44]. The change in CVRi was calculated from the three different ranges of end-tidal CO_2 levels: normocapnia to hypocapnia (NORM-HYPO), normocapnia to hypercapnia (NORM-HYPER), and hypocapnia to hypercapnia (HYPO-HYPER). The CVRi (HYPO-HYPER) was intended to represent cerebrovascular responsiveness to a wider range of end-tidal CO_2 fluctuation and to eliminate the potential effects of baseline neuronal activity and MCA-BFV on CVRi. In addition to CVRi, cerebrovascular conductance index (CVCi) was calculated in order to account for the effect of blood pressure on MCA-BFV.

Neuropsychological assessment. Participants completed a comprehensive battery of neuropsychological assessments, including standard clinical neuropsychological instruments with established reliability and validity. In order to reduce the number of multiple comparisons, neuropsychological measures were grouped into one of three domains: global cognition, memory, or attention-executive function. For the domain scores, raw test scores were converted to z-scores based on the study sample's mean and standard deviation. Timed test scores were multiplied by -1 so that higher scores indicate better performance. Domain scores were calculated for each participant by averaging the z-scores within the domain as follows: 1) *global*: MMSE[45] and WTAR[46]; 2) *memory*: CVLT-II immediate recall, delayed recall, and recognition discrimination[47]; 3) *attention-executive function*: Trail making A and B time to completion[48], COWAT[49], and WAIS-III Digit Span Subtest[50]. In addition, total cognitive composite score was calculated by averaging the z-scores of all the individual tests. All tests were administered and scored by a trained research assistant using standard administration and scoring criteria.

Statistical analyses

The distributions of all continuous variables were examined using the Shapiro-Wilk test of normality. According to the variable distributions, group differences in demographic and physiological variables were assessed using Mann-Whitney U or independent t-tests. Chi-square tests were used to compare group differences in categorical variables. Analysis of covariance was used to examine group differences in the measured variables after controlling for the potential covariates. Pearson's product moment and partial correlation analyses tested the associations among cardiopulmonary

fitness, cognitive function, and vascular reactivity measures after the variables with skewed distributions had been transformed to achieve normal distribution. In partial correlation analysis, age, sex, and education were entered as covariates. All statistical analyses were performed using SPSS 19 (SPSS inc., Chicago, IL). An α-level of 0.05 was set as the criterion for statistical significance.

RESULTS

Sedentary and endurance-trained subjects

There were no group differences in age, sex, ethnicity, and educational level (Table 2.1). As expected, endurance-trained subjects had significantly lower body mass index and body fat percentage and greater cardiopulmonary fitness level as measured by both subjective (i.e., Godin physical activity score) and objective (i.e., VO_2max and fitness percentile) scales. The cognitive function scores from total composite, memory, and attention-executive function were significantly higher in endurance-trained subjects than in sedentary subjects (Table 2.2).

Table 2.3 illustrates the basic cardiovascular and cerebrovascular parameters. There were no group differences in blood pressures, brachial arterial diameter, and MCA depth (all $P>0.05$). Endurance-trained subjects had lower heart rate at rest and greater normocapnic MCA-BFV and end-tidal CO_2 than sedentary subjects. Endothelium-dependent vasodilation as measured by brachial FMD was significantly higher in endurance-trained subjects than in sedentary subjects ($P<0.01$) (Figure 2.1). There were no group differences in the indices of cerebral CO_2 reactivity and conductance (Figure

2.2 and Figure 2.3). However, CVRi (HYPO-HYPER) showed a trend of higher level in endurance-trained subjects than in sedentary subjects (P=0.052).

The group differences in cognitive function disappeared after statistically controlling for vascular risk factors (i.e., HDL and BMI) and functions (i.e., FMD and CVRi (HYPO-HYPER) that showed the differences in the group comparison analysis (P>0.05).

Associations among cardiopulmonary fitness, cognitive function, and vascular reactivity

Pearson's product moment correlation analysis (Table 2.4) revealed that cardiopulmonary fitness percentile was correlated with cognitive function scores, including total cognitive composite (r=0.38), memory (r=0.32), and attention-executive function (r=0.28). After controlling for age, sex, and education using partial correlational analyses, VO_2max was significantly correlated with total cognitive composite and memory scores (r=0.36 and r=0.31, respectively).

Brachial FMD was significantly correlated with cardiopulmonary fitness percentile (r=0.37) whereas ΔCVRi (HYPO-HYPER) showed a trend of positive association with the fitness percentile (r=0.27, P=0.055) (Table 2.4). After controlling for age and sex, both FMD and ΔCVRi (HYPO-HYPER) showed significant correlations with VO_2max (r=0.33 and r=0.28, respectively). Indices of cerebral CO_2 conductance did not correlate with any of cardiopulmonary fitness measures.

Brachial FMD was significantly associated with total cognitive composite and attention-executive function scores (r=0.36 and r=0.38, respectively) (Table 2.4). However, these relations were abolished after controlling for age, sex, and education.

ΔCVRi (HYPO-HYPER) significantly correlated with total cognitive composite score (r=0.29) (Table 2.4), which remained significant even after controlling for age, sex, and education (r=0.32). No significant relations existed between indices of CVCi and cognitive function.

DISCUSSION

The primary findings from the present study are as follows. First, endurance-trained middle-aged adults demonstrated greater cardiopulmonary fitness, better cognitive function (i.e., total composite, memory, and attention-executive function), and higher brachial FMD than their sedentary peers. There was also a trend in ΔCVRi (HYPO-HYPER) to be greater in endurance-trained subjects than in sedentary subjects (P=0.052). Second, brachial FMD and ΔCVRi (HYPO-HYPER) were correlated with total cognitive composite scores. The correlation between ΔCVRi (HYPO-HYPER) and total cognitive composite score remained significant even after controlling for age, sex, and education. These results suggest that the beneficial effects of habitual aerobic exercise on cognitive function may be potentially mediated by cerebrovascular function. To the best of our knowledge, this is the first study to examine both cerebral and peripheral vascular reactivity in relation to cardiopulmonary fitness and cognitive function among middle-aged, healthy adults.

An accumulating body of epidemiological evidence suggests that vascular dysfunction and risk factors for vascular disease in midlife are associated with an elevated risk of late-life dementia, suggesting that an early exposure to vascular disease

risk may accelerate pathological alterations in the brain and contribute to the early onset of cognitive impairment[6, 30]. A potential mechanism that underlies the link between vascular disease risk and cognitive impairment is cerebral hypoperfusion[31, 32]. The human brain weighs only 2% of body mass, yet utilizes 20% of the body's oxygen consumption and 25% of total glucose utilization. Despite such high metabolic demand, the brain lacks intracellular energy storage or reserve and must heavily rely on a continuous supply of blood flow[8]. Therefore, cerebral hypoperfusion compromises the normal physiological functions of the brain and triggers a pathological cascade leading to cognitive decline. Indeed, cerebral hypoperfusion and the resultant hypoxia are associated with impaired protein synthesis and action potential generation and elevated cerebral amyloid production, which are all related to the pathological features of cognitive impairment and dementia[51, 52]. In contrast, habitual aerobic exercise is well accepted to improve vascular function and also increasingly recognized for its benefit on cognitive function[24, 39]. With this information as the rationale, we tested our hypothesis to determine whether better cognitive performance in endurance-trained middle-aged adults is associated with higher levels of cerebral and peripheral vascular reactivity measurements. The results from the present study were consistent with our hypothesis that endurance-trained middle-aged adults demonstrated greater levels of cognitive performance and brachial artery endothelium-dependent vasodilation. Although the group difference did not reach statistical significance, there was a trend (P=0.052) in endurance-trained subjects to have a better cerebral CO_2 reactivity. Moreover, these vascular reactivity measurements were

correlated with cognitive function, suggesting that the benefit of regular aerobic exercise on cognitive function may be, at least in part, mediated by improved vascular function.

We can only speculate on physiological mechanism by which greater endothelial function and cerebral CO_2 reactivity augment cognitive performance in endurance-trained adults. First, flow-mediated endothelium-dependent vasodilation plays an important role in modulating the extent of functional hyperemia[35, 36]. Functional hyperemia requires the vasodilatory responses in both the local neurovascular unit and remote feeding arterioles[33, 34, 37]. Because 50-60% of cerebrovascular resistance is controlled by the cortical pial arterioles located outside of the brain parenchyma[53], ascending or conducted vasodilation that propagates proximally to the remote feeding arterioles modulates blood flow to the local active neurons[33, 34]. The ability of vasodilation to ascend into proximal arteries is essential to achieving proper perfusion and the ascending vasodilation is eliminated with endothelial cell disruption. Second, vascular endothelial function may reflect the integrity of blood-brain barrier (BBB). The BBB is a single endothelial layer that is exclusively located at the level of cerebral capillaries and plays a crucial role in controlling the molecular trafficking between the brain and blood[54]. The disruption of BBB causes a leakage of the potentially hazardous molecules into the brain parenchyma and also impairs the clearance of cerebral amyloid from the intracellular space[20]. Third, cerebral CO_2 reactivity may assess the overall vascular health of intra- and extra-parenchymal arterioles. It is widely accepted that cerebral CO_2 reactivity measured at MCA is primarily mediated by vasodilation of the downstream arterioles[55, 56]. In addition, the vasodilation elicited by neuronal activation and hypercapnia takes place in the

arterioles of the similar size, suggesting that there may be a certain similarity in the microvascular adjustments responsible for two vasodilatory responses[33].

The strengths of the present study include the measurements of both peripheral and cerebral vascular reactivity, the use of neuropsychological assessments with established reliability and validity, and the clinically important finding that potentially provides a physiological support for the benefit of regular aerobic exercise on cognitive function. However, limitations from the present study also need to be underscored. First, endothelium-dependent vasodilation was measured on the brachial artery and may pose a limitation of site-specificity to the translation of this information to cerebral arteries. However, brachial endothelium-dependent vasodilation has been reported to reflect the systemic vascular endothelial function[57, 58] and may provide the condition of cerebrovascular endothelial function. Second, the cross-sectional study design does not address a cause-and-effect relationship. Such causal relationship can be revealed only with exercise intervention studies especially in middle-aged population since the pathological alterations in the brain is believed to proceed many years before the clinical symptoms. Third, a lack of neuroimaging in the present study precludes the elucidation of the mechanism underlying the association between vascular reactivity and cognitive function. Future studies should incorporate vascular neuroimaging markers such as calibrated blood-oxygen-level-dependent response to cognitive task[59]. Forth, a small sample size in our study limits the generalizability of our findings in different populations.

In summary, endurance-trained middle-aged adults demonstrated greater cognitive performance in total composite, memory, and attention-executive function than

the age-, sex-, and education-matched sedentary subjects. Cerebral CO_2 reactivity and vascular endothelial function as measured by brachial flow-mediated dilatation were associated with both cardiopulmonary fitness and cognitive function. These results suggest that better cognitive performance in endurance-trained subjects may potentially be mediated by the greater vascular functions. Future studies involving aerobic exercise training intervention and neuroimaging are warranted.

Table 2.1: Selected subject characteristics

	Sedentary	**Endurance-trained**	***P-values***
Males/Females (n)	10/17	11/21	0.83
Age (years)	54 ± 1	52 ± 1	0.30
Ethnicity (%)			0.85
Caucasian	69	78	
African-American	8	3	
Hispanic	8	3	
Asian	4	3	
Other	11	13	
Education (years)	16 ± 1	17 ± 1	0.29
Height (cm)	170 ± 2	167 ± 1	0.40
Body mass (kg)	76 ± 3	65 ± 2	**0.001**
Body mass index (kg/m^2)	26 ± 1	23 ± 1	**0.001**
Body fat (%)	36.7 ± 1.6	23.4 ± 1.5	**<0.001**
Lean tissue mass (kg)	45.6 ± 2.1	47.3 ± 1.8	0.48
Godin physical activity score (U)	18 ± 4	64 ± 4	**<0.001**
VO_2max (mL/min/kg)	26.0 ± 1.0	42.8 ± 1.5	**<0.001**
Cardiopulmonary fitness percentile (%)	15 ± 4	93 ± 5	**<0.001**
Maximal heart rate (bpm)	169 ± 3	170 ± 2	0.85
Maximal respiratory exchange ratio	1.10 ± 0.01	1.08 ± 0.01	0.19
Total cholesterol (mg/dl)	205 ± 7	191 ± 6	0.16
HDL-cholesterol (mg/dl)	60 ± 4	72 ± 4	**0.03**
LDL-cholesterol (mg/dl)	123 ± 6	108 ± 6	0.10
Glucose (mg/dl)	91 ± 2	93 ± 2	0.54

Values are means±SEMs. HDL=high density lipoprotein, LDL=low density lipoprotein, and VO_2max=maximal oxygen consumption.

Table 2.2: Neuropsychological assessment results

	Sedentary	Endurance-trained	*P-value*
Total cognitive composite score (z score)	-0.24 ± 0.10	0.20 ± 0.08	**0.001**
Global cognition (z score)	-0.19 ± 0.18	0.16 ± 0.10	0.24
MMSE	28.7 ± 0.2	29.0 ± 0.2	0.34
WTAR	41.2 ± 1.9	44.5 ± 0.8	0.40
Memory (z score)	-0.30 ± 0.17	0.26 ± 0.12	**<0.01**
CVLT-II immediate recall	10.6 ± 0.6	12.4 ± 0.4	**0.01**
CVLT-II delayed recall	10.8 ± 0.6	12.7 ± 0.4	**0.02**
CVLT-II discriminability index	2.7 ± 0.2	3.5 ± 0.4	0.09
Attention-executive function (z score)	-0.22 ± 0.11	0.18 ± 0.11	**0.02**
Trail Making Test A	32.4 ± 1.7	28.6 ± 1.4	0.08
Trail Making Test B	70.8 ± 4.0	58.2 ± 3.4	**0.02**
COWAT	43.1 ± 1.7	46.7 ± 2.6	0.37
WAIS-III Digit Span Subtest	18.0 ± 0.7	18.9 ± 0.7	0.35

Values are means±SEMs. COWAT=Controlled Oral Word Association Test, CVLT-II=California Verbal Learning Test-II, MMSE=Mini-Mental State Exam, WAIS-III=Wechsler Adult Intelligence Scale-III, and WTAR=Wechsler Test for Adult Reading.

Table 2.3: Basic vascular parameters

		Sedentary	**Endurance-trained**	***P-value***
Cardiovascular parameters at rest				
Heart rate (bpm)		64 ± 1	52 ± 1	**<0.001**
Systolic BP (mmHg)		120 ± 2	118 ± 2	0.32
Mean BP (mmHg)		90 ± 2	88 ± 1	0.28
Diastolic BP (mmHg)		73 ± 1	70 ± 1	0.11
Pulse pressure (mmHg)		48 ± 1	48 ± 1	0.77
Brachial artery mean diameter (mm)		3.54 ± 0.14	3.61 ± 0.13	0.69
Cerebrovascular parameters				
Mean blood flow velocity (cm/sec)				
	Normocapnia	58.9 ± 4.3	67.0 ± 2.4	**0.03**
	Hypocapnia	38.3 ± 2.9	39.5 ± 1.7	0.34
	Hypercapnia	77.2 ± 4.7	84.0 ± 2.8	0.20
End-tidal CO_2 (mmHg)				
	Normocapnia	42 ± 1	45 ± 1	**0.01**
	Hypocapnia	26 ± 1	28 ± 1	0.08
	Hypercapnia	51 ± 1	53 ± 0	**0.01**
Mean arterial pressure (mmHg)				
	Normocapnia	90 ± 2	87 ± 2	0.22
	Hypocapnia	80 ± 2	79 ± 2	0.79
	Hypercapnia	95 ± 2	92 ± 2	0.46

Values are means±SEMs. BP, blood pressure and CO_2, carbon dioxide.

Table 2.4: Pearson's product moment correlation coefficients and (P-values) illustrating the associations among cardiopulmonary fitness, cognitive function, and vascular reactivity measurements.

	Cardiopulmonary fitness measures			Cognitive function scores			
	Godin PAS	VO_2max	Fitness %ile	Total cognitive composite	Global cognition	Memory	Attention-executive function
Godin PAS		0.65* (<0.001)	0.67* (<0.001)	0.09 (0.52)	-0.08 (0.58)	0.18 (0.20)	0.05 (0.72)
VO_2max			0.93* (<0.001)	0.23 (0.08)	0.14 (0.29)	0.17 (0.20)	0.19 (0.15)
Fitness %ile				0.38* (<0.01)	0.22 (0.10)	0.32* (0.01)	0.28* (0.03)
FMD	0.25 (0.09)	0.33* (0.01)	0.37* (<0.01)	0.36* (<0.01)	0.10 (0.46)	0.26 (0.052)	0.38* (<0.01)
ΔCVRi (NORM-HYPO)	0.10 (0.51)	0.01 (0.97)	0.16 (0.27)	0.21 (0.13)	0.09 (0.50)	0.19 (0.17)	0.18 (0.19)
ΔCVRi (NORM-HYPER)	-0.03 (0.85)	0.01 (0.92)	-0.04 (0.77)	0.14 (0.30)	0.21 (0.12)	0.05 (0.74)	0.11 (0.44)
ΔCVRi (HYPO-HYPER)	0.11 (0.48)	0.20 (0.15)	0.27 (0.055)	0.29* (0.03)	0.23 (0.10)	0.25 (0.07)	0.20 (0.15)

PAS=physical activity score, VO_2max=maximal oxygen consumption, FMD=flow-mediated dilation, CVRi=cerebrovascular reactivity index, CVCi=cerebrovascular conductance index, NORM=normocapnia, HYPO=hypocapnia, and HYPER=hypercapnia.

Figure 2.1: Brachial endothelium-dependent vasodilation.

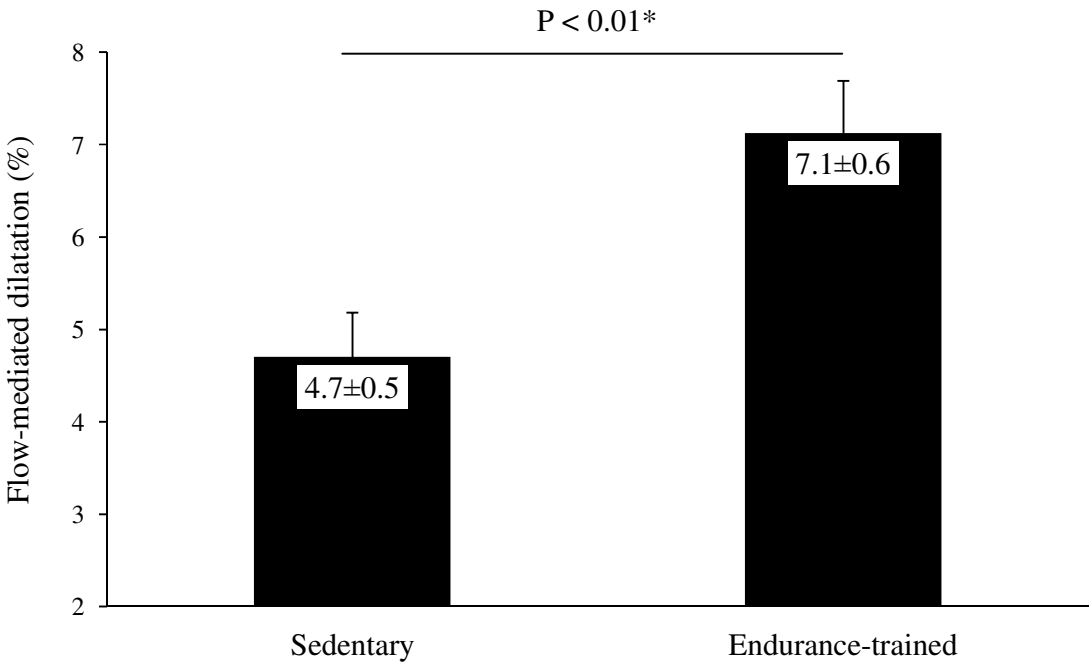
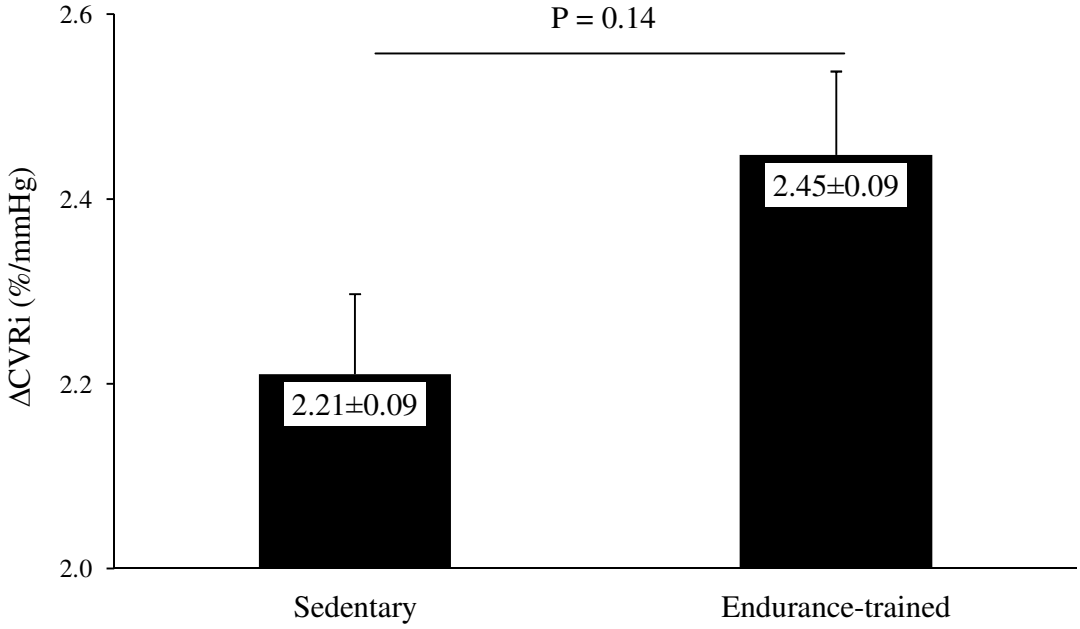
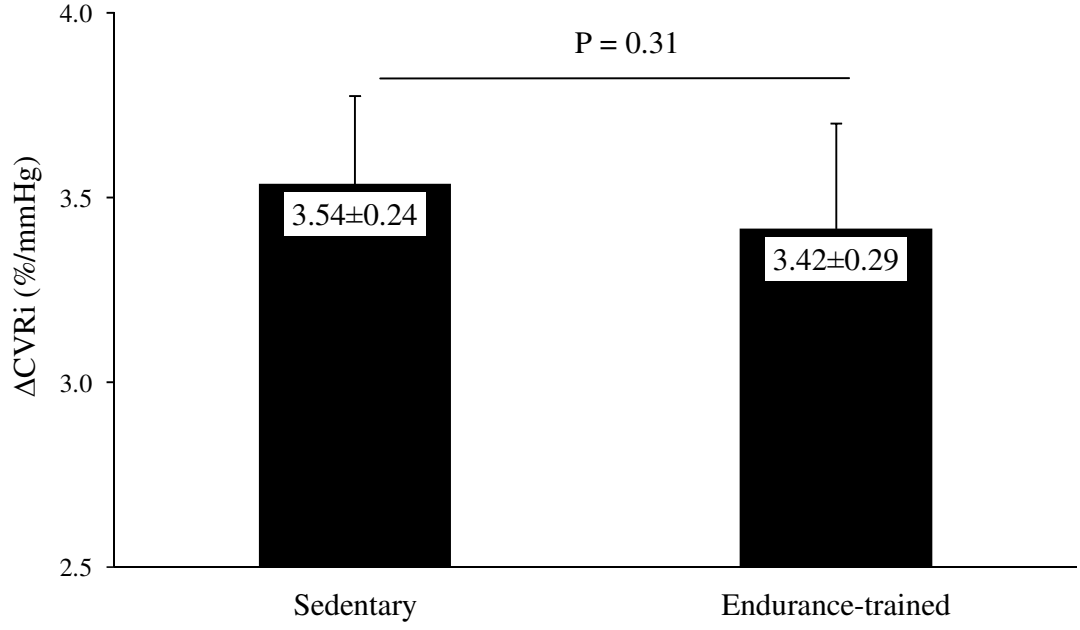


Figure 2.2: Cerebral CO_2 reactivity indices (CVRi).

a) Normocapnic to hypocapnic CVRi



b) Normocapnic to hypercapnic CVRi



c) Hypocapnic to hypercapnic CVRi

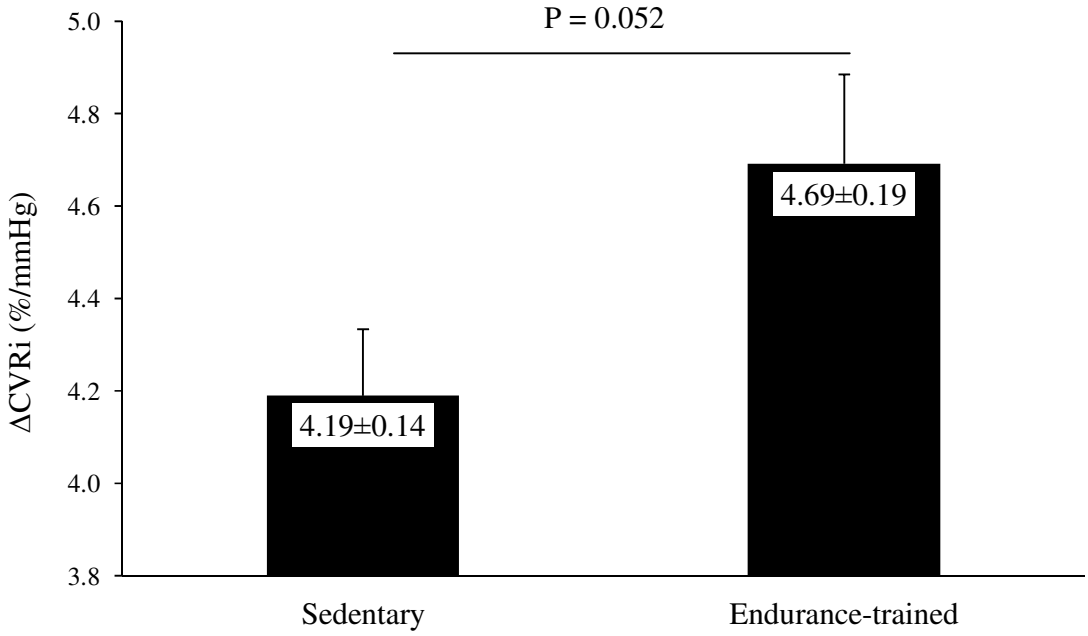
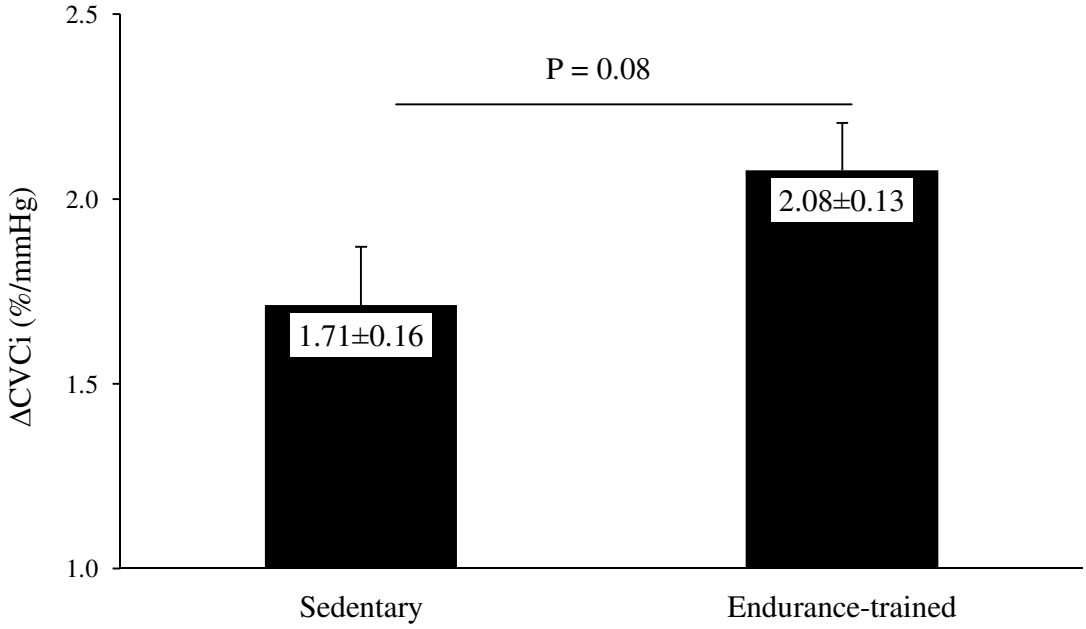
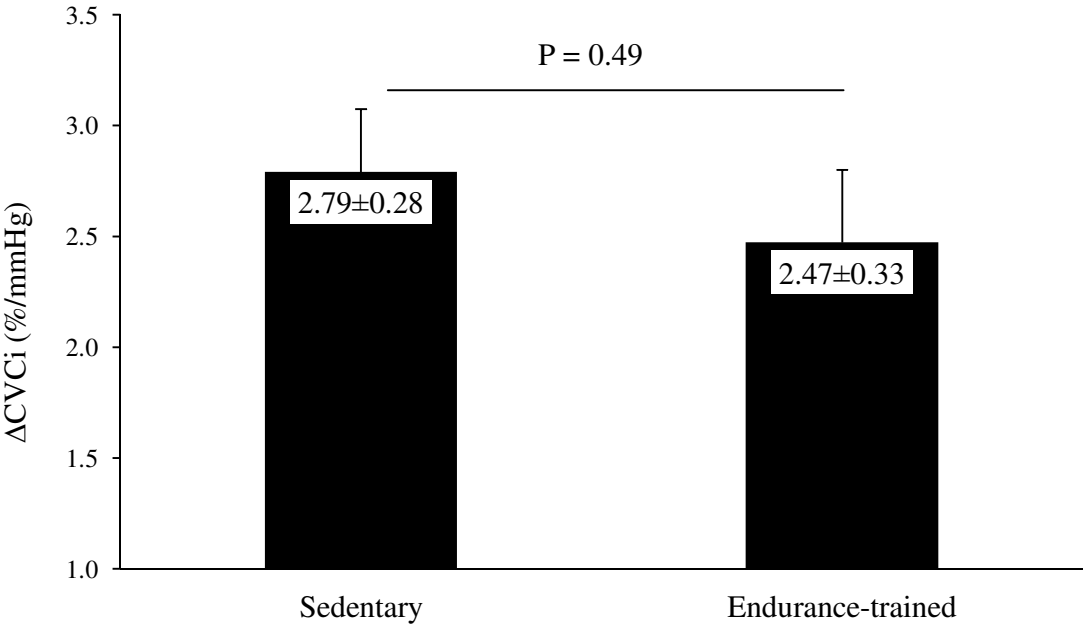


Figure 2.3: Cerebral CO_2 conductance indices (CVCi).

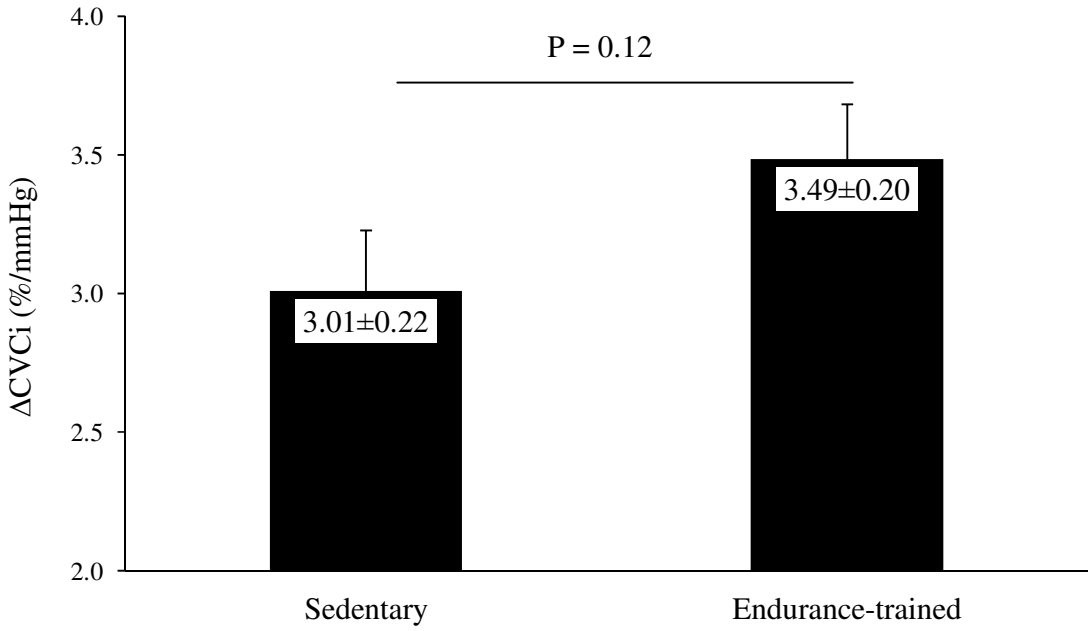
a) Normocapnic to hypocapnic CVCi



b) Normocapnic to hypercapnic CVCi



c) Hypocapnic to hypercapnic CVCi



Chapter 3: The Contributions of Central Artery Stiffness and Regional Cerebral Perfusion to Cognitive Function in Endurance-Trained and Sedentary Middle-Aged Adults

ABSTRACT

Central elastic arterial stiffness is associated with accelerated brain aging and an increased risk of cognitive impairment. In contrast, habitual aerobic exercise decreases central artery stiffness and is increasingly recognized to improve cognitive function. **Purpose**: To determine the associations among central arterial stiffness, cognitive function, and regional cerebral perfusion in sedentary and endurance-trained adults. **Methods**: Cardiopulmonary fitness, neuropsychological questionnaires, regional and local central arterial stiffness, and cerebral perfusion were measured in 27 healthy sedentary and 32 endurance-trained adults aged 43-65 years. **Results**: There were no group differences in age, sex, ethnicity, education, brachial and carotid blood pressures, and carotid intima-media wall thickness (all $P>0.05$). The endurance-trained group demonstrated significantly greater cardiopulmonary fitness, cognitive performance in total cognitive composite, memory, and attention-executive function, and occipito-parietal blood flow. Central artery stiffness was significantly lower in endurance-trained than in sedentary subjects. Controlling for age, sex, and education, carotid artery distensibility was significantly correlated with both VO_2max ($r=0.28$) and total cognitive composite score ($r=0.30$). Furthermore, regional and local measures of central artery stiffness were negatively correlated with occipito-parietal perfusion ($r=-0.41$-0.44,

P<0.05). **Conclusions**: Lower central artery stiffness in endurance-trained adults is associated with greater cognitive function and occipito-parietal perfusion. The findings from the present study suggest the potential physiological role of central arterial stiffness in promoting the relation between cardiopulmonary fitness and cognitive function.

INTRODUCTION

The prevalence of dementia is exponentially increasing due to rapid population aging and a lack of established treatment, and is expected to impose a major health problem in the Western societies[29]. While age and genetic predisposition are currently regarded as the established risk factors for dementia, mounting evidence indicates that vascular dysfunction and risk factors for vascular disease in midlife are associated with the greater risk, suggesting that the improvement in vascular profiles in midlife may slow down the pathophysiological process in the brain and attenuate cognitive decline[6, 30].

There is increasing recognition that central arterial stiffness is associated with accelerated structural brain aging and cognitive decline[11, 60]. The brain is a low impedance, high flow organ that is continuously exposed to flow pulsations generated from the left ventricle. In order for the brain to be protected from the deleterious impact of flow pulsations, elastic cardiothoracic arteries (e.g., common carotid artery) must effectively buffer the pulsatile energy and create a continuous flow in the microcirculation. However, with stiffening of central elastic arteries and increased aortic impedance with aging, pulsatile pressure energy penetrates into the delicate microcirculation and increases the risk of end-organ damage[9, 10]. Indeed, central elastic artery stiffness is associated with lower subcortical perfusion presumably due to an increased microvascular resistance[61].

Habitual aerobic exercise is regarded as an effective strategy to reduce central arterial stiffness. This form of lifestyle modification is increasingly recognized for its benefit on cognitive function as well [24, 39]. Given the link between arterial stiffness and

cognitive function, it is plausible to hypothesize that the beneficial influence of regular physical activity on cognition may be mediated by its effects on arterial stiffness. Accordingly, the primary purpose of this study was to determine the associations among cardiopulmonary fitness, central arterial stiffness, and cognitive function. We hypothesized that endurance-trained middle-aged adults would demonstrate lower central arterial stiffness and greater cognitive performance. Additionally, in a subset of participants, we also sought to investigate whether lower central arterial stiffness in endurance-trained adults is associated with greater cerebral blood flow. Our hypothesis is that lower central arterial stiffness in endurance-trained subjects is associated with greater cerebral perfusion in the occipito-parietal area.

METHODS

Subjects

Fifty-nine community-dwelling adults aged 43-65 years were recruited through flyers and newspaper advertisements posted in Austin, Texas. All subjects were nonsmoking, normotensive (<140/90 mm Hg), and free of overt cardiovascular, cerebrovascular, or neurological disease as assessed by medical health questionnaire, blood chemistry, and hematological evaluation. Individuals with MR imaging contraindications were excluded from participation. None of the subjects were taking cardiovascular-acting medications including hormone replacement therapy. Physical activity status was verified by a modified physical activity questionnaire[40] and maximal oxygen consumption. Endurance-trained subjects reported running, cycling, and/or

swimming at a moderate to vigorous exercise intensity for 7.5±0.6 hours/week. Sedentary subjects reported engaging in exercise less than once per week for the past year. Neuropsychological assessment and regional cerebral perfusion measurement and vascular function measurements (i.e., blood pressure and central artery stiffness) were conducted on separate days within a one-month period. Regional cerebral perfusion was measured in randomly selected sub-group of subjects. During the period of data collection, participants reported no major changes in their lifestyle, including dietary and exercise patterns. Vascular function measurements were conducted during the early follicular phase of the menstrual cycle in premenopausal women. Subjects fasted for at least 4 hours and abstained from alcohol, coffee, and exercise for at least 24 hours before all of the measurements. The Human Research Committee reviewed and approved all procedures, and written informed consent was obtained from all subjects.

Measurements

Body composition and cardiopulmonary fitness. Body composition was measured by dual-energy X-ray absorptiometry (Lunar DPX, General Electric Medical Systems, Fairfield, CT). Maximal oxygen consumption was measured during a modified Bruce protocol. After a 5-minute warm-up, subjects walked or ran while the treadmill slope was gradually increased 2% every 2 minutes until volitional exhaustion. Because maximal oxygen consumption can be influenced greatly by age and sex and potentially complicates the interpretation of one's cardiopulmonary fitness level, we additionally reported the fitness percentile calculated based on age- and sex-adjusted regression established by the American College of Sports Medicine[41].

Blood pressure and central elastic arterial stiffness. After at least 15 minutes of rest in the supine position, bilateral brachial and ankle blood pressures, carotid and femoral pulse pressure waveforms, and heart rate were simultaneously measured by an automated vascular testing device (VP-2000, Omron Healthcare Bannockburn, Illinois)[62]. Ankle-brachial index was calculated as ankle systolic blood pressure divided by brachial systolic blood pressure, and used to screen for peripheral arterial disease. Arterial applanation tonometry incorporating an array of 15 micropiezoresistive transducers recorded pulse pressure waveforms from the carotid and femoral arteries. The time it takes for the pulse wave to travel between the 2 tonometers was automatically measured based on the foot-to-foot method. The straight distance between the carotid and femoral arterial recording sites was measured on body surface and multiplied by 0.8 in order to adjust the measured distance close to the real pulse travel distance[63]. Subsequently, carotid-femoral pulse wave velocity (cfPWV) was calculated as pulse travel distance divided by the transit time. The average of at least 3 measurements that are recorded over 30-second periods was reported.

Arterial distensibility, β-stiffness index, and young's modulus were measured noninvasively by the simultaneous recordings of the common carotid artery diameter and the pulse pressure waveforms from the contralateral side[64]. Common carotid artery diameter was measured from the B-mode images on an ultrasound machine equipped with a high-resolution linear-array transducer (Phillips iE33 Ultrasound System, Bothel, WA). A longitudinal image of the cephalic portion of common carotid artery was acquired 1-2 cm proximal to the carotid bulb and optimized for diameter detection. All

images were analyzed on an offline computer using automated image analysis software (Carotid Analyzer, Medical Imaging Applications, Coralville, IA). The carotid artery pressure waveform was simultaneously obtained using an applanation tonometer incorporating an array of 15 micropiezoresistive transducers (VP-2000, Omron Healthcare Bannockburn, Illinois) placed on the contralateral side. Simultaneous measurements were made for 30 seconds (or at least 20 cardiac cycles). To correct for the hold-down pressure of applanation tonometry, carotid mean and diastolic blood pressures were calibrated to oscillometrically determined brachial mean and diastolic blood pressures as previously described[64]. Subsequently, carotid arterial distensibility, β-stiffness index, and young's modulus were calculated using the equations described in detail elsewhere[65, 66].

Regional cerebral perfusion. In the randomly selected sub-group of participants, regional cerebral perfusion was measured by the arterial spin labeling (ASL) technique described in detail elsewhere[67-69]. MRI data were acquired using a 3T GE Signa Excite scanner. Whole-brain T1-weighted images were collected for anatomical reference (spoiled gradient echo sequence, 256 × 256 matrix, FOV = 24 x 24 cm², 1 mm slice thickness, 0 gap). Perfusion imaging included an ASL sequence with a single-shot spiral readout, cerebrospinal fluid reference scan, and a minimum contrast scan[70, 71]. Cerebral blood flow was computed by subtracting the tag/control image series (CBFv3.2, Function Biomedical Informatics Research Network). These images were corrected for field inhomogeneities using the minimum contrast scan and converted to physiological units (ml/100 ml/min) using the reference signals[70, 71]. Average cerebral perfusion was

calculated for bilateral *a priori* regions of interest chosen for their documented susceptibility to cerebrovascular disease[72, 73]. Spherical regions of interest, 5 mm in diameter, were automatically created around the central coordinate for the chosen regions according to the Talairach and Tournoux atlas[74] using the Analysis of Functional NeuroImages (AFNI) software[75].

Neuropsychological assessment. Participants completed a comprehensive battery of neuropsychological assessments, including standard clinical neuropsychological instruments with established reliability and validity. In order to reduce the number of multiple comparisons, neuropsychological measures were grouped into one of three domains – global cognition, memory, or attention-executive function. For the domain scores, raw test scores were converted to z-scores based on the study sample's mean and standard deviation. Timed test scores were multiplied by -1 so that higher scores indicate better performance. Domain scores were calculated for each participant by averaging the z-scores within the domain as follows: 1) *global*: MMSE[45] and WTAR[46]; 2) *memory*: CVLT-II immediate recall, delayed recall, and recognition discrimination[47]; 3) *attention-executive function*: Trail making A and B time to completion[48], COWAT[49], and WAIS-III Digit Span Subtest[50]. In addition, total cognitive composite score was calculated by averaging the z-scores of all the individual tests. All tests were administered and scored by a trained research assistant using standard administration and scoring criteria.

Statistical analyses

All variable distributions were examined using the Shapiro-Wilk test of normality recommended for small samples. Group differences in demographic and physiological

variables were assessed using non-parametric chi-square or Mann-Whitney U tests because some of the measured variables showed naturally skewed distributions. ANCOVA was performed to determine the group difference in cognitive function after covarying for arterial stiffness measures. The association among cardiopulmonary fitness, cognitive function scores, central arterial stiffness, and regional cerebral perfusion were assessed by Pearson's simple correlation analysis after skewed variables were log transformed and achieved normal distributions. Partial correlation analyses were performed to examine the association between central arterial stiffness and cognitive function after controlling for the basic covariates (i.e., age, sex, and education level). All statistical analyses were performed using SPSS 19 (SPSS inc., Chicago, IL). An α-level of 0.05 was set as the criterion for statistical significance.

RESULTS

Endurance-trained versus sedentary subjects

There were no group differences in age, sex, ethnicity, education, blood pressures, and carotid intima-media wall thickness (Table 3.1 and Table 3.2). As expected, endurance-trained subjects had significantly lower body mass index and body fat percentage and greater maximal oxygen consumption and cardiopulmonary fitness percentile than sedentary subjects. The cognitive function scores from total composite, memory, and attention-executive function were significantly higher in endurance-trained subjects than in sedentary subjects (Figure 3.1). Endurance-trained subjects demonstrated significantly lower cfPWV (central artery stiffness) than sedentary subjects but there was

no group difference in faPWV (peripheral artery stiffness; Figure 3.2). Figure 3.3 exhibits the local measures of arterial stiffness taken at the common carotid artery. Carotid distensibility was significantly greater and β-stiffness index and Young's elastic modulus were significantly lower in endurance-trained subjects than in sedentary subjects. The group difference in attention-executive function was abolished after covarying for the measures of carotid arterial stiffness, which included carotid distensibility (P=0.10), beta stiffness index (P=0.08), and Young's modulus (P=0.13).

Associations among cardiopulmonary fitness, cognitive Function, and arterial stiffness

Pearson's simple correlation analysis revealed that cardiopulmonary fitness percentile is significantly correlated with cognitive functions in total composite (r=0.40), global cognition (r=0.29), and memory (r=0.34) (Table 3.3). After controlling for age, sex, and education using partial correlation analysis, VO_2max was significantly correlated with total cognitive composite (r=0.38) and memory scores (r=0.33).

Among the measures of central arterial stiffness, all of the carotid stiffness parameters were significantly correlated with both VO_2max (r=0.30-0.38) and total cognitive composite scores (r=0.28-0.33). In specific to cognitive domains, carotid distensibility correlated with memory performance (r=0.27, P<0.05), and the Young's modulus was associated with attention-executive function (r=-0.30, P<0.05). Furthermore, partial correlation analysis controlling for age, sex, and education revealed that carotid distensibility and Young's modulus are significantly associated with total cognitive composite score (r=0.30 and r=-0.30, respectively).

Central arterial stiffness and cerebral perfusion

Table 3.4 shows the group comparison between the randomly selected sedentary and endurance-trained subjects who underwent regional cerebral perfusion measurement. A total of 36 subjects (19 sedentary and 17 endurance-trained) were included in this analysis. Demographic variables, cognitive function scores, and cardiovascular measurements, including central arterial stiffness, exhibited the similar results as observed from the entire subject population. Among the *a priori* regions where cerebral blood flow measurement was performed, endurance-trained subjects exhibited significantly higher perfusion in the occipito-parietal area than in sedentary subjects. Furthermore, the occipito-parietal perfusion was significantly correlated with cfPWV (r=-0.44) and carotid distensibility (r=0.46) and Young's modulus (r=-0.41), indicating that lower central arterial stiffness is associated with greater occipito-parietal perfusion. Occipito-parietal perfusion was not correlated with any of the cognitive function scores.

DISCUSSION

The primary findings from the present study are as follows. First, middle-aged adults who participated in regular aerobic exercise demonstrated greater cognitive performance in total composite, memory, and attention-executive function and lower central arterial stiffness than sedentary subjects. Second, central arterial stiffness measured locally from carotid artery was negatively correlated with total cognitive composite score. Moreover, after controlling for age, sex, and education, VO_2max was correlated positively with higher cognitive functions in total composite and memory and negatively with carotid arterial stiffness. These results suggest a potential role of carotid

arterial stiffness in mediating the relation between cardiopulmonary fitness and cognitive function. Third, in the randomly selected subgroup of subjects, occipito-parietal perfusion was greater in endurance-trained subjects than in sedentary subjects and was related to measures of central arterial stiffness, indicating that lower central arterial stiffness in endurance-trained subjects is associated with greater occipito-parietal perfusion. To the best of our knowledge, this is the first study to examine the physiological role of central arterial stiffness in the relation between cardiopulmonary fitness and cognitive function.

Mounting evidence indicates that vascular dysfunction and risk factors for vascular disease accelerate structural brain aging and cognitive decline[6, 30]. In particular, stiffening of cardiothoracic arteries is associated with lower subcortical perfusion, higher prevalence of subcortical infarcts, and an increased risk of cognitive impairment[60]. The brain, which accounts for only 2% of body mass, is a high flow, low impedance organ that receives 15% of cardiac output[8]. As a result, microcirculation in the brain is continuously exposed to deleterious flow pulsations generated from the left ventricle. With stiffening of central elastic arteries and the failure to buffer flow pulsations, the risk of end-organ damage increases due to repeated barotrauma and elevated cerebrovascular resistance[9, 10]. On the other hand, habitual aerobic exercise decreases central elastic arterial stiffness and is recognized for its potential benefit on cognitive function[24, 39, 64]. With this information serving as the conceptual basis, we tested our hypothesis that lower central arterial stiffness in endurance-trained adults is associated with greater cognitive performance. Furthermore, in order to provide a potential physiological link between

lower central arterial stiffness and higher cognitive function in endurance-trained subjects, we tested our secondary hypothesis of an inverse relationship between central arterial stiffness and cerebral perfusion. Our overall results generally support our hypotheses. After controlling for age, sex, and education, we found that lower central elastic arterial stiffness, measured particularly by carotid distensibility, was associated with greater cognitive performance. Furthermore, lower central arterial stiffness was associated with greater occipito-parietal perfusion in randomly selected subjects.

How could lower central arterial stiffness and higher occipito-parietal perfusion augment cognitive performance in endurance-trained middle-aged adults? The available evidence points to reduced flow pulsation (discussed above) and lower level of cerebral amyloid-β (Aβ) accumulation[60, 76]. Cerebral Aβ is produced by proteolytic cleavage of the amyloid precursor protein and accumulates primarily in the extracellular space of the brain parenchyma and increases the risks of oxidative stress, neurotoxicity, and abnormal cerebral vasoconstriction[77]. Importantly, the accumulation of cerebral Aβ is a pathological hallmark of Alzheimer's disease that is thought to precede neurodegeneration and cognitive impairment[77]. In the normal physiological condition, the rates of Aβ production and clearance are homeostatically balanced so that the net accumulation is minimized[78]. However, with cerebral hypoperfusion and the resultant hypoxia, the rate of production may be upregulated and become greater than that of clearance[51, 79]. Furthermore, normal cerebral perfusion combined with distensible cerebral arteries plays an important role in providing a motive force for the clearance of Aβ from the perivascular interstitial space[80-82]. Although our study did not include the

measurement of intracranial arterial stiffness, the previous autopsy study reported that intracranial and extracranial arteries concomitantly stiffen with aging and suggests the possibility that our endurance-trained subjects may have less stiffening of the intracranial arteries[83]. In addition, the finding from the present study demonstrating lower cerebral perfusion in occipito-parietal area in sedentary subjects spatially coincides with a region particularly susceptible to Aβ accumulation[84]. Taken together with recent evidence showing that regular engagement in physical activity moderates the effect of genetic risk on amyloid disposition[76], lower central arterial stiffness and greater occipito-parietal perfusion in our endurance-trained group may help maintain the homeostatic balance of cerebral Aβ, which may in turn translate to better cognitive function.

Clearly, the strength of the present study is the interdisciplinary nature of the research that include the state of the art measurements for cardiopulmonary fitness, cognitive function, and central elastic arterial stiffness combined with a non-invasive regional cerebral perfusion measurement. However, in any other research investigations, there are several limitations that should be mentioned. First, due to the cross-sectional nature of the study, we cannot draw causal relationships among regular aerobic exercise, central arterial stiffness, regional cerebral perfusion, and cognitive function. Second, a small sample size in our study may limit our ability to generalize our findings to other populations (e.g., older adults, cardiac patients).

In conclusion, the primary finding of the present study is that endurance-trained middle-aged adults demonstrated better cognitive performance and lower central elastic arterial stiffness. Controlling for the potential covariates, carotid arterial stiffness was

negatively correlated with cognitive performance. Moreover, in the randomly selected subjects, lower central arterial stiffness was associated with greater occipito-parietal perfusion, suggesting a potential physiological link between lower central arterial stiffness and greater cognitive performance in our endurance-trained subjects. These results suggest that habitual aerobic exercise and the maintenance of lower central arterial stiffness in midlife may attenuate the risk of cognitive decline and impairment.

Table 3.1: Selected subject characteristics

	Sedentary	**Endurance-trained**	***P-values***
Males/Females (n)	10/17	11/21	0.83
Age (years)	53 ± 1	52 ± 1	0.40
Ethnicity (%)			0.70
Caucasian	18	25	
African-American	3	1	
Hispanic	2	1	
Asian	1	1	
Other	3	4	
Education (years)	16 ± 1	17 ± 1	0.19
Height (cm)	170 ± 2	167 ± 1	0.22
Body mass (kg)	77 ± 3	65 ± 2	**0.001**
Body mass index (kg/m^2)	27 ± 1	23 ± 1	**<0.01**
Body fat (%)	36.9 ± 1.7	23.4 ± 1.5	**<0.001**
Lean tissue mass (kg)	45.8 ± 2.0	47.3 ± 1.8	0.54
Godin physical activity score (U)	18 ± 4	64 ± 4	**<0.001**
VO_2max (mL/min/kg)	25.7 ± 1.0	42.8 ± 1.5	**<0.001**
Cardiopulmonary fitness percentile (%)	13 ± 4	93 ± 5	**<0.001**
Maximal heart rate (bpm)	168 ± 3	170 ± 2	0.96
Maximal respiratory exchange ratio	1.10 ± 0.01	1.08 ± 0.01	0.25

Values are means±SEMs. VO2max=maximal oxygen consumption.

Table 3.2: Cardiovascular parameters at rest

	Sedentary	**Endurance-trained**	***P-values***
Heart rate (bpm)	64 ± 2	52 ± 1	**<0.001**
Systolic BP (mmHg)	120 ± 2	118 ± 2	0.35
Mean BP (mmHg)	91 ± 2	88 ± 1	0.23
Diastolic BP (mmHg)	73 ± 1	70 ± 1	0.12
Pulse pressure (mmHg)	48 ± 1	48 ± 1	0.75
Carotid systolic BP (mmHg)	112 ± 2	113 ± 2	0.81
Carotid pulse pressure (mmHg)	40 ± 2	43 ± 2	0.30
Carotid lumen area (mm^2)	26.1 ± 1.1	23.2 ± 0.7	**0.04**
Carotid intima media thickness (mm)	0.59 ± 0.01	0.60 ± 0.02	0.59
Carotid IMT/lumen diameter	0.104 ± 0.004	0.113 ± 0.004	0.08
Carotid distension (%)	16.1 ± 0.7	20.8 ± 1.0	**<0.01**

Values are means±SEMs. BP=blood pressure and IMT=intima-media thickness.

Table 3.3: Pearson's product moment correlation coefficients and (P-values) illustrating the associations among cardiopulmonary fitness, cognitive function, and central arterial stiffness

	Cardiopulmonary fitness measures			Cognitive function scores			
	Godin PAS	VO_2max	Fitness %ile	Total cognitive composite	Global cognition	Memory	Attention-executive function
Godin PAS		0.69* (<0.001)	0.70* (<0.001)	0.13 (0.34)	-0.07 (0.64)	0.20 (0.14)	0.09 (0.54)
VO_2max			0.94* (<0.001)	0.26 (0.051)	0.22 (0.10)	0.19 (0.14)	0.17 (0.19)
Fitness %ile				0.40* (<0.01)	0.29* (0.03)	0.34* (<0.01)	0.26* (0.05)
cfPWV	-0.17 (0.23)	-0.30* (0.02)	-0.29* (0.03)	-0.14 (0.28)	0.01 (0.98)	-0.14 (0.30)	-0.15 (0.27)
Carotid distensibility	0.35* (0.01)	0.38* (<0.01)	0.32* (0.02)	0.32* (0.02)	0.22 (0.11)	0.27* (0.048)	0.23 (0.09)
Carotid β-index	-0.32* (0.02)	-0.34* (0.01)	-0.25 (0.07)	-0.28* (0.04)	-0.23 (0.10)	-0.19 (0.17)	-0.23 (0.09)
Carotid Einc	-0.33* (0.02)	-0.30* (0.03)	-0.25 (0.07)	-0.33* (0.01)	-0.17 (0.20)	-0.25 (0.06)	-0.30* (0.03)

PAS=physical activity score, VO_2max=maximal oxygen consumption, cfPWV=carotid-femoral pulse wave velocity, and Einc=Young's modulus.

Table 3.4: Regional cerebral perfusion measurements

	Sedentary control (n=20)	**Exercise-trained (n=17)**	***P-value***
Male/Female (n)	7/12	11/6	0.92
Age (years)	53 ± 1	51 ± 2	0.45
Education (years)	16 ± 1	18 ± 1	0.15
Height (cm)	171 ± 2	169 ± 2	0.36
Body mass (kg)	77 ± 3	67 ± 2	**0.04**
Body mass index (kg/m2)	26 ± 1	24 ± 1	0.11
VO_2max (mL/min/kg)	25.2 ± 1.1	42.2 ± 2.0	**<0.001**
Heart rate (bpm)	65 ± 2	52 ± 2	**<0.001**
Mean arterial pressure (mmHg)	92 ± 2	87 ± 2	0.09
Carotid pulse pressure (mmHg)	40 ± 2	43 ± 3	0.66
Carotid-femoral PWV (cm/sec)	855 ± 28	769 ± 30	0.07
Carotid distensibility ($\times 10^{-3}$/mmHg)	4.08 ± 0.22	5.29 ± 0.38	**0.01**
Carotid β-stiffness	5.76 ± 0.32	4.80 ± 0.29	**0.04**
Carotid young's elastic modulus (mmHg/cm)	902 ± 58	712 ± 61	**0.01**
Neuropsychological measures (z score)			
Total cognitive composite score	-0.23 ± 0.13	0.26 ± 0.10	**<0.01**
Global cognition	-0.23 ± 0.24	0.26 ± 0.11	0.27
Memory	-0.27 ± 0.19	0.32 ± 0.16	**0.02**
Attention-executive function	-0.19 ± 0.13	0.21 ± 0.15	0.11
Grey matter areas (mL/100g/min)			
Hippocampus	40.6 ± 5.8	38.3 ± 5.0	0.89
Caudate nucleus	62.4 ± 5.0	59.6 ± 6.7	0.71
Occipitoparietal area	29.6 ± 6.4	45.8 ± 6.5	**0.045**
White matter areas (mL/100g/min)			
Frontal	46.5 ± 7.0	48.1 ± 5.9	0.82
Centrum semiovale	46.9 ± 5.8	43.6 ± 5.7	0.94
Parietal	67.6 ± 5.4	63.1 ± 5.7	0.78

PWV=pulse wave velocity and VO2max=maximal oxygen consumption. Values are means±SEMs.

Figure 3.1: Neuropsychological assessments in sedentary and endurance-trained adults.

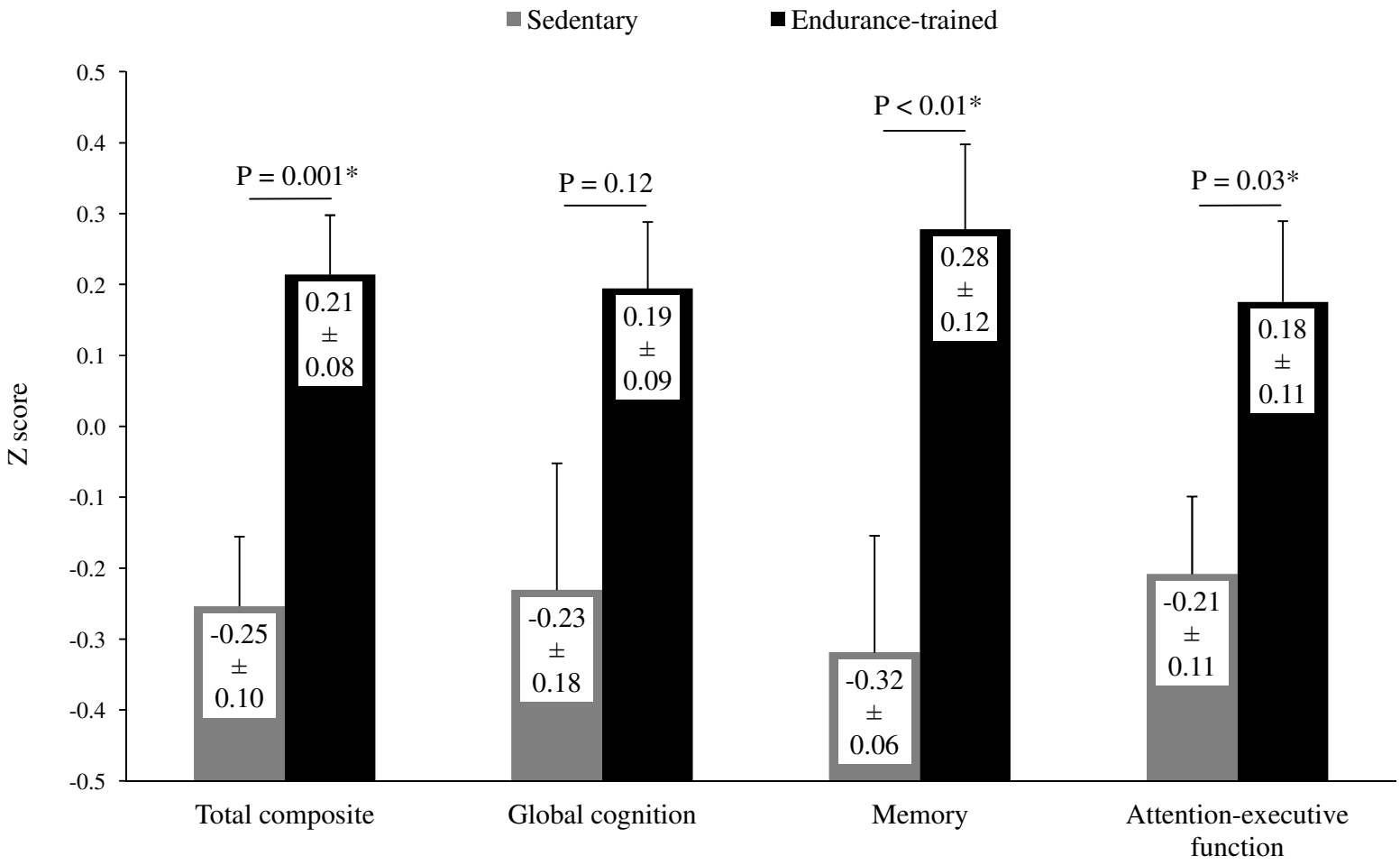


Figure 3.2: Central and peripheral pulse wave velocities.

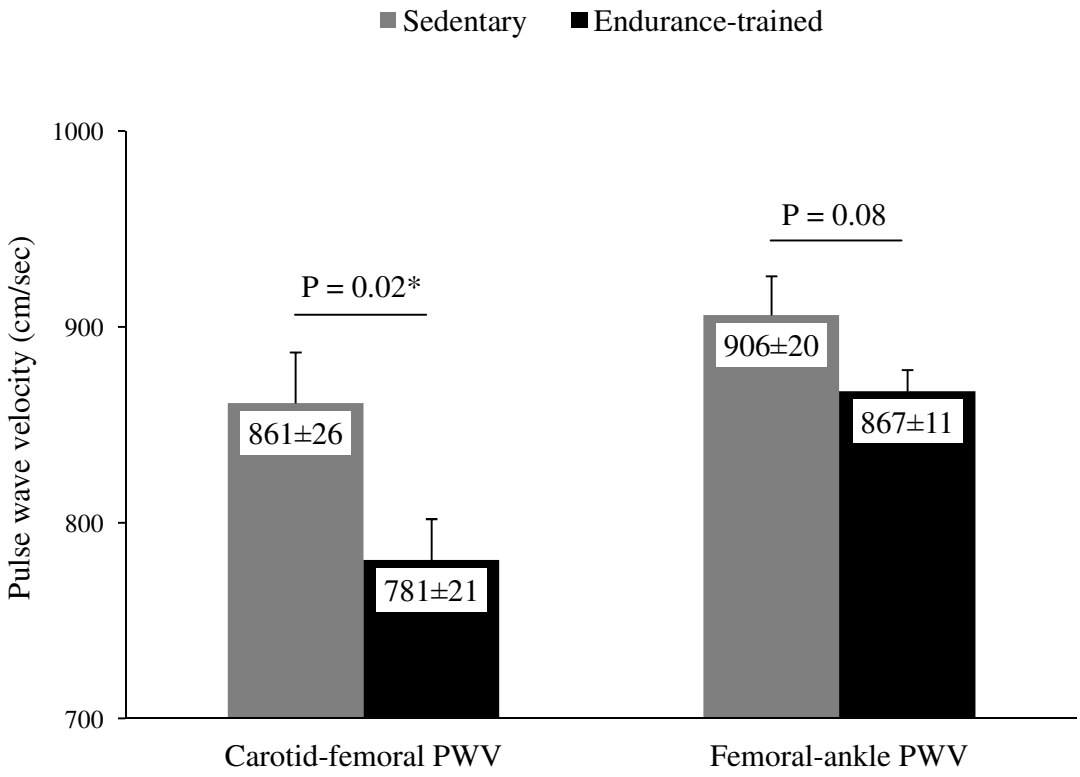
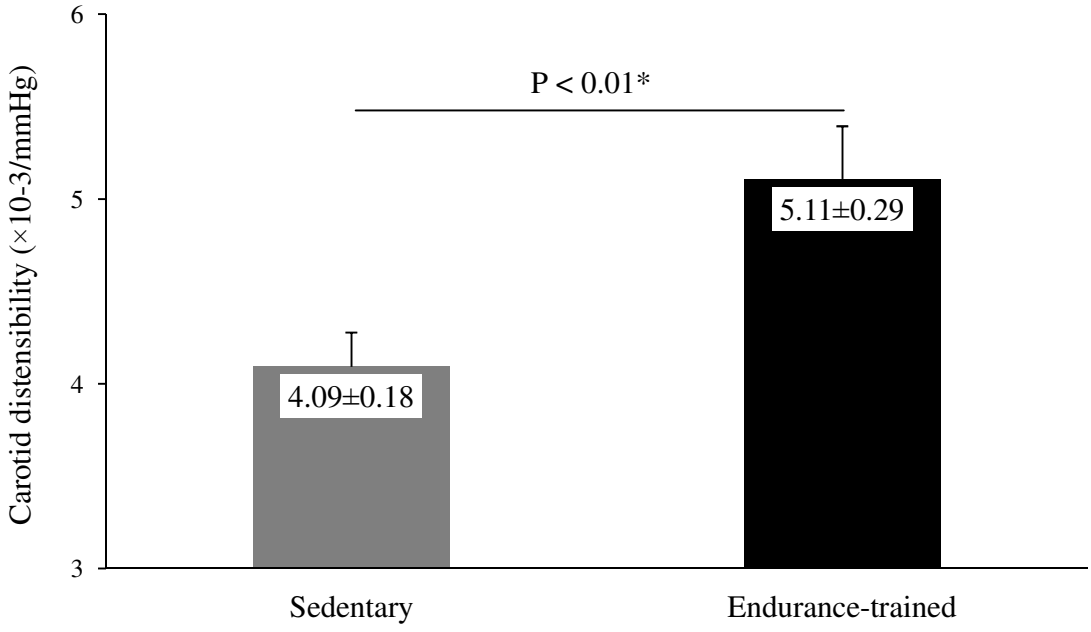
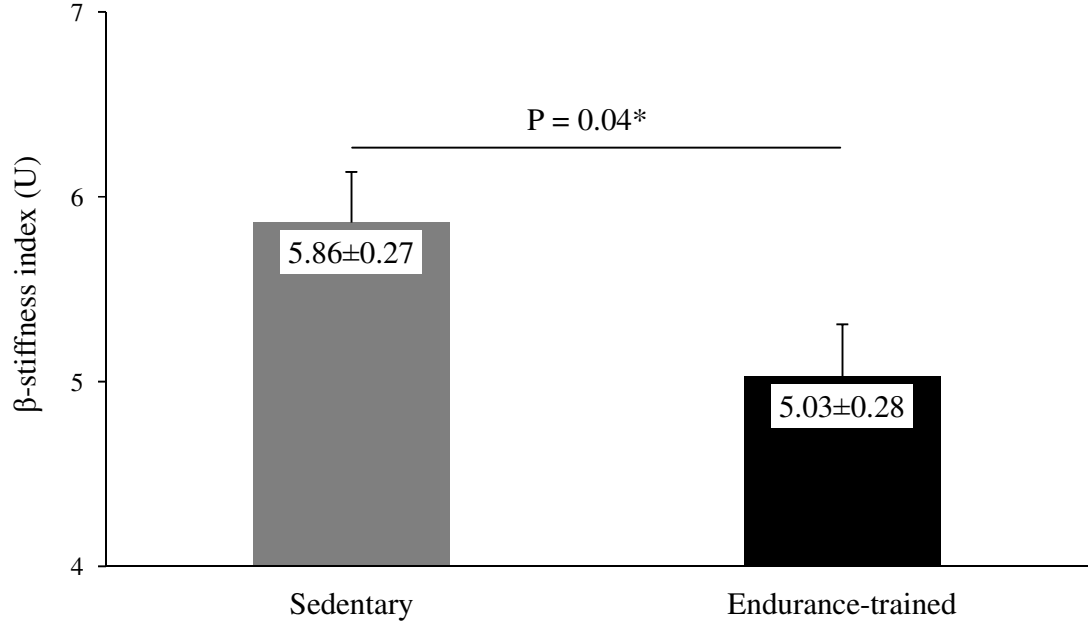


Figure 3.3: Carotid arterial stiffness measures.

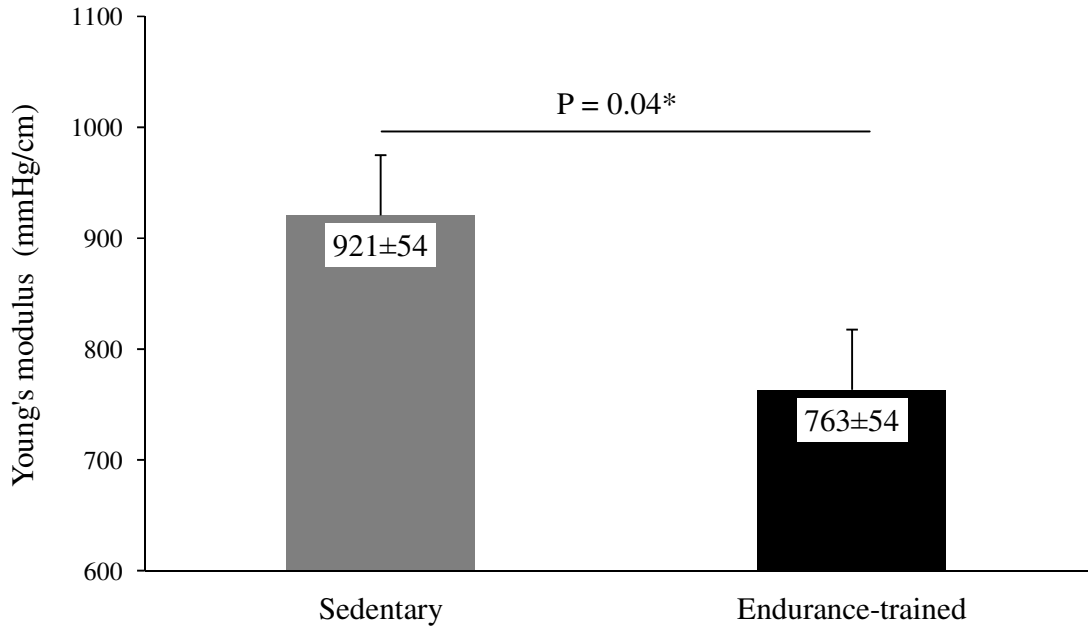
a) Distensibility coefficient



b) β-stiffness index



c) Young's modulus



Chapter 4: Cardiopulmonary Fitness and Cognitive Function: An Association Mediated by Plasma Insulin Concentration

ABSTRACT

Insulin resistance is associated with an increased risk of cognitive impairment. In contrast, regular aerobic exercise ameliorates insulin resistance and is increasingly recognized to improve cognitive function. **Purpose**: To determine the role of plasma insulin in mediating the relation between cardiopulmonary fitness and cognitive function. **Methods**: Fifty-eight middle-aged adults underwent the measurements of plasma insulin, cardiopulmonary fitness, and neuropsychological assessment. **Results**: Endurance-trained subjects demonstrated significantly lower plasma insulin concentrations and greater cardiopulmonary fitness and cognitive function than sedentary subjects. Greater maximal oxygen consumption was significantly related to higher memory performance (β=0.35) and lower plasma insulin level (β=-0.51). The significant association between maximal oxygen consumption and memory performance was abolished when the effect of plasma insulin was statistically removed (β=0.24, P=0.17). **Conclusion**: Plasma insulin has an indirect role in mediating the relationship between cardiopulmonary fitness and memory performance and potentially serves as a modifiable risk factor for cognitive impairment.

INTRODUCTION

An exponential increase in the prevalence of dementia is expected to impose a major health problem in the Western societies [29]. While the currently established risk factors for dementia (i.e., age and genetic predisposition) are not modifiable in nature, mounting evidence indicates that vascular disease risk is associated with accelerated brain aging and cognitive decline[6, 30]. Among the risk factors for vascular disease, insulin resistance and hyperinsulinaemia have been shown to exhibit the pathological phenotype resembling the Alzheimer's brain[85]. In contrast, habitual aerobic exercise is an effective strategy to ameliorate insulin resistance and increasingly recognized to improve cognitive function[26, 86]. However, it remains elusive whether the common association between regular aerobic exercise and greater cognitive function may be mediated by lower insulin resistance.

Accordingly, the primary aim of the present study was to determine the role of plasma insulin in mediating the association between cardiopulmonary fitness and cognitive function. Middle-aged men and women were specifically tested because the pathological alterations in the brain are thought to proceed years before the clinical onset of cognitive impairment and the associations among the measured variables could be negated by the effects of primary aging. The primary hypothesis was that endurance-trained middle-aged subjects would demonstrate lower plasma insulin level and greater cognitive function than sedentary subjects. We further hypothesized that plasma insulin concentration would indirectly mediate the relation between greater cardiopulmonary fitness and cognitive function.

METHODS

Subjects

Fifty-eight community-dwelling adults aged 43-65 years were recruited through flyers and newspaper advertisements posted in Austin, Texas. All subjects were nonsmoking, normotensive (<140/90 mm Hg), non-diabetic (fasting blood glucose<126 mg/dL), and free of overt vascular or neurological disease as assessed by medical health questionnaire, blood chemistry, and hematological evaluation. None of the subjects were taking cardiovascular-acting medications. Physical activity status was verified by maximal oxygen consumption. Endurance-trained subjects reported running, cycling, and/or swimming at a moderate to vigorous exercise intensity for 7.6±0.6 hours/week. Sedentary subjects reported engaging in exercise less than once per week for the past year. Neuropsychological assessment and blood collection and maximal oxygen consumption test were conducted on separate days within a one-month period. During the period of data collection, participants reported no major changes in their lifestyle, including dietary and exercise patterns. The Human Research Committee reviewed and approved all procedures, and written informed consent was obtained from all subjects.

Measurements

Cardiopulmonary fitness. Maximal oxygen consumption (VO_2max) was measured during a modified Bruce protocol. Because VO_2max is influenced by age and sex and potentially complicates the interpretation of one's cardiopulmonary fitness level, we additionally

reported the fitness percentile calculated based on age- and sex-adjusted regression established by the American College of Sports Medicine[41].

Blood sample collection and analysis. Subjects fasted for at least 12 hours and abstained from alcohol, caffeine, and exercise for at least 24 hours. Whole blood samples were drawn from the antecubital vein into EDTA plasma tubes and subsequently centrifuged at 3,500 rpm (Eppendorf 5702R, Westbury, NY) for 10 minutes at 4°C. Plasma aliquots were dispersed into microcentrifuge tubes and stored at -80°C for later analysis. Plasma insulin was measured in duplicate using commercially available RIA kit (MP Biomedicals, Orangeburn, NY). Homeostatic assessment of insulin resistance (HOMA-IR) was calculated using the equation described in detail elsewhere[87].

Neuropsychological assessment. Participants completed a comprehensive battery of neuropsychological assessments, including standard clinical neuropsychological instruments with established reliability and validity. In order to reduce the number of multiple comparisons, neuropsychological measures were grouped into one of three domains: global cognition, memory, or attention-executive function. For the domain scores, raw test scores were converted to z-scores based on the study sample's mean and standard deviation. Timed test scores were multiplied by -1 so that higher scores indicate better performance. Domain scores were calculated for each participant by averaging the z-scores within the domain as follows: 1) *global*: MMSE[45] and WTAR[46]; 2) *memory*: CVLT-II immediate recall, delayed recall, and recognition discrimination[47]; 3) *attention-executive function*: Trail making A and B time to completion[48], COWAT[49], and WAIS-III Digit Span Subtest[50]. In addition, total cognitive composite score was calculated by

averaging the z-scores of all individual tests. All tests were administered and scored by a trained research assistant using standard administration and scoring criteria.

Statistical analyses

The variable distributions were examined using the Shapiro-Wilk test of normality. Group differences in continuous variables were assessed using Mann-Whitney U test or independent t-test as appropriate. Chi-square test compared group differences in categorical variables. Analysis of covariance was used to examine group differences in the measured variables after controlling for the potential covariates. Linear regression analysis examined the association between VO_2max and cognitive function after controlling for age, sex, and education. The direct and indirect effects of VO_2max on cognitive function through plasma insulin were tested using both the traditional causal steps approach[88] and non-parametric bootstrapping procedures[89]. All statistical analyses were performed using SPSS 19 (SPSS inc., Chicago, IL). An α-level of 0.05 was set as the criterion for statistical significance.

RESULTS

Sedentary versus endurance-trained subjects

Endurance-trained subjects showed significantly lower body mass index and greater VO_2max and cardiopulmonary fitness percentile (Table 4.1) than sedentary subjects. While fasting blood glucose was not different between the groups, plasma insulin and HOMA-IR were significantly lower in endurance-trained subjects than in sedentary subjects. Endurance-trained subjects demonstrated significantly higher

cognitive performance in total cognitive composite, memory, and attention-executive function than sedentary subjects. The group differences in cognitive function disappeared after statistically controlling for the effect of plasma insulin (P>0.05).

Associations between VO_2max and cognitive function

Greater VO_2max was significantly related to higher scores in total cognitive composite (β=0.38, P<0.01) and memory (β=0.34, P=0.01) independent of age, sex, and education, but not global cognition (β=0.24, P=0.09) and attention-executive function (β=0.23, P=0.09). Based on these results, a mediation model was constructed including memory as a dependent variable.

Mediation analysis

We examined the potential role of plasma insulin in mediating the relation between VO_2max and memory by testing each path illustrated in Figure 4.1. As described above, VO_2max had a significant association with memory performance (path c: β=0.34, P=0.01), independent of age, sex, and education. The concentration of plasma insulin was significantly related to both VO_2max (path a: β=0.-0.51, P<0.001) and memory performance (path b: β=0.-0.38, P<0.01). Finally, the significant association between VO_2max and memory performance was abolished when plasma insulin was entered into the model (path c': β=0.24, P=0.17), indicating successful mediation. This result was further confirmed by the non-parametric bootstrapping procedure, which demonstrated significant indirect effects of plasma insulin on memory performance (95% CI: 0.0044 - 0.0274).

DISCUSSION

The primary findings from the present study were as follows. First, endurance-trained subjects demonstrated greater cognitive performance in total composite, memory, and attention-executive function and lower concentrations of plasma insulin than sedentary subjects. Second, greater VO_2max was associated with higher cognitive performance in total cognitive composite and memory and lower plasma insulin level. Third, the association between VO_2max and memory performance was significantly attenuated when the effect of plasma insulin was statistically removed, indicating the indirect role of plasma insulin in mediating this association. To the best of our knowledge, this is the first study to demonstrate that plasma insulin may be involved in mediating the association between cardiopulmonary fitness and cognitive function in middle-aged healthy adults.

There are at least two physiological events that support an indirect role of plasma insulin in mediating the association between cardiopulmonary fitness and cognitive function. First, regular aerobic exercise increases the rate of glucose uptake from skeletal muscle and improves insulin sensitivity[26]. Skeletal muscle is the major site of insulin-mediated glucose disposal, and its adaptations to chronic aerobic exercise would enhance the disposal process. Indeed, habitual aerobic exercise promotes angiogenesis that enhances blood flow and delivery of glucose to skeletal muscle for disposal, upregulates the synthesis of insulin-dependent glucose transporter proteins (i.e., GLUT-4), and increases the activity of mitochondrial and oxidative enzymes[90, 91]. Second, insulin resistance is associated with the pathological hallmarks of neurodegenerative dementia,

including the reduction in cerebral glucose metabolism and the accumulation of cerebral amyloid-β (Aβ). A recent study demonstrated that the extent of insulin resistance in diabetic patients is inversely associated with metabolic rate of cerebral glucose utilization within similar regions where Alzheimer's disease patients expresses a reduced metabolic rate[21]. Moreover, hyperinsulinaemia compromises the ability of insulin-degrading enzyme (IDE) to remove cerebral Aβ from the extracellular space[92]. IDE is highly expressed in the brain and degrades substrates including insulin, insulin-like growth factors, and amylin, Aβ[92]. Hyperinsulinaemia increases the level of Aβ because elevated insulin competes with Aβ for IDE.

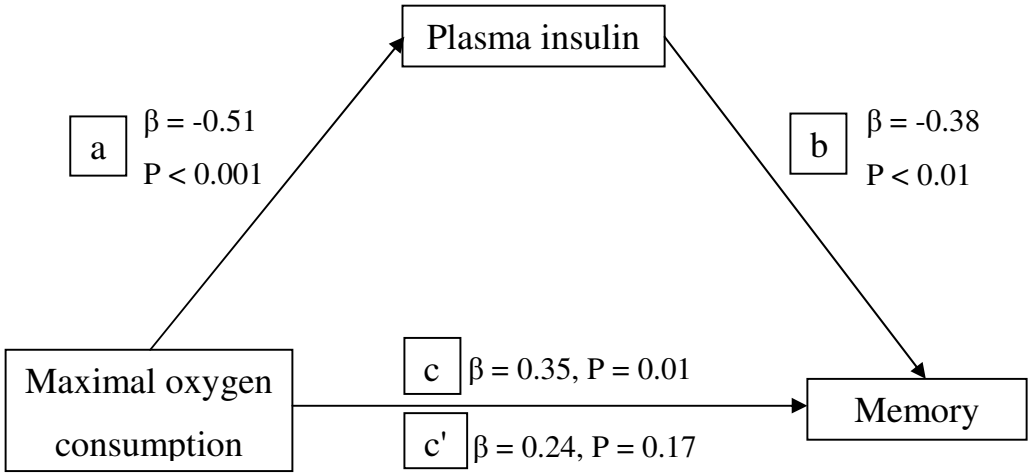
In conclusion, the present study demonstrated that plasma insulin mediates at least indirectly the association between greater cardiopulmonary fitness and memory performance. Future studies should determine whether the exercise-related reduction in plasma insulin increases cerebral metabolic rate of glucose utilization and decreases cerebral Aβ level. Moreover, the results from the present study necessitate further investigation using a prospective interventional study design with a larger sample size in order to establish a causal relation.

Table 4.1: Selected subject characteristics

	Sedentary	**Endurance-trained**	***P-values***
Males/Females (n)	10/16	11/21	0.75
Age (years)	53 ± 1	52 ± 1	0.50
Education (years)	16 ± 1	17 ± 1	0.25
Body mass index (kg/m2)	27 ± 1	23 ± 1	**<0.001**
VO_2max (mL/min/kg)	25.7 ± 1.1	42.8 ± 1.5	**<0.001**
Cardiopulmonary fitness percentile (%)	12 ± 4	93 ± 5	**<0.001**
Glucose (mg/dl)	91 ± 2	93 ± 2	0.51
Insulin (ulU/mL)	21.3 ± 1.5	12.6 ± 0.6	**<0.001**
HOMA-IR	4.85 ± 0.40	2.87 ± 0.14	**<0.001**
Neuropsychological assessment			
Total cognitive composite score (z score)	-0.26 ± 0.10	0.21 ± 0.08	**0.001**
Global cognition (z score)	-0.21 ± 0.19	0.17 ± 0.10	0.21
MMSE	28.6 ± 0.3	29.0 ± 0.2	0.26
WTAR	41.6 ± 1.7	44.5 ± 0.8	0.33
Memory (z score)	-0.35 ± 0.17	0.29 ± 0.12	**<0.01**
CVLT-II immediate recall	10.3 ± 0.6	12.4 ± 0.4	**<0.01**
CVLT-II delayed recall	10.5 ± 0.6	12.7 ± 0.4	**<0.01**
CVLT-II discriminability index	2.7 ± 0.2	3.5 ± 0.4	0.09
Attention-executive function (z score)	-0.21 ± 0.11	0.17 ± 0.11	**0.02**
Trail Making Test A	32.2 ± 1.8	28.6 ± 1.4	0.11
Trail Making Test B	70.8 ± 4.2	58.2 ± 3.4	**0.02**
COWAT	43.0 ± 1.7	46.7 ± 2.6	0.34
WAIS-III Digit Span Subtest	18.1 ± 0.7	18.9 ± 0.7	0.40

Values are means±SEMs. BP=blood pressure, HDL=high density lipoprotein, HOMA-IR=homeostatic assessment model of insulin resistance, LDL=low density lipoprotein, and VO2max=maximal oxygen consumption.

Figure 4.1: A model illustrating that plasma insulin indirectly mediates the relation between maximal oxygen consumption and memory performance.



Chapter 5: Effects of Aerobic Exercise Training Intervention on Vascular and Cognitive Functions in Healthy Middle-Aged Adults

ABSTRACT

Mounting evidence suggests that vascular disease risk in midlife is associated with accelerated brain aging and cognitive decline. One potential mechanism underlying such association is cerebral hypoperfusion which attributes to impaired vascular function. Habitual aerobic exercise is an efficacious lifestyle strategy that improves vascular function and its effect may translate into cognitive function. **Purpose**: To determine the effects of aerobic exercise training on vascular and cognitive functions. **Methods**: The key features of vascular structure and function, including central arterial stiffness and cerebral and peripheral vascular reactivity, and neuropsychological assessment were measured before and after 3 months of moderate intensity aerobic exercise training. Eighteen middle-aged adults completed the aerobic exercise training program, and 10 subjects in a control group completed the relaxation exercise program designed to match the exercise-training program for time and subject attention. **Results**: After aerobic exercise training, central systolic blood pressure decreased and carotid distensibility coefficient and brachial flow-mediated vasodilation improved (all $P<0.05$) while cerebral CO_2 reactivity remained unaltered ($P>0.05$). Although the cognitive function scores remained unchanged after aerobic exercise training (all $P>0.05$), changes in memory score were associated with the corresponding changes in carotid systolic blood pressure after aerobic exercise training ($r=-0.49$, $P<0.05$). **Conclusions**: The 3-month moderate

intensity aerobic exercise training program produced the improvements in peripheral vascular functions while cerebral reactivity and cognitive function remained unchanged. A significant association between the exercise-related improvement in memory score and the reduction in central systolic blood pressure suggest that exercise training may improve cognitive function at least in part through its effect on the vasculature.

INTRODUCTION

An exponential increase in the prevalence of dementia is expected to impose a major health problem in the Western societies[29]. While the currently established risk factors for dementia (i.e., age and genetic predisposition) are not modifiable in nature, mounting evidence indicates that vascular disease risk in midlife is associated with accelerated brain aging and cognitive decline[6, 30]. One hypothetical mechanism underlying such association is cerebral hypoperfusion which attributes to impaired vascular function[31]. Because the brain heavily relies on a continuous supply of cerebral blood flow, any extent of cerebral hypoperfusion that causes a mismatch between the energy demand and supply may trigger a pathological cascade leading to cognitive decline and impairment[8]. Indeed, cerebral hypoperfusion is associated with impaired protein synthesis and generation of action potential, excitotoxicity caused from glutamate, and accumulation of cerebral amyloid, which are all related to the pathological features of dementia[20, 51, 52].

Vascular disease risk is modifiable through lifestyle changes and potentially provides an effective strategy to reduce the risk and future prevalence of dementia. In particular, regular aerobic exercise has widely been recommended to lower risks of vascular disease and improve vascular function including central arterial stiffness and endothelium-dependent vasodilatory function[24]. Physiologically, central arterial stiffness is associated with reduced cerebral perfusion and higher prevalence of subcortical infarct[93, 94]. Endothelium-dependent vasodilation or vascular endothelial function plays an important role to maintain the normal cerebrovascular tone at rest and during neuronal

activation, protect cerebral arteries from atherogenesis, and sustain the integrity of blood-brain barrier[95, 96].

Previous investigations which examined the biological benefits of regular aerobic exercise on cognitive function in humans primarily focused on the effect mediated through neurotrophic factor (e.g., brain-derived neurotrophic factor)[97] despite physiological evidence demonstrating that cerebral perfusion and vascular function also play an important role for the normal cognitive function[20]. Accordingly, the primary purpose of the present study was to determine whether exercise-related improvement in vascular function is associated with cognitive function. We specifically recruited middle-aged men and women because the pathological alterations in the brain is thought to precede many years before the clinical onset of cognitive impairment and the potential benefits of aerobic exercise training on vascular and cognitive functions could be negated by the effects of primary aging. In order to obtain comprehensive information about vascular health, arterial structure and function were noninvasively measured from middle cerebral, common carotid, and brachial arteries. We hypothesized that 3 months of moderate-intensity aerobic exercise training will reduce central arterial stiffness, augment vascular reactivity of cerebral and brachial arteries, and improve cognitive function.

METHODS

Subjects

Healthy sedentary subjects aged 45-65 years were tested before and after 3 months of aerobic exercise training. All subjects were free of overt cardiovascular,

cerebrovascular, and neurological diseases as assessed by medical history, physical examination, and complete blood chemistry and hematological evaluations. Before exercise training, all subjects were evaluated by ECG and blood pressure during rest and incremental treadmill exercise performed under the supervision of a physician. Candidates who were taking cardiovascular-acting medications were excluded from participation. No subjects had performed regular exercise during the preceding year. All subjects gave their written informed consent to participate. All procedures were reviewed and approved by the Human Research Committee of the University of Texas at Austin.

After baseline measurements, subjects were randomly assigned to aerobic exercise training or attention and time-control (i.e., relaxation and stretching exercise) group. During the course of this investigation, subjects in the 2 groups were instructed to maintain their usual lifestyle and dietary habits, aside from their prescribed experimental training regimes.

Exercise Intervention

After a week of supervised orientation, subjects in the exercise intervention group underwent 3 months of home-based aerobic exercise training program (i.e., walking or jogging). Subjects were asked to exercise at least three times a week, and each exercise session consisted of a 10-minute warm up, a ≥30-munite exercise at target heart rate, and a 10-minute cool down. During weeks 1-3 of the exercise training program, the target heart rate was set to ~50% of their individually determined heart rate reserve (HRR). As their exercise tolerance improved, the target heart rate was increased to 50-60% HRR during weeks 4-6 and 60-70% HRR after the sixth week of the exercise training program.

HRR was calculated as: resting heart rate + [percent of the difference between maximal heart rate and resting heart rate]. All subjects were provided with a heart rate monitor, provided continuous feedback of their exercising heart rate, and recorded the heart rate data during each session. Adherence to the exercise prescription was documented through the use of heart rate monitors (Polar Heart Rate Monitor) and physical activity logs that were returned to the laboratory biweekly[98].

Subjects in the attention control group underwent a week of supervised orientation and thereafter performed a mixture of relaxation and stretching exercises at home using a DVD instruction. Adherence to the relaxation and stretching exercise program was documented using an activity log that was returned to the laboratory every 4 weeks.

Measurements

Subjects abstained from alcohol, caffeine, and exercise for at least 24 hours and fasted for at least 4 hours (a 12-hour overnight fast was used for determination of metabolic risk factors) before vascular function measurements. Neuropsychological assessment was conducted on a separate day. Subjects were instructed to continue intervention program until the completion of both vascular function measurements and neuropsychological assessment. In premenopausal women, vascular function measurements were collected during the early follicular phase of the menstrual cycle.

Cardiopulmonary fitness and body composition. Maximal oxygen consumption (VO_2max) was measured during a modified Bruce protocol. After a 5-minute warm-up, subjects walked or ran while the treadmill slope was gradually increased 2% every 2

minutes until volitional exhaustion. Body composition was measured by dual-energy X-ray absorptiometry (Lunar DPX, General Electric Medical Systems, Fairfield, CT).

Central elastic arterial stiffness. Carotid arterial distensibility was non-invasively measured by the simultaneous recordings of the diameter and pulse pressure waveforms[64]. Common carotid arterial diameter was measured from the B-mode images on an ultrasound machine equipped with a high-resolution linear-array transducer (Phillips iE33 Ultrasound System, Bothel, WA). A longitudinal image of the cephalic portion of common carotid artery was acquired 1-2 cm proximal to the carotid bulb and optimized for diameter detection. All images were analyzed on an offline computer using automated image analysis software (Carotid Analyzer, Medical Imaging Applications, Coralville, IA). Carotid pulse pressure waveform was simultaneously obtained using an applanation tonometry incorporating an array of 15 micropiezoresistive transducers (VP-2000, Omron Healthcare Bannockburn, Illinois) placed on the contralateral side. Simultaneous measurements were made for 30 seconds (or at least 20 cardiac cycles). To correct for the hold-down pressure of applanation tonometry, carotid mean and diastolic blood pressures were calibrated to oscillometrically determined brachial mean and diastolic blood pressures (VP-2000, Omron Healthcare Bannockburn, Illinois) as previously described[64]. Subsequently, the distensibility coefficient of common carotid artery was calculated using the equation described in detail elsewhere[65, 66].

Brachial flow-mediated dilatation (FMD). Endothelium-dependent vasodilatory function was assessed by brachial FMD using a noninvasive, standardized procedure[42]. The left (non-dominant) arm was extended and placed in a customized arm support system to

prevent movement of the arm and to standardize the position of an ultrasound transducer. Brachial arterial diameter and blood flow velocity were measured from images derived from a Doppler ultrasound machine equipped with a high-resolution linear array transducer (Philips iE33 Ultrasound System, Bothel, WA). Once the subject was resting in a comfortable position, the pneumatic arm cuff was placed on the forearm, 3-5 cm distal to the antecubital fossa and connected to a rapid cuff inflator (E20 Rapid Cuff Inflator, D.E. Hokanson; Bellevue, WA). Once a longitudinal image of the brachial artery, 5-10 cm proximal to the antecubital fossa was obtained, the transducer was stabilized in secure position. One minute each of baseline brachial artery diameter and blood flow velocity were recorded prior to cuff inflation. The arm cuff was then inflated to 100 mmHg above resting systolic blood pressure (measured prior to baseline image capture) for 5 minutes. Blood flow velocity was recorded10 seconds prior to cuff deflation and continued until 20 seconds after cuff deflation. Then, the ultrasound was switched to 2D mode to optimize the image for brachial artery diameter measurements for the next 160 seconds. The image files were transferred to an offline computer and stored for later data analysis. Brachial arterial diameter during end-diastole, as determined from the ECG trace, was measured by the same investigator using automated image analysis software (Brachial Analyzer, Medical Imaging Applications; Coralville, IA). FMD was calculated using the following equation: [(peak diameter - baseline diameter)/baseline diameter] × 100. The baseline end-diastolic diameter was calculated by taking the average of at least 20 cardiac cycles before cuff inflation. Peak end-diastolic diameter was taken from the

average of 3 consecutive cardiac cycles demonstrating the largest brachial artery dilation after cuff deflation.

Cerebral CO_2 reactivity. Blood flow velocity (BFV) of middle cerebral artery (MCA) was measured by transcranial color-coded duplex ultrasonography (iE 33 Ultrasound System, Philips, Bothell, WA) during hypocapnic , normocapnic, and hypercapnic steady states. MCA was insonated from the left posterior temporal window using a 1.6 MHz transcranial Doppler probe which was mounted on a custom-made probe fixation device attached to a commercially available headgear (Dia Mon, DWL Compumedics, Charlotte, NC)[43]. Subjects wore a nose clip and breathed only through a mouthpiece with a Y-way valve (Hans-Rudolph, Shawnee, KS), one end connected to a 5-liter air reservoir containing a mixture of 5 % CO_2 and 21 % O_2 balanced with nitrogen and another end open to room air. End-tidal CO_2, an estimate of arterial CO_2 tension, was measured from expired air and analyzed by a capnograph (Capnocheck Plus, Smiths Medical, Waukesha, WI). Non-invasive beat-by-beat blood pressure was recorded by Portapres (Finapres Medical, Amsterdam, Netherlands) throughout the protocol.

After at least15 minutes of rest in the supine position, three minutes of baseline recording was made during spontaneous breathing of room air. Next, subjects underwent 1 minute of maximal voluntary hyperventilation with a duty cycle of 1 second. This short period of hyperventilation was intended to reduce end-tidal CO_2 level to ~25 mmHg but not to cause respiratory muscle fatigue or central hypoxia possibly associated with a prolonged hyperventilation. The MCA-BFV was recorded during the last 20 seconds of hyperventilation. From the pilot study which was conducted prior to data collection, 30-

40 seconds of maximal hyperventilation effectively decreased end-tidal CO_2 to the near minimal level (~25 mmHg). After the MCA-BFV returned to the baseline following hyperventilation, a respiratory valve was switched to an air reservoir containing 5% CO_2 and 21% O_2 and the subjects were asked to breathe spontaneously for 3 minutes. The air reservoir was continuously filled from a cylinder whose air pressure was manually adjusted to subject's respiratory volume. The MCA-BFV was recorded during the last minute of hypercapnia.

The MCA-BFV waveform was manually traced by a single investigator who was blinded to subject characteristics and study design. Time-averaged peak velocity (i.e., area under curve of BFV waveform) was recorded from at least 10 cardiac cycles in normocapnic, hypocapnic, and hypercapnic steady states. Because transcranial Doppler does not measure blood flow per se, cerebral CO_2 reactivity index (CVRi) was calculated as a percent change in MCA-BFV over an absolute change in end-tidal CO_2[43]. The percent change in MCA-BFV has been reported to have a strong correlation with an absolute change in cerebral blood flow measured by intravenous Xenon dilution technique[44]. The CVRi was calculated from the three different ranges of end-tidal CO_2 levels: normocapnia to hypocapnia (NORM-HYPO), normocapnia to hypercapnia (NORM-HYPER), and hypocapnia to hypercapnia (HYPO-HYPER). The CVRi (HYPO-HYPER) was intended to examine cerebrovascular responsiveness to a wider range of end-tidal CO_2 fluctuation and to eliminate the potential effects of baseline neuronal activity and MCA-BFV on CVRi. In addition to CVRi, cerebrovascular conductance

index (CVCi) was calculated in order to account for the effect of blood pressure on MCA-BFV.

Neuropsychological assessment. Participants completed a comprehensive battery of neuropsychological assessment, including standard clinical neuropsychological instruments with established reliability and validity. In order to reduce the number of multiple comparisons, neuropsychological measures were grouped into one of three domains: global cognition, memory, or attention-executive function. For the domain scores, raw test scores were converted to z-scores based on the study sample's mean and standard deviation. Timed test scores were multiplied by -1 so that higher scores indicate better performance. Domain scores were calculated for each participant by averaging the z-scores within the domain as follows: 1) *global*: MMSE[45] and WTAR[46]; 2) *memory*: CVLT-II immediate recall, delayed recall, and recognition discrimination[47]; 3) *attention-executive function*: Trail making A and B time to completion[48], COWAT[49], and WAIS-III Digit Span Subtest[50]. In addition, total cognitive composite score was calculated by averaging the z-scores of all the individual tests. Variable distributions were examined using the Shapiro-Wilk test of normality. All tests were administered and scored by a trained research assistant using standard administration and scoring criteria.

Statistical analyses

Data were analyzed using analysis of variance with repeated measures. For a significant F value, least significant difference post hoc analysis was used to determine mean differences. Pearson's product moment correlation analysis was performed to determine the associations between variables of interest. All statistical analyses were

performed using SPSS 19 (SPSS inc., Chicago, IL). An α-level of 0.05 was set as the criterion for statistical significance.

RESULTS

Eighteen middle-aged adults completed the aerobic exercise training program and 10 subjects in control group completed the relaxation exercise program. Subjects in the aerobic exercise training program exercised for an average of 13.0±0.5 weeks, 3.2±0.2 days/week, and 35±2 min/session at 59±2% HRR. Before the intervention period (at baseline), there were no significant differences in any physical and physiological variables between the aerobic exercise intervention and attention control groups (Table 5.1).

After the intervention period, body fat percentage, total and LDL-cholesterols, and fasting blood glucose decreased in the aerobic exercise training group. Although there was no change in VO_2max after aerobic exercise training, heart rate at the same absolute submaximal intensity of exercise (~70% of baseline maximal oxygen consumption) decreased significantly after exercise training (Table 5.1). Aerobic exercise training significantly decreased heart rate, brachial systolic and mean blood pressures, and carotid systolic blood pressure at rest (Table 5.2). Among the measures of arterial structure and function, carotid distensibility coefficient (Figure 5.1) and brachial FMD (Figure 5.2) increased significantly after aerobic exercise training whereas the indices of cerebral CO_2 reactivity remained unchanged (Table 5.3).

Table 5.4 exhibits the results from neuropsychological assessment measured before and after the interventions. Aerobic exercise training did not produce any changes in cognitive function score when measured globally between the exercise and control groups. However, individual changes in memory score after aerobic exercise training correlated with the corresponding change in carotid systolic blood pressures ($r = -0.49$, $P<0.05$).

DISCUSSION

Mounting evidence indicates that vascular dysfunction and risk factors for vascular disease in midlife are associated with accelerated brain aging and cognitive decline[6, 30],while regular aerobic exercise improves vascular function[24] and whose effects may translate into cognitive function. The primary findings from the present study were as follows. First, a 3-month aerobic exercise training reduced carotid arterial stiffness and increased brachial endothelium-dependent vasodilation but did not alter cerebral CO_2 reactivity. Second, none of the cognitive function scores measured from total cognitive composite, global cognition, memory, and attention-executive function improved after aerobic exercise training. Third, the reduction in central systolic blood pressure after aerobic exercise training was correlated with the improvement in memory. The results from the present study were consistent with our hypothesis regarding the benefits of regular aerobic exercise on central arterial stiffness and endothelium-dependent vasodilation. However, we did not observe improvements in cerebral CO_2 reactivity and cognitive function. Unlike our previous cross-sectional studies, the present interventional

study did not support the influence of vascular function in mediating the association between cardiovascular fitness and cognitive function.

Effect of regular aerobic exercise on vascular function

Central arterial stiffness and impaired endothelium-dependent vasodilation and cerebrovascular responsiveness to CO_2 are associated with the greater risks of both vascular disease[24, 99] and dementia[100, 101]. Physiologically, the elastic property of cardiothoracic arteries buffers arterial blood pressure generated from the heart, creates a continuous blood flow in the peripheral microcirculation, and protects the delicate capillary system from end-organ damage.[9, 10] The brain is a high flow, low impedance organ which comprises the vast network of capillary system and highly susceptible for microcirculatory barotrauma. Indeed, an increasing number of studies report a strong association between central arterial stiffness and cerebral leukoaraiosis or white matter hyperintensity on T2 weighted MRI images[11, 94, 102, 103]. In addition, normal vascular endothelial function and endothelium-dependent vasodilation maintain proper cerebrovascular tone during rest and functional hyperemia and inhibit atherogenesis[95, 96]. Indeed, endothelial dysfunction and atherosclerosis are prominent features of the Alzheimer's disease patients[104, 105]. Moreover, cerebral CO_2 reactivity is associated with the risk of ischemic stroke and impaired in the patients with Alzheimer's disease[100, 106]. Physiologically, cerebral vasodilation elicited by hypercapnia occurs in resistance arterioles of the similar size where functional hyperemia takes place, suggesting the potential similarities in the mechanism between these vasodilatory responses[33].

The beneficial effects of regular aerobic exercise on central arterial stiffness and vascular endothelial function have been demonstrated in middle-aged and older adults whose basic physical characteristics are similar to our present subjects[64, 107]. Moreover, regular aerobic exercise has recently been demonstrated to improve cerebral CO_2 reactivity in the ischemic stroke patients[108]. Therefore, our findings from the present study showing the improvements in central arterial stiffness and endothelium-dependent vasodilation after aerobic exercise training are consistent with the previous studies. However, we did not observe improvement in cerebral CO_2 reactivity after aerobic exercise training. Such discrepancy between the present and previous studies may be attributing to the selection of different subject population and the duration of aerobic exercise training program. In contrast to the present study which included only healthy participants who do not exhibit any overt vascular disease and prescribed a 3-month aerobic exercise training, Ivey et al. tested stroke survivors with residual mild to moderate hemiparetic gait and prescribed a 6-month of aerobic exercise training[108]. Therefore, normal healthy subjects in the present study may possibly have posed a ceiling effect on the improvement in cerebral CO_2 reactivity. Alternatively, exercise-related adaptation in cerebrovascular system may require a longer period of training than that in peripheral vascular system.

Effect of regular aerobic exercise on cognitive function

There is an emerging body of evidence suggesting that regular physical activity improves cognitive function in both healthy adults and the patients with mild cognitive impairment who are at risk for Alzheimer's disease. A meta-analysis has concluded that

exercise training increases the major domains of cognitive performance, regardless of the type of cognitive task, the exercise training program, or participants' characteristic[86]. In particular, executive-control function showed the greatest benefit from increased physical activity[86]. Regarding exercise training regimen, a combination of strength and aerobic training elicited a greater improvement in cognition than aerobic training alone. A participation in relatively short-term training programs provided at least as much benefit as moderate-length training, but not quite as much as a long-term training program. Moreover, participants in the younger-old age category (66-70 years old) benefited more than the middle-aged (55-65 years old) and older (71+ years old) groups[86].

The discrepancy in the results from the present and previous studies can be attributed to the three primary issues. First, the subjects in the present study demonstrated the normal cognitive function and risk factors for vascular disease at baseline. Although our vascular function measurements demonstrated the positive changes after aerobic exercise training, the normal baseline level of cognitive function may have limited subject's capacity for improvement. Second, the duration and intensity of our exercise training program could have been inadequate to introduce the exercise-related adaptations in cerebrovascular and cognitive functions. Previous interventional studies which demonstrated the beneficial effects of exercise training on cognitive function employed a 6-12 months of exercise training[39, 97, 109]. Moreover, a greater intensity of aerobic exercise training such as high intensity interval training is well recognized to increase cardiopulmonary fitness that is associated with a better cognitive function[110]. Third, the

concurrent observations showing the lack of improvements in both cerebrovascular and cognitive functions indicate the potential link between these two measurements[33].

The potential mechanism underlying the association between reduced central blood pressure and improved memory score

Central systolic blood pressure generated from the left ventricle is buffered by the elastic property of cardiothoracic arteries, thereby protecting the delicate microcirculation from the repeated barotrauma[9, 10]. However, stiffening of the compliant cardiothoracic arteries (e.g., common carotid artery) increases left ventricular afterload and allows the penetration of damaging pulsatile pressure into the cerebral capillaries[111]. The brain is a high flow, low impedance organ, composed of delicate nervous tissue, and highly susceptible for end-organ damage[9, 10, 53]. Indeed, central arterial stiffness and pulse pressures are associated with greater risks of cerebral leukoaraiosis, subcortical infarct, stroke, and cognitive impairment[11, 94, 112].

Regular aerobic exercise decreases central arterial stiffness and aortic impedance, which in turn leads to the reductions in central systolic blood pressure[24]. Therefore, the observed association between reduced central systolic blood pressure and improved memory function provides us with a further support for the importance of central blood pressure on cognitive function.

In conclusion, the primary findings from the present study include the improvements in central arterial stiffness and brachial endothelium-dependent vasodilation after a 3-month moderate intensity aerobic exercise training. In contrast to our hypothesis, we did not observe improvements in cerebral CO_2 reactivity and

cognitive function after the exercise training. Unlike our previous cross-sectional study, a role of vascular function in mediating the association between regular exercise and cognitive function was not supported by the present exercise intervention study. However, an association between reduced central systolic blood pressure and improved memory function with aerobic exercise training suggests at least some role of central arterial stiffness as a potential determinant of cognitive function.

Table 5.1: Basic subject characteristics

	Attention control group		Exercise-training group	
	Before	**After**	**Before**	**After**
Males/Females	2/8		5/13	
Age (years)	56 ± 2		53 ± 1	
Ethnicity (%)				
Caucasian	60		76	
African-American	10		6	
Hispanic	10		6	
Other	20		12	
Education (years)	16 ± 1		16 ± 1	
Height (cm)	167 ± 4		169 ± 2	
Body mass (kg)	74 ± 5	74 ± 6	78 ± 4	77 ± 4
Body mass index (kg/m^2)	26 ± 2	26 ± 2	27 ± 1	27 ± 1
Body fat (%)	37.4 ± 2.7	37.6 ± 2.7	38.1 ± 2.0	36.6 ± 1.7*
Lean tissue mass (kg)	42.6 ± 2.9	42.6 ± 3.0	44.7 ± 2.3	45.6 ± 2.3
VO2max (mL/min/kg)	26.6 ± 2.1	26.5 ± 2.3	26.3 ± 1.2	28.3 ± 1.2
Maximal heart rate (bpm)	173 ± 4	172 ± 6	168 ± 4	169 ± 3
Maximal respiratory exchange ratio	1.10 ± 0.02	1.06 ± 0.03	1.09 ± 0.02	1.08 ± 0.03
Heart rate at 70%VO_2max (bpm)	128 ± 4	132 ± 4	130 ± 3	124 ± 3*
Total cholesterol (mg/dl)	211 ± 8	216 ± 10	198 ± 9	179 ± 8*
HDL-cholesterol (mg/dl)	60 ± 8	61 ± 7	61 ± 4	56 ± 5
LDL-cholesterol (mg/dl)	125 ± 12	135 ± 10	125 ± 8	112 ± 6*
Glucose (mg/dl)	90 ± 4	99 ± 4*	94 ± 4	87 ± 3*
Insulin (ulU/mL)	21.4 ± 3.0	24.4 ± 2.7	17.7 ± 1.7	16.1 ± 2.0
HOMA-IR	4.95 ± 0.88	6.01 ± 0.80	4.25 ± 0.52	3.62 ± 0.55*

Values are means±SEMs. HDL=high density lipoprotein, LDL=low density lipoprotein, PPE=rate of perceived exertion, and VO2max=maximal oxygen consumption. *: P≤0.05 versus before.

Table 5.2: Cardiovascular parameters at rest

	Attention control group		Exercise-training group	
	Before	**After**	**Before**	**After**
Heart rate (bpm)	62 ± 2	64 ± 3	63 ± 2	58 ± 2*
Systolic BP (mmHg)	116 ± 4	119 ± 4	122 ± 2	116 ± 2*
Mean BP (mmHg)	87 ± 3	90 ± 3	91 ± 2	87 ± 2*
Diastolic BP (mmHg)	71 ± 3	71 ± 3	73 ± 2	71 ± 2
Pulse pressure (mmHg)	45 ± 2	48 ± 2	48 ± 1	46 ± 1
Carotid systolic BP (mmHg)	109 ± 5	116 ± 6	112 ± 3	108 ± 3*
Carotid pulse pressure (mmHg)	38 ± 4	45 ± 5	39 ± 2	38 ± 2
Carotid intima media thickness (mm)	0.59 ± 0.02	0.63 ± 0.02	0.56 ± 0.02	0.60 ± 0.03
Carotid lumen area (mm^2)	24.7 ± 1.2	24.7 ± 1.4	26.4 ± 1.1	25.5 ± 1.4
Carotid distension (%)	16.7 ± 1.4	16.4 ± 1.1	15.5 ± 1.0	16.5 ± 1.1
Brachial mean diameter (mm)	3.44 ± 0.22	3.58 ± 0.16	3.60 ± 0.18	3.60 ± 0.19

Values are means±SEMs. BP=blood pressure and IMT=intima-media thickness. *: $P \leq 0.05$ versus before.

Table 5.3: Hemodynamic measures of middle cerebral artery during cerebral CO2 reactivity test

		Attention control group		Exercise-training group	
		Before	After	Before	After
Mean blood flow velocity (cm/sec)					
	Normocapnia	56.6 ± 4.5	53.7 ± 1.9	57.1 ± 3.9	54.3 ± 3.7
	Hypocapnia	36.1 ± 2.5	32.2 ± 0.8	36.6 ± 2.8	35.5 ± 2.8
	Hypercapnia	72.2 ± 5.1	67.9 ± 2.4	78.3 ± 5.4	76.8 ± 4.3
End-tidal CO_2 (mmHg)					
	Normocapnia	43 ± 1	43 ± 1	41 ± 1	39 ± 2
	Hypocapnia	27 ± 1	26 ± 1	25 ± 1	25 ± 1
	Hypercapnia	52 ± 1	53 ± 1	51 ± 1	50 ± 1
Mean arterial pressure (mmHg)					
	Normocapnia	86 ± 4	86 ± 2	91 ± 3	86 ± 3
	Hypocapnia	83 ± 4	76 ± 2	84 ± 3	76 ± 3
	Hypercapnia	95 ± 1	88 ± 3	99 ± 4	91 ± 3
ΔCVRi (normocapnia-hypocapnia) (%/mmHg)		2.28 ± 0.28	2.34 ± 0.17	2.32 ± 0.11	2.48 ± 0.16
ΔCVRi (normocapnia-hypercapnia) (% /mmHg)		3.15 ± 0.42	2.76 ± 0.26	3.79 ± 0.17	3.94 ± 0.28
ΔCVRi (hypocapnia-hypercapnia) (% /mmHg)		3.98 ± 0.22	4.13 ± 0.19	4.47 ± 0.17	4.86 ± 0.22
ΔCVCi (normocapnia-hypocapnia) (% /mmHg)		2.05 ± 0.31	1.75 ± 0.10	1.99 ± 0.15	1.90 ± 0.20
ΔCVCi (normocapnia-hypercapnia) (% /mmHg)		2.10 ± 0.61	2.84 ± 0.41	2.73 ± 0.26	3.02 ± 0.37
ΔCVCi (hypocapnia-hypercapnia) (% /mmHg)		3.05 ± 0.38	3.12 ± 0.16	3.33 ± 0.28	3.31 ± 0.26

Values are means±SEMs. CO2=carbon dioxide, CVCi=cerebrovascular conductance index, CVRi=cerebrovascular reactivity index, and MCA=middle cerebral artery.

Table 5.4: Neuropsychological assessment results

	Attention control group		Exercise-training group	
	Before	After	Before	After
Total cognitive composite score (z score)	0.10 ± 0.22	0.15 ± 0.21	-0.13 ± 0.13	0.00 ± 0.15
Global cognition (z score)	0.10 ± 0.31	0.09 ± 0.30	-0.17 ± 0.20	0.06 ± 0.20
MMSE	29.1 ± 0.4	29.2 ± 0.5	28.4 ± 0.4	28.9 ± 0.3
WTAR	42.8 ± 3.4	42.0 ± 3.4	42.6 ± 2.2	43.4 ± 2.0
Memory (z score)	0.07 ± 0.36	0.08 ± 0.27	-0.12 ± 0.24	0.08 ± 0.19
CVLT-II immediate recall	12.2 ± 1.0	12.0 ± 0.8	11.2 ± 0.7	12.1 ± 0.5
CVLT-II delayed recall	12.3 ± 1.0	12.4 ± 0.8	11.4 ± 0.8	12.7 ± 0.5
CVLT-II discriminability index	2.9 ± 0.3	2.9 ± 0.2	3.0 ± 0.2	2.8 ± 0.2
Attention-executive function (z score)	0.09 ± 0.20	0.20 ± 0.21	-0.09 ± 0.17	-0.07 ± 0.20
Trail Making Test A	27.8 ± 2.0	27.1 ± 2.4	31.3 ± 2.3	31.1 ± 3.0
Trail Making Test B	61.4 ± 2.7	59.1 ± 4.7	62.6 ± 3.6	67.4 ± 5.0
COWAT	43.6 ± 4.3	45.5 ± 5.2	44.1 ± 2.4	46.2 ± 2.9
WAIS-III Digit Span Subtest	19.4 ± 1.3	19.8 ± 1.3	17.9 ± 1.3	18.8 ± 0.9

Values are means±SEMs. COWAT=Controlled Oral Word Association Test, CVLT-II=California Verbal Learning Test-II, MMSE=Mini-Mental State Exam, WAIS-III=Wechsler Adult Intelligence Scale-III, and WTAR=Wechsler Test for Adult Reading.

Figure 5.1: Changes in carotid distensibility in the aerobic exercise intervention group (n = 18) and attention control group (n = 10). Values are means ± SEMs. *: P<0.05 versus before.

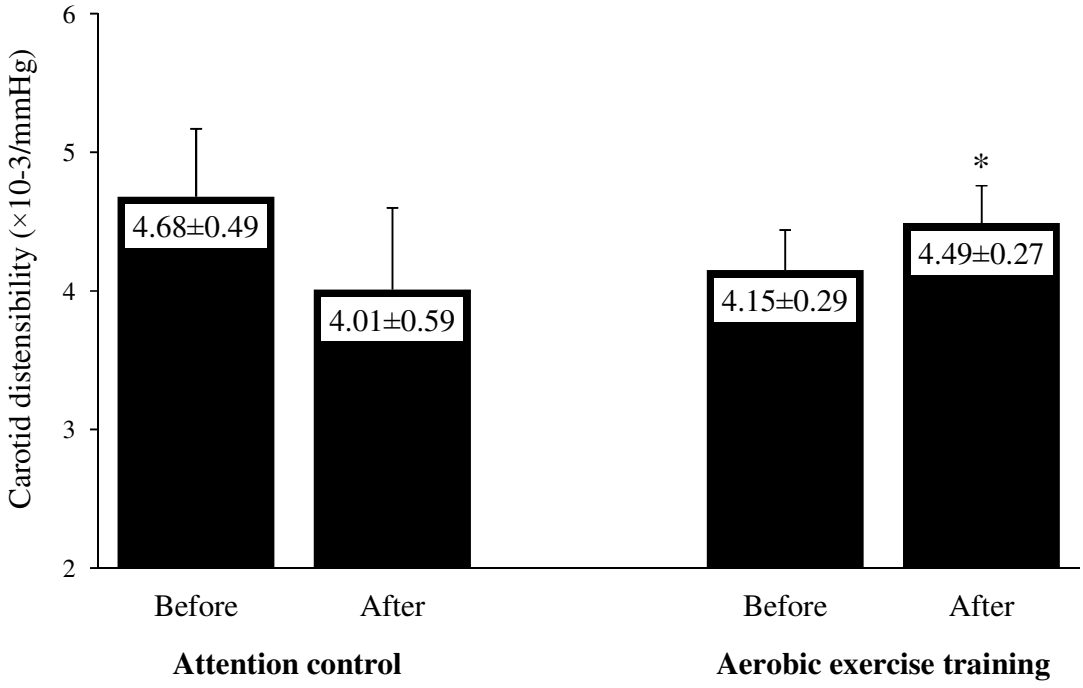


Figure 5.2: Changes in brachial flow-mediated dilatation in the aerobic exercise intervention group (n = 18) and attention control group (n = 10). Values are means ± SEMs. *: P<0.05 versus before.

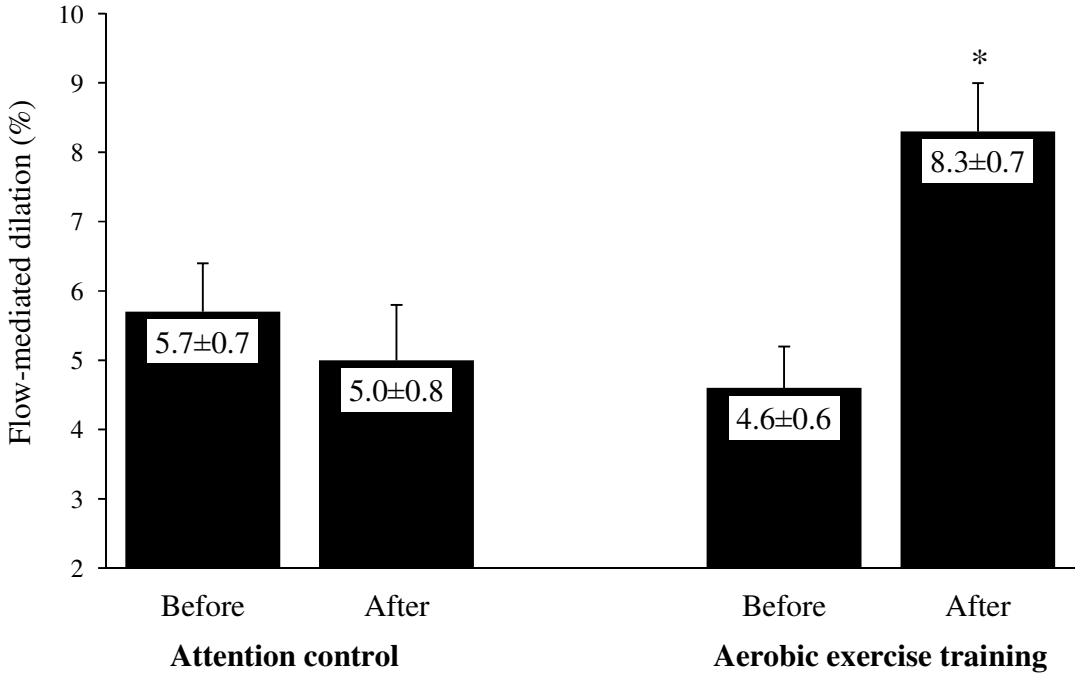
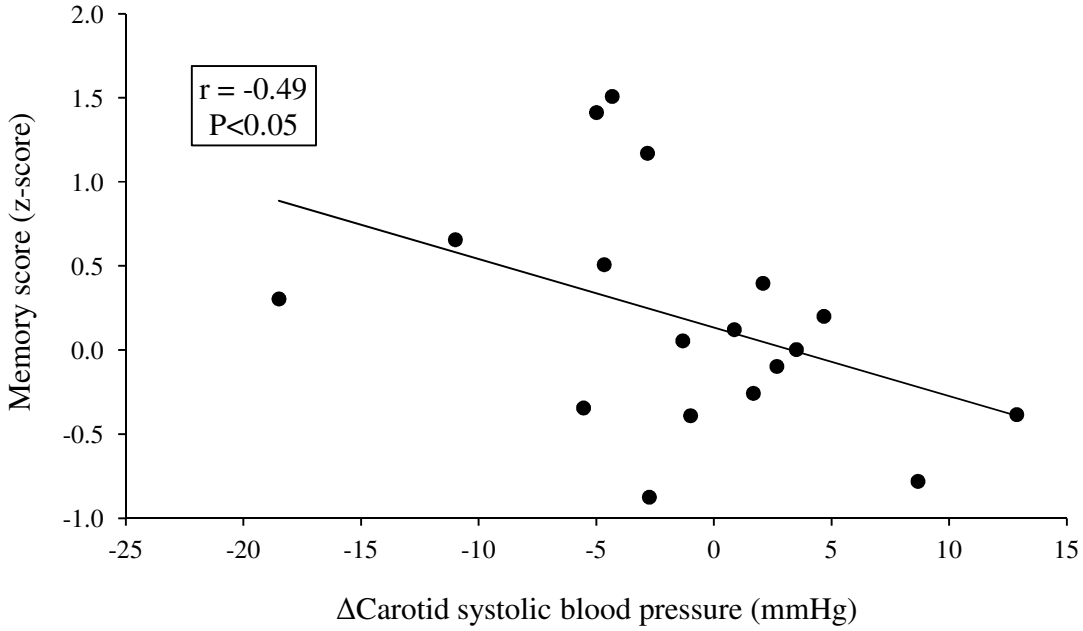


Figure 5.3: A scatter plot illustrating the association between the changes in memory performance and carotid systolic blood pressure after aerobic exercise training (n = 18).



Chapter 6: Review of Literature

Epidemiology of Dementia and Cerebrovascular Disease

Prevalence and Pathology

Cognition is the most important determinant for autonomy, quality of life, and functional ability in older age.[1] Cognitive decline begins as early as 20's and progressively affect multiple cognitive domains, including memory, reasoning, spatial visualization, and speed.[2] With increasing life expectancy and older population, the prevalence of dementia is expected to double in the next two decades and imposes a major health problem in the Western countries. The United Nations predicted that the number of demented people in the world will increase from 25.5 million in 2000 to 63 million in 2030 and to 114 million in 2050.[3, 113] Currently, there is no established treatment for dementia, including vascular and neurodegenerative subtypes.[114] Hence, primary prevention provides the greatest potential for reducing the burden of disease.

Alzheimer's disease and vascular dementia are the most common forms of cognitive disorder.[3] The cerebral atrophy of medial temporal lobe is a pathological hallmark of Alzheimer's disease and primarily affects memory function.[115] On the other hand, vascular dementia, which is attributed to cerebral ischemia, often causes abnormal structural alterations in frontal-striatal circuit and impairs attention-executive function.[116] Traditionally, Alzheimer's disease and vascular dementia were considered different entities because the cerebral atrophy in Alzheimer's disease is uniquely attributed to a deposition of amyloid-β as opposed to cerebral infarct observed in vascular dementia.[116,

[117] However, while this "amyloid hypothesis" still holds a prevailing view that Alzheimer's disease may be different from vascular dementia, accumulating evidences suggest that cardiovascular risk factors and cerebrovascular dysfunction are the common pathological pathway in both diseases.[37] The direct evidence supporting this view comes from studies on human cerebral arteries showing that atherosclerotic plaque, which is a pathological hallmark of vascular disease, is considerably more prominent in Alzheimer's disease patient than in non-demented subjects.[104, 118]

Risk Factors

Cardiovascular risk factors and cerebrovascular dysfunction are thought to play an important role for the pathogenesis of dementia. The Framingham Stroke Risk Profile (FSRP), which consists of age, systolic blood pressure, antihypertensive therapy, diabetes, cigarette smoking and presence of cardiovascular disease, provides a multivariate assessment of an individual's probability of a stroke.[119] Using this profile, a 10 % increase in the 10-year risk of stroke was associated with 1 % reduction in total brain volume, which was significantly related to lower performance in the tests of multiple cognitive domains.[120] Moreover, the Framingham study revealed that longitudinally measured blood pressure and chronicity of hypertension were inversely related to composite cognitive score and measures of attention and memory.[121] Specifically, every 10 mmHg increment in blood pressure declined the standardized composite cognitive (z) score by 0.04 to 0.07 over the two years of follow-up period.[121] The previous history of stroke also increases the risk of dementia. The recent evidence indicated that an incident

stroke doubles the risk of subsequent dementia, independently from prestroke level of cognitive function and prestroke rate of cognitive decline.[122, 123]

Multiple lines of evidence suggest that atherosclerosis, arterial disease which develops as a cumulative result of cardiovascular risk factors, may be more directly linked with the risk of dementia. A higher degree of common carotid artery intima-media thickness was associated with 1.9-3.2 times the risk of vascular dementia, 1.3-1.8 times the risk of Alzheimer's disease, and 1.3-1.9 times the risk of all dementia.[124] Moreover, the study, in which the Circle of Willis was autopsied from the Alzheimer's patients, provided the direct evidence that the degree of atherosclerotic plaque was significantly more severe compared with non-demented control subjects.[104, 118] It is also worthwhile to mention that cerebral atherosclerosis increases the risk of stroke. A prospective multicenter study revealed that an increment of 1 standard deviation in common carotid artery intima-media thickness increases the risk of stroke 25-33% after adjustment for the conventional cardiovascular risk factors.[125]

Taken together, cardiovascular risk factors play significant role in the pathogenesis of both dementia, including Alzheimer's disease and vascular dementia, and stroke. These epidemiological observations strongly suggest that vascular risk factors accelerate the formation of atherosclerosis in cerebral artery. In turn, cerebral atherosclerosis seems to be directly associated with morphological change in the brain and its pathology.

Effect of Physical Activity

Physical activity improves cognitive function. Meta-analytic study has reported that exercise training increased the major domains of cognitive performance by 0.5 standard deviations, regardless of the type of cognitive task, the exercise training program, or participants' characteristic.[86] In particular, executive-control function showed the greatest benefit from increased physical activity. Regarding exercise training regimen, a combination of strength and aerobic training elicited a greater improvement in cognition than aerobic training alone. Participation in relatively short-term training programs provided at least as much benefit as moderate-length training, but not quite as much as long-term training program. Short bouts of exercise less than 30 minutes had very little impact on cognitive function. Finally, participants in old category (66-70 years old) benefited more than the middle-aged (55-65 years old) and older (71+ years old) groups. There was no difference in improved cognitive function between clinical (e.g., depression) and non-clinical (healthy) populations.[86]

A majority of the studies which investigated the relation between a level of physical activity and stroke incidence yielded an inverse association,[126-130] whereas some studies showed a U-shape [131] or even a positive association.[132] Despite the general consensus that higher physical fitness prevents future stroke, the reason for some discrepancies is thought to stem from a limited number of the incidence during follow-up periods. To overcome the limitation, a meta-analysis that combined the results from 18 cohort and 5 case-control studies demonstrated that moderately active individuals had a 20 % lower risk and highly active individuals had 27 % lower risk of stroke incidence or mortality compared with the low-active individuals.[133]

Cerebrovascular Anatomy

The brain is critically dependent on a continuous blood supply in order to maintain the functional and structural integrity. For this reason, the cerebral vasculature uniquely works in concert with nervous tissue (termed "neurovascular coupling") and precisely regulate blood flow to the active neurons. An understanding of cerebral blood flow regulation requires the basic knowledge about the anatomy of neurovascular unit.

Large Cerebral Arteries and the Circle of Willis

The Circle of Willis is the primary site of anastomosis where the blood supply from the large cerebral arteries merges and protects the brain from regional blockage of vascular supply. Cerebral perfusion in each brain hemisphere arises from internal carotid and vertebral arteries, which originate from common carotid and subclavian arteries, respectively. In both right and left hemispheres, the internal carotid artery enters the cranium, gives off a posterior communicating artery, and divides into the two terminal branches: anterior and middle cerebral arteries. While the middle cerebral artery branches into progressively smaller arteries and supplies most of the cortical gray and subcortical white matter, the anterior cerebral and posterior communicating arteries form the anterolateral and posterolateral portions of the Circle of Willis, respectively. The anterior portion of the Circle of Willis is formed by anterior communicating artery, which connects the right and left sides of anterior cerebral arteries.[8, 134]

The right and left vertebral arteries merge to form the basilar artery. The basilar artery, in turn, gives rise to the right and left posterior cerebral arteries and forms the posterior portion of the Circle of Willis.[8, 134]

Smaller Cerebral Arteries and Neurovascular Unit

The large cerebral arteries arising from the Circle of Willis become progressively smaller while branching and traveling on the brain surface.[8, 134] These small surface vessels, named pial (extraparenchymal) arteries, give rise to the penetrating (intraparenchymal) arteries and enter the substance of the brain at right-angles to the surface. The pial and the proximal portion of penetrating arteries consist of an endothelial layer, a smooth muscle cell layer, and adventitia, containing collagen, fibroblasts, and perivascular nerves. In addtion, the proximal portion of penetrating arteries is separated from the brain tissue by the Virchow-Robin space, the prolongation of the pia mater filled with cerebrospinal fluid. As the penetrating arteries enter deeper into the brain substance and transform into the capillaries, the Virchow-Robin space disappears; the arterial wall loses the smooth muscle cell layer and consists of endothelial cells, pericytes, and the capillary basal lamina; and the vascular basement membrane comes into the direct contact with the astrocytic end feet. [73]

Astrocytes, a type of glial cells whose name derived from the Greek word for glue, plays critical role in neurovascular coupling. Astrocytes are the most abundant glial cells in the central nervous system and histologically characterized by their star-like shape and the broad end-feet on their processes. Because these end-feet put the astrocyte into direct

contact with both cerebral capillaries and neurons, astrocytes are thought to regulate the local blood flow during the neural activation.[8, 135, 136]

The endothelium in small cerebral arterioles and capillaries also exhibit the unique characteristic compared with the peripheral blood vessels of equivalent size such that the individual endothelial cells are connected by tight cellular junctions rather than the fenestrated connections observed in the other vasculature.[19] Such structure known as "blood-brain barrier (BBB)" limits free exchanges of solutes (e.g., plasma components, red blood cells, and leukocytes) between blood and cerebral interstitial fluid. Thus, the transcellular transport of nutrient and metabolic byproducts across BBB requires the specialized protein transporters such as carrier-mediated transport, ion-transport, active efflux transport, receptor-mediated transport, and caveolae-mediated transport.[19]

Brain Metabolism during Neuronal Activation

Action potential is an energy demanding process that allows neurons to synaptically communicate. Action potential is a short-lasting event during which the electrical potential of a cell rapidly rises and falls. However, the restoration of resting membrane potential requires ATP to move the intra- and extra-cellular ions against the electrochemical gradient.[8] For this reason, the brain energy consumption accounts for 20% of total blood oxygen and 25% of total body glucose utilization although it weighs only 2 to 3% of total body weight in adult human.[8]

The brain has limited amount of energy storage and accordingly requires the continuous supply of blood glucose and oxygen. Moreover, it has been long believed that

glucose is consumed directly and solely by neurons and that oxidation of glucose provides almost all of the energy necessary for the neuronal activation.[137] However, the latter notion has recently been challenged by the study showing the effective segregation between the neuronal oxidative phosphorylation and the astrocytic glycolysis. Thus, the existence of long-disputed astrocyte-neuron lactate shuttle hypothesis has recently been strengthened.[136] The astrocyte-neuron lactate shuttle hypothesis suggests that the energetic demand of the neural activation is supplied by oxidative phosphorylation fueled with both blood glucose and extracellular lactate pool.[136, 138] The metabolic pathway of the lactate shuttle begins from a normal neuronal excitation in which excitatory presynaptic neuron releases glutamate and induces an excitatory postsynaptic potential (EPSP). With a sufficiently strong EPSP, the propagation of membrane depolarization opens the voltage-gated Na+ channels and eventually activates the Na+/K+ ATPase, leading to an increased demand of ATP. In response, the rapid activation of the mitochondrial oxidative phosphorylation replenishes the cytoplasmic ATP using blood glucose as the main substrate.[136, 138] However, a limited amount of the glycolytic enzyme (e.g., phosphofructokinase) in neurons as well as an active downregulation of the glycolysis slow down the production of the TCA cycle substrate and the rate of the glucose oxidation.[139] For this reason, even minor or short-lasting neural activity (~10 seconds) cannot energetically be maintained solely by blood glucose unless the additional source of substrate is available for the oxidative phosphorylation.[138]

Immediately after the neural depletion of glycolytic substrates, the active neurons predominantly start utilizing the extracellular lactate. Kaisischke et al. made an important

observation that the astrocytes located in the close proximity to the active neurons are presynaptically stimulated by glutamate and concurrently increases the rate of the astrocytic glycolysis.[136] As a result, increased intra-astrocytic lactate replenishes the extracellular lactate pool which may sustain the late phase of neural activation.[138] Overall, blood glucose still serves as the main energy substrate for neural activation and restoration of the resting membrane potential, but extracellular lactate also provides the substrate especially during a prolonged neural activity.

Cerebral Blood Flow Regulation

The Control of Cerebrovascular Resistance

Blood pressure is controlled by a gradient between upstream and downstream vascular resistance. Therefore, systemic cerebral perfusion is established by the overall difference in vascular resistance between large cerebral arteries (e.g., internal carotid and vertebral arteries) and veins (e.g., jugular veins).[53] However, the brain is a heterogeneous organ that requires precise distribution of blood flow matched temporally and spatially with neural activity. In order to accomplish this, the cerebrovasculature needs to uniquely control the regional as well as the remote vascular resistance relative to the site of neural activation.[140, 141]

Inside a cranium, extracerebral large arteries and pial arterioles account for 2/3 of the vascular resistance while intracerebral penetrating arterioles and capillaries account for the remaining 1/3.[53] As such, blood pressure in the largest extracerebral vessel (e.g., basilar artery) has approximately 80% of aortic pressure, and the pial arterioles on the

brain surface have only 50-60% of systemic pressure.[142-144] Moreover, for an artery or arteriole of a given diameter, microvascular pressure is lower in the brain[142] than in the heart,[145] mesentery,[146] or skeletal muscle[147]. This indicates that, vascular resistance is greater in the brain than in other organs despite the differences in a relative diameter, length, and branching pattern of the arteries. Therefore, vessels residing outside of the brain have the greatest impact on parenchymal blood flow. From the functional perspective, it is critically important that dilations of both downstream and upstream cerebral vessels are temporally coordinated and reduce their vascular resistance during neural activation.[37, 141] In pathological conditions, compromised vasodilatory responses either at the local site of neural activation or at the remote resistance arteries bring about the inadequate supply of energy substrate and may cause functional impairment and tissue damage.[37, 140, 148]

Cerebral Autoregulation

Arterial blood pressure markedly changes during normal daily activities, and these fluctuations in blood pressure may potentially cause substantial changes in cerebral blood flow. In order to effectively counteract this, cerebral vasculature is equipped with a unique vascular function called the cerebral autoregulation.[149] Cerebral autoregulation is the physiological regulatory mechanism that maintains a constant cerebral blood flow over wide range of perfusion pressure, normally 60-150 mmHg mean arterial pressure. Specifically, during autoregulatory response, cerebral arterioles constrict to increase the vascular resistance when systemic blood pressure increases and dilates to decrease the resistance when blood pressure decreases.[149] Therefore, the brain is protected from the

acute hyper- or hypo-perfusion of blood, which may potentially damage delicate nervous tissue.

The mechanism of cerebral autoregulation, including the possible roles of neuronal and endothelial functions, has extensively been explored in the past. These studies reported that pressure-mediated regulation of vascular tone is unaltered even when perivascular nerves or vascular endothelium was denuded, suggesting that cerebral autoregulation is independent of endothelial function and is accomplished by an intrinsic property of vascular smooth muscle (termed myogenic response). [150]

Myogenic response is initiated by an increased intracellular concentration of calcium ions. Specifically, an elevated intravascular pressure results in the sustained depolarization of vascular smooth muscle cell membranes, subsequently leading to an opening of voltage-dependent calcium ion channels followed by the rapid influx of extracellular calcium ions.[151] The crossbridge cycling of vascular smooth muscle will then be activated by calcium-regulated phosphorylation of myosin.[152] Cerebral autoregulation, characterized as an intrinsic property of vascular smooth muscle, play a critical role to protect the brain from an acute episode of hypotension or hypertension that may lead to cerebral hypoperfusion or hyperperfusion, respectively. Because the brain is critically dependent on blood energy supply and is physically delicate when exposed to overperfusion of blood, cerebral autoregulation provides the first line of defense against changes in perfusion pressure.

Endothelial Regulation

The important roles of cerebral vascular endothelium is to homeostatically maintain basal cerebral blood flow[38, 153], inhibit atherogenesis by reducing aggregation and adhesion of platelets or leukocytes[154], build blood-brain barrier to limit a free exchange of plasma particles into the intracellular space[19], and indirectly assist functional hyperemia during neural activation.[33] In normal conditions, endothelium-derived vasoactive factors interactively regulate resting cerebral vascular tone and blood flow.[38, 153] Previous studies have reported that pharmacological agonists of endothelium-derived NO (e.g., acetylcholine, L-arginine, bradykinin, ATP, and ADP) evoked endothelium-dependent vasodilation of cerebral arteries and that antagonists (e.g., L-NMMA, indomethacin) attenuated the agonist-mediated vasodilatory responses as well as the basal vascular tone.[95, 155-163] These results suggest that endothelium-derived vasoactive factors are thought to act as a background for the other dilator and constrictor systems, for example, during neural activation.

In pathological conditions, structural and functional disruptions of cerebral vascular endothelium play a role in the pathogenesis of cerebrovascular disease. Structural damage in cerebral endothelium increases the aggregation and adhesion of platelets and leukocytes at the injured site as well as at a distant upstream site.[154] When combined with a reduced bioavailability of endothelium-derived substances, particularly NO, the aggregation/adhesion of leukocytes and platelets is enhanced.[154] When the endothelial injury is locally treated with L-arginine, a substrate for NO synthesis, an increased synthesis and release of NO inhibit the leukocytes and platelet aggregation and adhesion.[154] The meta-analytic studies have demonstrated that lacunar ischemic stroke is

linked with impaired endothelial function.[99, 164] Moreover, a recent study reported that lacunar stroke patients, particularly with white matter lesions, had a greater level of endothelial activation compared with the other types of lacunar stroke.[165] White matter lesions are attributed to a disruption of BBB that causes components of plasma to freely enter intracellular space, elicit inflammatory response, and form edema in the white matter.[19] Taken together, these evidences suggest that an impaired endothelial function may lead to an abnormal basal cerebrovascular tone, an increased level of atherosclerosis as well as cerebral hypoperfusion, and a breakdown of BBB which causes neuronal damage.

Endothelium-Derived Vasoactive Factors

Nitric oxide (NO). NO plays particularly important role in inhibiting atherosclerosis in the brain. NO is synthesized by an enzymatic action of NO synthase (NOS), which catalyzes oxygen-dependent conversion of L-arginine to NO.[166] There are three isoforms of NOS: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). Shear stress is the primary stimulus to activate eNOS while synaptic activity increases nNOS activity.[167, 168] iNOS is not normally expressed in the healthy brain; however, pathological stimuli such as inflammation can induce its expression in the blood vessels.[169, 170] Under basal conditions, tonic release of NO is a significant regulator of resting cerebral blood flow such that NOS inhibition constricts cerebral arteries and reduces cerebral blood flow.[95] While eNOS is the primary source of the NO that contributes to the basal tone of resistance pial arteries, neuronally derived-NO produced from parenchymal neurons or perivascular nerves can also induce cerebral artery

relaxation.[171] Up to approximately 2 % of all neurons are thought to possess NOS throughout many regions of the brain.[172] Currently, the relative importance of eNOS and nNOS in regulating cerebral blood flow remains unknown.

Cardiovascular risk factors such as hypertension and diabetes mellitus are associated with the reduction in NO-mediated dilation of middle cerebral artery assessed by hypercapnia in human.[173] Mechanistically, increased production of reactive oxygen species is considered as the pathway linking vascular pathology and reduced NO bioavailability.[174] Under pathological states, endothelial cells, neurons, glial cells, and invading leukocytes all produce reactive oxygen species (e.g., superoxide), which can, in turn, scavenge NO and form potent oxidant such as peroxynitrite.[175] Peroxynitrite in turn exacerbates oxidative stress, reduces NO bioavailability (endothelial dysfunction), and alter vascular tone.[174]

Endothelium-Derived Hyperpolarizing Factor (EDHF). EDHF-mediated cerebral artery vasodilation is characterized by endothelium-dependent hyperpolarization of smooth muscle, and activation of potassium ion channels. [176] It was previously demonstrated in cerebral circulation that EDHF complements the vasodilation elicited by endothelial-derived NO especially when the diameter of cerebral vessels decreases.[177, 178] Moreover, EDHF-mediated vasodilation is upregulated after stroke whereas vasodilation evoked by endothelium-derived NO is compromised.[179] Therefore, during pathological conditions, upregulation of EDHF may compensate the reduced NO-mediated endothelium-dependent vasodilation to maintain basal levels of normal cerebral blood flow.

Prostacyclin. Prostacyclin is a major endothelial metabolite of arachidonic acid that is produced by the two rate-limiting cyclooxygenase enzymes (COX-1 and COX-2).[180] In general, COX-1 is stably and constitutively expressed in adults; however, endothelial expression can be increased by stimuli such as shear stress.[181] The expression of COX-2 can be stimulated by inflammatory and the other pathological conditions, including oxidative stress.[170] After synthesis, prostacyclin diffuses to the cerebral vascular smooth muscle where it activates adenylate cyclase through G-protein-coupled receptors, causes potassium ion channels to open, and produces smooth muscle hyperpolarization. The hyperpolarization of the vascular smooth muscle, in turn, closes voltage-dependent calcium ion channels, decreases the intracellular calcium ion concentration, and causes vasodilation.[182, 183]

COX-1 and COX-2 have been shown to exert opposite influences on ischemic brain damage. While COX-2 null mice display a reduction in the infarct produced by middle cerebral artery occlusion.[184], COX-1 null mice exhibit attenuations in selected vasodilatory response to bradykinin and hypercapnia and increased susceptibility to ischemic brain injury.[185] These studies suggested that while COX-2 reduces a susceptibility to the brain damage through the beneficial effect attributed to preserved cerebral blood flow, COX-1 promotes the vulnerability in regions at risk for infarction.

Endothelin (ET)-1. ET-1 is characterized as a potent endothelium-derived constrictor of large and small cerebral arteries.[186, 187] There are two types of ET-1 receptors that are expressed in endothelium and smooth muscle: ETA and ETB.[188] Whereas the primary role of ET-1 is to profoundly cause constriction of cerebral arteries

via ETA receptor[189], the ETB receptor can conversely produce endothelium-derived NO-mediated vasodilation, depending on the concentration of ET-1 and the type of cerebral vessels.[190] For example, low concentration of ET-1 dilates, rather than constricts, pial arterioles of rats.[190]

Under normal conditions, ET-1 does not appear to contribute to cerebral blood flow, suggesting that ET-1 is not normally released from cerebrovascular endothelium.[191] In pathological states, however, the ET-1 system is activated and may contribute to vascular dysfunction and cerebral ischemia. For example, ET-1 synthesis and expression of ET-1 receptors are upregulated in cerebral vascular endothelium after brain injury, ischemia, and edema.[192, 193]

Functional Hyperemia

Role of Astrocyte. Functional hyperemia is the cerebrovascular mechanism that acts to ensure each brain region to receive blood flow matched to their neural activity level and is accomplished by a rapid relaxation of precapillary arterioles adjacent to active neurons and a delayed vasodilation of the feeding resistance vessels.[135] In vivo, vasodilatory and blood flow responses during functional hyperemia normally reach a peak in seconds and gradually decays, lasting for 30-40 seconds until returning to the baseline.[194] A number of investigations have attempted to identify the predominant vasoactive substance responsible for functional hyperemia. However, inhibition of any one of the factors failed to completely block the hemodynamic response.[135] Thus, a widely accepted hypothesis suggests that functional hyperemia is triggered by a variety of vasoactive substances produced from post-synaptic neuron in response to the activation

of glutamate receptors and the subsequent elevation in the intracellular calcium ion level. Even though this hypothesis seems reasonable based on the evidence showing that synaptic communication produces vasoactive substances (e.g., NO, prostaglandins, and adenosine), neurons are rarely in direct contact with vascular smooth muscle, and extracellular diffusion of the vasoactive agents from synapse to the local arterioles cannot explain the rapidity of hemodynamic response.[135] Thus, this evidence collectively suggests that the other mechanisms such as cellular structures that physically connect neurons and blood vessels may play a role during functional hyperemia.

There is an increasing recognition that astrocyte plays a critical role in initiating functional hyperemia especially at the onset of neural activation. A previous investigation provided the direct evidence showing that electrical stimulation of cortical slices increases the level of intracellular calcium ions in astrocytes, temporally followed by vasodilation of small arterioles.[194] This observation was further extended using pharmacological blockade in vivo to show that the increased level of calcium ions in the astrocyte produces the metabolic byproduct of the COX-1 (e.g., prostaglandin), which in turn plays the primary role in triggering the rapid vasodilation at the onset of functional hyperemia.[194] The same authors also demonstrated that COX-1 is densely expressed in astrocytic endfeet using immunohistochemical analysis.[194] Therefore, these findings suggest that astrocyte, particularly its metabolic byproduct of COX-1, triggers functional hyperemia at the onset of neural activity.

Role of Endothelium. Functional hyperemia is remotely assisted by endothelium-dependent vasodilation of the upstream resistance arterioles in an ascending fashion.[37]

Since 50-60% of cerebrovascular resistance is controlled at the level of pial arterioles, temporally-coordinated vasodilations in intraparenchymal precapillary arterioles and extraparenchymal feeding arterioles prevent vascular steal.[53] Vascular steel diverts blood flow from inactive tissue to active region, thereby increasing the risk of cerebral ischemia in the inactive region.[53] Importantly, vascular steal occurs in deep subcortical white matter regions where elderly patients often develop leukoaraiosis or white matter hyperintensity.[195] Iadecola et al. clearly demonstrated in vivo that electrical stimulation of neurons whose blood flow is supplied by the 3rd and 4th order intraparenchymal arterioles evoked vasodilation progressively in the lower-order parent branches, through the 1st order main branch.[33] Moreover, the time it takes for blood flow to reach a peak or plateau after neural stimulation, the duration of the plateau, and the time it takes for the blood flow to return to the baseline were all significantly longer at the remote site than at the local site.[33] Thus, these observations in hemodynamics suggest that the vasodilation in the lower-order branch arterioles are governed by mechanism(s) different from the local vasodilation adjacent to the stimulated neurons. There are currently two hypothetical mechanisms responsible for the upstream propagation of the vasodilation (termed retrograde vasodilation). The first mechanism is flow-mediated dilation, in which downstream vasodilation during neural activation leads to an increased blood flow in the upstream feeding vessels.[35, 36] An increased shear stress on the vascular endothelium leads to a release of vasoactive factors and causes vasodilation of the feeding vessels and amplification of the flow response.[35, 36] Flow-mediated dilation in isolated intracerebral arterioles has been found to be mediated by NO.[35] The second mechanism indicates that

vasodilation may be propagated through conduction of metabolic or electrical signal via gap junctions between endothelial cells and vascular smooth muscle cells.[196]

Therefore, functional hyperemia critically requires an increased vasodilation of the upstream feeding arterioles.[141] Physiologically, endothelium-dependent vasodilation seems to play a key role in the vascular adjustment.[35, 36] In pathological states, reduced endothelial function may lead to vascular steal, which increases the risk of cerebral ischemia in the inactive neural tissue. In the long-term, such repetitive ischemia may lead to cell mortality and impaired cognitive function.[195]

Hypertension and Cerebrovascular Dysregulation

Morphological Alterations

Microvascular Remodeling. Hypertension induces abnormal structural alterations in both large and small cerebral arteries.[197] Specifically, the media-to-lumen ratio of smaller cerebral arteries (e.g., pial arterioles) increases in hypertensive human subjects.[198] There are mainly two types of microvascular remodeling: hypertrophic and eutrophic.[199] In hypertrophic remodeling, the lumen cerebral arterioles grows inward via hypertrophy or hyperplasia of the vascular smooth muscle cells, increasing the wall thickness, and reducing the lumen size. In eutrophic remodeling, cerebral arterioles undergo a rearrangement of vascular smooth muscle cells that leads to a reduction in vessel lumen size without changes in total vascular mass or wall thickness.[199] It was previously demonstrated that chronic hypertension specifically induces hypertrophic remodeling of pial arterioles which led to the reduced vasodilatory capacity in response to the

application of active vasodilatory stimuli.[200] In contrast, the distensibility of the arterioles was preserved or even greater during passive stretching compared with the normal healthy control.[13] The wall composition analysis using a light microscope also supported this observation that a ratio of distensible (elastin and smooth muscle) to non-distensible (collagen and basement membrane) elements was greater in the cerebral pial arterioles that were exposed to chronically elevated blood pressure.[13]

Large Artery Stiffening. Chronic hypertension induces vascular stiffening of large cerebral arteries that is accompanied with the similar morphological alterations observed in the microvasculature, including reduced vessel lumen size and increased wall thickness.[12, 14] The analyses of pressure-internal radius and pressure-distension ratios revealed that chronic hypertension significantly reduces the distensibility of posterior cerebral artery, a large cerebral artery that feeds vascular supply to the Circle of Willis.[12] The wall composition analyses also support the observation that when exposed to high blood pressure, the posterior cerebral artery exhibited a greater ratio of collagen to elastin compared with the artery that was exposed to normal blood pressure.[12]

An increased stiffness of large cerebral arteries allows the penetration of pulse pressure into the deep intraparenchymal capillaries and raises the risk of structural damage such as BBB breakdown.[9, 10] In cardiovascular system, when the heart contracts, the resulting forward pressure wave travels down the aorta. As the forward wave propagates distally, it encounters regions of impedance mismatch (the concept analogous to difference in vascular resistance) created by variable wall properties and diameter and produces a partial wave reflection. Whereas reflected waves from throughout the arterial

system return to the central aorta, augment diastolic pressure, and help maintain coronary perfusion, the remaining forward wave perfuses the distal capillaries.[111] In the pathological condition when a large cerebral artery becomes stiffened, the regions of impedance mismatch moves distally or even disappear.[201] As a result, a greater magnitude of forward pressure wave penetrates into the fragile cerebral capillaries and may damage the vessel wall structure.[9, 10] For example, if the BBB is damaged, the ability to limit a free exchange of plasma particles will be lost, and the subsequent inflammatory response may facilitate the development of edema. Importantly, the formation of edema in the substance of the brain is believed as a major pathological cause for white matter hyperintensity observed in T2-weighted MR images.[19]

Taken together, while the structural alterations of cerebral microvasculature (e.g., inward remodeling) may protect the intraparenchymal capillaries from the increased flow pulsation, an elevated vascular resistance may reduce resting cerebral blood flow.[12, 200] Moreover, mechanically compromised vasodilatory capacity may decrease hemodynamic response during functional hyperemia.[15] In previous studies, hypertensive patients demonstrated the preserved or only slightly reduced resting cerebral perfusion, indicating that cerebral autoregulation may be offsetting the increased cerebrovascular resistance at rest.[202, 203] However, the magnitude of functional hyperemia during cognitive task has been reported to decline, thereby increasing the occurrences of vascular steal.[195, 204] Therefore, although the morphological changes in cerebral arteries attempt to protect resting cerebral perfusion during chronic hypertension, the risks for capillary damage (e.g., blood-brain barrier breakdown) and cerebral ischemia are still elevated. These

structural damages may be clinically associated with the development and progression of stroke and vascular dementia.

Functional Alterations

Cerebral autoregulation. Cerebral autoregulation maintains the constant levels of brain blood flow in the face of fluctuating perfusion pressure. The cerebral autoregulatory function in humans is typically assessed by monitoring the changes in cerebral blood flow velocity in response to a transient systemic hypotension induced by a lower limb vascular occlusion technique.[43] The excellent time-resolution and validated capability of using transcranial Doppler (TCD) imaging to detect changes in cerebral blood flow allow investigators to measure one's cerebral autoregulatory function.[44]

In chronic hypertension, the operating range of cerebral autoregulation shifts to a higher pressure.[149] As a result, while the brain is protected from overperfusion of blood in the face of chronically elevated blood pressure, higher perfusion pressures are constantly required to maintain the normal level of cerebral blood flow. A susceptibility to cerebral hypoperfusion is also increased due to a greater chance of systemic blood pressure to fall below a low end of the autoregulatory operating range (e.g., orthostatic hypotension).[149] The shift in the autoregulatory relation is perhaps explained by the structural remodeling of the cerebral arteries.[202, 203] As discussed earlier, chronic hypertension causes stiffening of large cerebral artery and hypertrophic remodeling of the microvasculature.[12-14, 197] These structural alterations may lessen their mechanical sensitivity to transduce changes in perfusion pressure.[202, 203] Along with an elevated basal vascular resistance, an increased risk of cerebral hypoperfusion during orthostatic hypotension imposes a greater

risk of ischemia particularly in deep white matter region (e.g., periventricular white matter).[205, 206] Indeed, an attenuated perfusion and an increased prevalence of silent lacunar infarct have been reported in the periventricular white matter.[206] Although the observed adaptation in cerebral autoregulation may provide a short-term protection against deleterious impact of elevated blood pressure, it may negatively impact brain perfusion in the longer term.

Endothelial Function and Atherosclerosis. Vascular endothelium maintains the vascular homeostasis by sensing changes in hemodynamic forces and blood-borne signals and by releasing a number of autocrine and paracrine factors.[16] Among such factors, endothelium-derived NO plays an important role in preventing atherosclerosis.[207, 208] In some conditions, including hypertension, hypercholesterolemia, hyperglycemia, cigarette smoking, etc., structural and functional impairment of the vascular endothelium precedes atherosclerosis, which is now thought to be inflammatory disease of arterial wall.[209]

The atherogenic process is generally divided into two parts. In the first part, endothelial dysfunction increases the adhesion of leukocytes and platelets to the lesion site and permeability to plasma molecules (e.g., LDL cholesterol). When this initial inflammatory response fails to effectively neutralize or remove harmful agents, it stimulates migration and proliferation of the local vascular smooth muscle cells that become intermixed with the area of inflammation to form an intermediate lesion.[209] In the second part, continuous inflammation results in increased numbers of macrophages and lymphocytes, which enter from the blood circulation and multiply within the lesion. The activation of these cells leads to the release of molecules that have a proteolytic effect

and eventually causes focal necrosis. As a consequence, a vicious cycle of mononuclear cell accumulation, smooth muscle cell migration and proliferation, and fibrous tissue formation leads to restructuring of the lesion and development of a fibrous cap that overlies a core of lipid and necrotic tissue.[209]

Atherosclerosis occurs primarily in large- and medium-sized elastic and muscular arteries and leads to ischemia of the heart[210], brain[104, 118], or extremities[211], eventually resulting in infarction.[125] The post-mortem study revealed that a degree of atherosclerotic plaque in the Circle of Willis is associated with elevated incidence of Alzheimer's disease.[104, 118] Therefore, maintenance of healthy vascular endothelium is crucial to prevent the pathogenesis of dementia as well as cerebrovascular disease. To date, endothelial function has been assessed primarily using endothelium-derived NO-mediated vasodilation, based on the assumption that the impaired vasodilation reflects alterations of the other functions of the endothelium.[42, 212, 213]

Endothelial Function: Evidence from Animal Studies. Cerebral vascular endothelium is susceptible to both structural and functional damages during hypertension. In acute hypertension in which blood pressure exceeds the operating range of cerebral autoregulation, cerebral blood vessels forcefully dilate, and endothelium of pial arterioles exhibits morphological damage.[214] The cerebral vascular endothelium develops numerous dome-like lesions that project into the lumen of the vessel and subsequently undergo necrosis.[214] Interestingly, the structural damage is limited to endothelium and the underlying vascular smooth muscle cells remain undamaged.[214] This is perhaps because

endothelium is a single cell layer that is directly exposed to the increased hemodynamic stress.

Both acute and chronic hypertension reduce endothelium-derived NO bioavailability via increased production of free oxygen radials (e.g., superoxide).[160, 215] Oxygen free radicals are known to scavenge NO and promote the formation of potent oxidants (e.g., peroxynitrite).[174, 216] Consistent with this, the studies that investigated the association between hypertension and endothelium-dependent dilation of cerebral artery reported that NO-mediated dilations of the large (e.g., middle cerebral and basilar arteries) and small (e.g., pial arteriole) cerebral arteries were significantly attenuated while endothelium-independent vasodilation was unaffected.[157-161, 215] Reduced bioavailability of NO that potentially coexists with necrotic endothelium may reduce vasomotor response to metabolic stimuli and create a favorable environment for atherosclerotic formation. The attenuated endothelium-dependent vasodilation induced by acute hypertension is partially restored following the application of superoxide dismutase, an antioxidant that outcompete the damaging reaction of superoxide.[157, 215]

Endothelial Function: Evidences from Human Studies. Cerebral blood flow response to hypercapnia and hypocapnia (termed cerebrovascular CO_2 reactivity) has been recognized as a useful technique to assess the vasomotor function of cerebral arteries in human.[217-219] The vascular response is evoked by multiplicity of vasoactive factors, including NO and prostacyclin, as an infusion of the exogenous donor (i.e., sodium nitroprusside) increased and an inhibition of the synthesis attenuated the vascular reactivity.[173, 220, 221] A recent meta-analysis reported the positive association between

cerebrovascular reactivity and endothelium-dependent vasodilation assessed from peripheral arteries.[99, 164]

Cerebrovascular CO_2 reactivity is impaired among the patients with hypertension and stroke. The results from hypertensive subjects indicated that hypertension does not only reduce cerebrovascular CO_2 reactivity, but also accompanies an impaired peripheral endothelial function.[173, 219] Impaired cerebrovascular CO_2 reactivity and peripheral endothelial function have been observed among the patients with cerebral ischemia and vascular risk factors.[99] Importantly, when the ischemic stroke patients are compared with the control subjects matched for vascular risk factors, the observed differences in cerebrovascular and peripheral endothelial function are less clear, suggesting that ischemic stroke manifests as a cumulative result of vascular risk factors.[99] In summary, this evidence suggests that cerebrovascular vasomotor and endothelial function is impaired among the patients with hypertension and ischemic stroke.

Effect of Regular Aerobic Exercise on Vascular Function

Central Elastic Artery

The primary function of the central elastic (cardiothoracic) artery is to convert pulsatile flow to continuous steady flow. It is accomplished by an expansion of the artery during systole and its elastic recoil during diastole.[111] The stiffness or compliance of the large central artery can be measured noninvasively in human using pulse wave velocity (PWV) and arterial compliance techniques.[222, 223] PWV is a measure of the traveling velocity of pulse pressure along the arterial tree, and higher arterial stiffness is

represented by faster PWV. Arterial compliance is a direct measure of arterial distensibility at a given transmural pressure. This provides a buffering capacity of the central artery against left ventricular ejection pressure.[222, 223] With cardiovascular risk factors, particularly advancing age and hypertension, the central elastic artery stiffens, allowing the left ventricular pulsation to reach the peripheral microcirculation and potentially cause end-organ damage. In particular, the brain is considered particularly susceptible to such damage because it is continuously and passively perfused at high-volume flow throughout systole and diastole.[9, 10]

Regular aerobic exercise is associated with an attenuated age-related stiffening of central elastic artery. Aortic PWV in senior endurance athletes is about 25% less than that in the age-matched sedentary peers, and is even similar to the level observed among young sedentary healthy controls.[224] Likewise, carotid artery compliance is 20-35% higher in middle-aged and older endurance-trained groups than in the age-matched sedentary and recreationally-active groups.[64] In the same study, 3 months of progressive aerobic exercise program increased carotid artery compliance by 25%, which was the similar level observed in the endurance-trained athletes.[64] Moreover, regular aerobic exercise has been shown to reduce central artery stiffness in postmenopausal sedentary women.[225]

In contrast to the benefit of regular aerobic exercise on age-related central artery stiffening, an increasing amount of evidence suggests that regular aerobic exercise may not reduce hypertension-related central arterial stiffness. In one previous study, despite a reduction in resting blood pressure, central arterial stiffness did not change after 12 weeks

of progressive aerobic exercise program among hypertensive postmenopausal women.[226] Consistent with this finding, 8 weeks of aerobic exercise training also failed to improve mechanical properties of central elastic artery in middle-aged and older patients with isolated systolic hypertension.[227] Moreover, higher peak aerobic capacity was not associated with lower central artery stiffness.[228] Collectively, these data suggest that, among patients with chronic hypertension, the central elastic artery may be resistant to morphological change after aerobic exercise training. However, these lines of evidence bring up an important question as to whether a participation in longer-term aerobic exercise training improves features of central elastic artery in hypertensives.

The mechanism by which regular aerobic exercise attenuates central elastic artery stiffness remains to be elucidated due to inaccessibility of the arteries in humans, both physically and with respect to experimental manipulation of potential signaling pathways. Despite the experimental constraints, a limited number of human as well as animal studies indicated that both functional and structural alterations in the arterial wall property are responsible for decreasing central artery stiffness. In general, functional changes are attributed to reduced vascular smooth muscle tone controlled by neural, hormonal, and local vasoactive factors while the structural changes include quantitative and/or qualitative modifications of elastic component of the arterial wall. In humans, the pharmacological blockade study demonstrated that increased central artery compliance after a 3-month moderate endurance training was accompanied by the reduced basal α-adrenergic sympathetic vasoconstrictor tone.[229] Although this result seems unexpected considering the evidence that peripheral sympathetic nervous activity rises after

endurance exercise training, changes in sympathetic nervous system activity may be heterogeneous in different bodily parts.[230, 231] Indeed, exercise training was found to reduce basal spillover of plasma norepinephrine from kidney but not heart.[231] In addition to neural control of vascular smooth muscle tone in central artery, basal release of endothelium-derived vasoactive factors may also change following aerobic exercise training. A 12-week aerobic exercise program in middle-aged and older adults significantly decreased the concentration of plasma ET-1, a potent endothelium-derived vasoconstrictor peptide, which was accompanied by reduced central artery stiffness.[232] Moreover, 8 weeks of detraining from aerobic exercise reverses the exercise-related downregulation of ET-1 to the baseline level.[233] While this study did not include arterial stiffness measurement, these findings are consistent with the notion that the ET-1 production is influenced by one's physical activity level. Currently, it remains unclear whether upregulated production of NO contributes to improved central artery compliance after regular aerobic exercise because, despite the upregulated protein expression of endothelial NO synthase, pharmacological blockade of the NO synthesis did not affect arterial compliance.[229, 234]

Structural adaptation of central elastic artery wall may occur after a participation in years of aerobic exercise training. There is only one animal study, which investigated the effect of a 17-21-week aerobic exercise training on structural composition of central elastic artery. In that study, exercise-induced reductions in aortic stiffness were not associated with quantitative alterations in arterial wall elastin and collagen.[235] This finding, together with the evidence showing that functional adaptations occur after a

similar duration of exercise training, suggests that the arterial wall is more resistant to structural modification than to functional change, and may involve some other mechanism(s) that was/were not examined in this study. For example, aerobic exercise training may induce qualitative, rather than quantitative, modifications in the arterial wall such that fracture and fragmentation of elastin may be minimized or reduced after exercise training.[236] Further, elevated pulse pressure, distending pressure for arterial wall, during exercise may stretch and modify cross-linking and/or break advanced glycation endproducts.[237]

Peripheral Conduit and Resistance Arteries

The primary roles of peripheral muscular conduit and resistance arteries are to deliver and control blood flow to metabolically active tissue by adjusting arterial caliber and vascular resistance. In contrast to the central elastic artery, peripheral muscular arteries undergo more prominent functional adaptations than the mechanical changes following regular aerobic exercise.[24, 238, 239] Endothelial function, particularly reflected by NO-mediated endothelium-dependent vasodilation (EDV), is improved with regular aerobic exercise.[238] Improvemenmts in endothelial function are considered systemic in nature based on the evidence that EDV's measured from different vasculature beds (e.g., coronary and brachial arteries) are related within the same subject.[240] Indeed, a lower body aerobic exercise training increases basal production of NO and EDV, measured in upper limb arteries.[107, 241, 242] This finding may be surprising given the prevailing view that blood flow to inactive muscles decreases during exercise due to increased sympathetic nervous activity[243, 244]. However, a recent study study demonstrated an

increase in blood flow and shear stress in inactive upper limb muscles when leg exercise is performed.[245]

Regular aerobic exercise attenuates or even completely restores age-related decline in EDV. Old endurance-trained athletes aged between 50-76 years exhibited 25% greater EDV than the age-matched sedentary peers, which was similar to the level observed in young endurance-trained athletes.[107] These cross-sectional finding is consistent with an intervention study, in which a 3-month aerobic exercise training completely abolished the observed difference in EDV between the old sedentary and the age-matched endurance-trained individuals.[107] Regular aerobic exercise is also effective in increase EDV in individuals with cardiovascular risk factors. A 12-week aerobic exercise program, which consisted of 30 minutes of brisk walking 5 to 7 times per week, increased EDV by 25% among hypertensive patients.[242] Moreover, in metabolic syndrome patients, aerobic exercise training improved their EDV by 35%.[246] The observed increases in EDV after aerobic exercise training are primarily thought to reflect heightened bioavailability of NO because pharmacological blockade of NO synthase abolished the improved EDV at post-exercise training.[242, 247]

Regular aerobic exercise increases bioavailability of NO by upregulating the protein expression of endothelial NO synthase (eNOS) and reducing oxidative stress in the vascular cells. The repeated increase in mechanical shear stress on vascular endothelium is thought to be a potent stimulus to upregulate mRNA and protein expression of eNOS.[248, 249] Moreover, oxidative stress, which is characterized as an imbalance in favor of generating reactive oxygen species (ROS) over endogenous

antioxidant enzyme (e.g., superoxide dismutase; SOD), scavenges NO.[216] ROS are highly bioactive, short living molecules that are derived from reduction of molecular oxygen. One of the most important ROS in vasculature is superoxide, which is produced by several enzymatic systems, including NADPH oxidase and xanthine oxidase. When the level of antioxidant enzyme activity is low, superoxide reacts with NO to generate peroxynitrite.[216] The formation of peroxynitrite negates the protective effect of NO on the vascular cells and promotes atherosclerotic formation.[216] The recent animal study, which examined the molecular effects of regular aerobic exercise on age-related reduction in EDV, revealed that decreased protein expression of eNOS can be completely restored to the same level observed among the young exercising mice.[248] Furthermore, exercise training increases aortic SOD activity and decreases nitrotyrosine level, in vivo marker of peroxynitrite[250], accompanied by reduced protein expression of NADPH oxidase.[248] Taken together with the evidence from a human study showing that exogenous administration of antioxidant ascorbic acid (Vitamin C) restores EDV only in old sedentary but not old and young endurance-trained individuals[251], the evidence strongly supports the hypothesis that regular aerobic exercise corrects the imbalance that favors ROS generation over antioxidants, setting up the vascular environment where a production of NO is greater than the removal.

Cerebral Artery

Regular aerobic exercise attenuates any age-related reduction in resting cerebral blood flow and functional hyperemia during a cognitive task.[109, 252] Functional MRI imaging showed a greater hemodynamic response to a cognitive task in physically fit old

adults than the age-matched unfit peers.[109] Additionally, a 6-month aerobic exercise program improved the previously-reduced hemodynamic response among the unfit subjects.[109] Moreover, using transcranial Doppler imaging of middle cerebral artery, endurance-trained athletes in all age groups exhibited ~17% greater resting cerebral blood flow velocity than the age-matched sedentary healthy subjects.[252] In addition, a recent study demonstrated that a lower level of systemic oxidative stress is associated with higher basal cerebrovascular conductance among physically fit postmenopausal women.[253] These studies, however, should be interpreted cautiously since transcranial Doppler does not have capability to measure "absolute" flow volume across subjects with different arterial diameters.[44]

Mechanistically, attenuated microvascular remodeling and enhanced EDV may explain improved cerebrovascular hemodynamics after regular aerobic exercise. The age- and cardiovascular risk factor-related increase in central artery stiffness is known to augment pulse pressure in peripheral microcirculation and cause cerebral arterioles to undergo vascular wall remodeling.[10, 15] As a result, vascular resistance to the blood flow increases and dilatory capacity of the artery is compromised.[14, 15, 197, 198, 200] Thus, reduced central artery stiffness after aerobic exercise training may lessen a degree of the vascular remodeling and mechanical constraint imposed on cerebral blood flow.[64] Cerebral artery EDV may be enhanced with exercise training in two additional ways. First, regular aerobic exercise may elevate the protein expression of eNOS in cerebral arteries, thereby upregulating the production of NO.[174] It was previously reported that total and regional cerebral blood flow increases along with elevated neuronal activity during exercise.[254]

Since eNOS protein expressions is thought to be regulated, in part, by mechanical shear stress on vascular endothelium[248, 249], regular exercise may increase the production of endothelium-derived NO in cerebral arteries. Second, cardiovascular fitness is associated with lower level of systemic oxidative stress. A favorable balance between ROS generation and antioxidant defense reduce the harmful reactions of ROS, especially with NO.[248, 251] Therefore, regular aerobic exercise may set up the environment where vasomotion of cerebral artery is enhanced with increased bioavailability of NO and reduced mechanical constraint against relaxation of vascular smooth muscle.

So far, no study investigated the effect of regular aerobic exercise on cerebrovascular vasomotion in human. This is due, primarily, to limited availability of the measurement technique. In recent years, the use of cerebrovascular response to CO_2 has been increasingly recognized to test the vasomotor function as well as NO bioavailability in cerebral vessels.[173, 255] Importantly, the cerebrovascular reactivity is significantly attenuated among the patients with cerebrovascular disease.[99, 164] Moreover, no study has yet examined the effect of regular aerobic exercise on the association between cerebrovascular vasomotion and cognitive function. The elucidation of the potential mechanism by which regular aerobic exercise improves cognitive function may contribute to the development of a novel and effective therapeutic modality for dementia.

Summary

Cognitive functions decline with aging progressively as early as 20's.[2] With increasing life expectancy, the prevalence of dementia will be expected to double in the

next two decades and imposes a major health issue in the Western countries.[3, 113] Currently, there is no established treatment for dementia, hence primary prevention provides the greatest potential for reducing the disease burden.[114]

Alzheimer's disease and vascular dementia are the most prevalent forms of dementia.[3] Accumulating evidences suggest that cerebral hypoperfusion is the overlapping pathway for the pathogenesis of both Alzheimer's disease and vascular dementia.[37] Midlife hypertension is the powerful, yet modifiable, independent risk factor for dementia.[256-258] If untreated, large cerebral arteries stiffen and the wall of the smaller arteries becomes hypertrophied.[12-14]As a result, an operating range of cerebral autoregulation shifts to a higher pressure [149, 202, 203], and the dilatory capacity of cerebral arteries is reduced.[15] The penetration of pulse pressure into the parenchymal microcirculation causes damage.[10, 11] Moreover, endothelial dysfunction increases the risk of developing atherosclerosis.[19, 140, 157, 159, 161] These abnormal alterations lead the brain to a hypoperfused state and creates metabolic imbalance between neural demand and vascular supply of energy substrate.

Physical activity improves cognitive function.[86] However, the underlying mechanism remains to be elucidated. In cardiovascular system, regular aerobic exercise is known to attenuate age-related reductions in central artery compliance and endothelial function.[64, 107] A limited number of evidence indicates that regular aerobic exercise prevents age-related reductions in resting cerebral blood flow and hemodynamic response to cognitive task. The future investigation of whether regular aerobic exercise can reverse deleterious effects of vascular risk factors on cerebrovascular function is needed.

Chapter 7: Summary and Future Directions

Summary

There is an increasing recognition that vascular disease risk is associated with a greater incidence of cognitive impairment and dementia[4-6]. Physiologically, such recognition is supported by the fact that cerebral metabolism heavily relies on the vascular supply of oxygen and energy substrates. The brain weighs only 2% of body mass but utilizes 20-25% of the body's total oxygen and glucose consumptions. Due to the lack of energy reserve, the brain depends on cerebral blood flow as the primary energy source and indeed receives 15% of cardiac output[8]. Cerebral hypoperfusion which results from vascular dysfunction causes a mismatch between energy supply and demand, and is associated with the pathological features of dementia, including the impairments of action potential generation and protein synthesis, glutamatergic excitotoxicity, and the deposition of cerebral amyloid-β proteins[51, 52, 259]. In contrast, habitual aerobic exercise is an established strategy to ameliorate the risk factors for vascular disease and is increasingly recognized to improve cognitive function[24, 39].

Accordingly, the primary purpose of this dissertation study was to investigate whether the exercise-related improvement in cognitive function is attributable to ameliorated vascular function and risk factors for vascular disease. Specifically, we hypothesized that regular aerobic exercise would demonstrate the improvement in cognitive function which is associated with the concomitant increases in cerebral and peripheral vascular reactivity and reductions in central arterial stiffness and plasma

insulin. In order to systematically address our study aim, we used cross-sectional and interventional study approaches. Middle-aged men and women were specifically recruited because pathological alterations in the brain is thought to precede years before the clinical onset of cognitive impairment, and because the association between vascular disease risk and cognitive function could be negated by the effects of primary aging[27, 28].

The primary findings from the present study were as follows. In the cross-sectional study, greater cognitive performance observed in endurance-trained adults was associated with higher levels of cerebral CO_2 reactivity and brachial endothelium-dependent vasodilation and lower levels of central arterial stiffness and plasma insulin. In the interventional study, a 3-month aerobic exercise training did not improve cognitive function while central arterial stiffness and brachial endothelium-dependent vasodilation made favorable changes. Interestingly, we however found that the improvement in memory performance after aerobic exercise training is correlated with the reduction in central systolic blood pressure.

Taken together, better cognitive performance observed in endurance-trained subjects (Study 1) may not directly be attributable to greater vascular function because there were discrepant changes in cognitive and vascular functions after a 3-month aerobic exercise training (Study 2). The correlation between the changes in memory performance and central systolic blood pressure is interesting but needs further investigation using a larger sample size. The discrepancy in the results from the cross-sectional and interventional studies could be explained by the duration of exercise training and/or the

time it takes for the effect of improved vascular function to translate into cognitive function.

Future Directions

The effects of regular aerobic exercise on cognitive function need further investigation using neuroimaging which has the reported predictability for future cognitive impairment and dementia. These include the measurements of cerebral metabolic rate of glucose utilization and cerebral deposition of amyloid-β protein[260, 261]. It is of great interest to investigate whether exercise-related improvements in vascular function provide the favorable outcomes in these measurements. For example, the recent finding which demonstrated the association between physical activity and lower level of cerebral amyloid-β deposition could be mediated by exercise-related improvements in vascular function[76], given the fact that cerebral amyloid primarily deposits within the wall of leptomeningeal arteries[262]. Moreover, the selection of subject population needs careful consideration. Although the pathological alterations in the brain are believed to precede years before the clinical onset of cognitive impairment[28], the future studies should target the individuals who are at risk for dementia. Such population includes the patients with mild cognitive impairment and/or chronic vascular disease risk (e.g., hypertension and diabetes). Finally, translational studies which incorporate both human and animal models may further advance our understanding of the mechanism underlying the association between regular aerobic exercise and the reduced risk of dementia. For example, the formation and receptor activation of advanced glycation endproduct (AGE)

has recently been demonstrated to play an important role to upregulate the production of cerebral amyloid[263]. Because AGE is the common pathological features of both central elastic arterial stiffness and insulin resistance[237, 264], it may provide the potential mechanism that connects the link between vascular disease risk and the increased risk of dementia.

Appendix A: Definition of Terms

Arterial distensibility: relative change in lumen area from diastole to systole for a given pulse pressure.

Arterial spin labeling (ASL): a non-invasive technique to measure regional cerebral blood flow in the living human brains using magnetic resonance imaging.

Arterial stiffness: progressive hardening of arterial wall and change in ability to buffer the pulsatile flow from the heart to steady flow through the arterial tree. Arterial stiffness was measured as pulse wave velocity, arterial compliance and distensibility, and β-stiffness.

β-stiffness: a measure of arterial stiffness independent of the blood pressure effect.

Cerebral carbon dioxide (CO_2) reactivity: the ability of cerebral arterioles to dilate and constrict in response to increased and decreased arterial CO_2 levels respectively.

Cognitive function: the mental information processes that include attention, memory, language, solving problems, and making decisions. The paper-based neuropsychological assessments measured global cognition, memory, and attention-executive function which are particularly vulnerable to aging and dementia.

Endothelial function: the ability of the thin cells lining the inside of a blood vessel to respond to changes in blood flow, stretch, circulating substances, and inflammatory mediators

Endothelium-dependent vasodilation: increase in arterial diameter in response to increased shear stress or acetylcholine infusion. Shear stress increases production of nitric

oxide in endothelial cells. Nitric oxide diffuses to adjacent vascular smooth muscle cells and causes them to relax (vasodilation).

Exercise training: regularly engaging in planned physical activity for at least 30 minutes a day, 3 days per week, for 6 months.

Flow mediated dilatation: a change in blood flow through a vessel causes a change in the arterial diameter. An increase in blood flow increases shear stress acting on the vessel wall and causes dilation to maintain perfusion. See endothelial-dependent vasodilation.

Insulin resistance: a physiological condition where the natural hormone insulin becomes less effective at lowering blood sugars.

Middle-age: age between 45-65 years

Nitric Oxide (NO): one of the endothelium-derived relaxing factors (EDRF). Nitric oxide is synthesized from arginine by nitric oxide synthase (NOS). NO formed by the endothelial isoform of NOS is a vasodilator and inhibits platelet activity, vascular smooth muscle cell growth, and leukocyte adhesion.

Pulse wave velocity: speed of pulse pressure traveling along an arterial segment. Pulse wave velocity is calculated as an arterial path length divided by the transit time.

Sedentary: description of an individual who has participated in less than 1 hour a week of physical activity for one year prior to this study.

Vascular function: general term to describe changes in the arterial tree including arterial stiffness and endothelial-dependent vasodilation.

Appendix B: Questionnaires

Medical Health Questionnaire

Participant #_____

Welcome to the *Physical Fitness, Cardiovascular & Brain Health* Study

Thank you very much for your interest in our study! This is to confirm your study visit, which is scheduled for _____

_____ at _____ am/pm. Your appointment will last approximately 2-3 hours.

Directions are provided in this mailing.

If you wear hearing aids or eyeglasses, please bring them with you to the appointment.

Please **complete the enclosed questionnaire** to the best of your ability and **bring it with you to your appointment**. The following questions are designed to provide us with information concerning your medical history.

Please answer the questions honestly and completely. All information **will be kept confidential**.

If it is necessary for you to change your appointment, please call The UT Clinical Neuroscience Laboratory at (512) 471-7926 **at least 48 hours in advance**.

Thank you for your cooperation, and we look forward to seeing you.

Physical Fitness, Cardiovascular and Brain Health Study
MEDICAL HISTORY QUESTIONNAIRE

Please answer each question as honestly and accurately as possible, and **bring this completed questionnaire with you to your appointment**. Your answers will be kept strictly confidential.

Today's Date:_____/_____/_____

General Information

Age:_____ Date of Birth: _____/_____/_____ Sex (please circle): Male Female

Relationship Status (please circle):

Married Domestic Partnership Single Divorced Separated Widowed

Race (please circle): Caucasian African American Latino Asian Other

Current Height:_____ Current Weight:_____

Women: Are you post-menopausal?_____

Mailing Address:_____

_____

Primary Telephone Number:_____ (please circle: home work cell)

Alternate Telephone Number:_____ (please circle: home work cell)

Emergency Contact Information

Name of closest relative or friend (for emergency contact): _____

Relationship to you: _____ Telephone: _____

Physician Information

Primary Care Physician Name: _____ Telephone:_____

Primary Care Physician Address:_____

_____

Cardiologist Name:_____ Telephone:_____

Cardiologist Address:_____

_____

IMPORTANT: Do you experience adverse reactions to nitroglycerin?

(please circle) Yes No Unsure

Family History

Has someone in your immediate family (that is, your biological mother, father, brother, sister, or any of your children) experienced any of the following problems: heart attack, bypass, angioplasty/stent placement, dementia, **hypertension BEFORE age 60**?

YES or NO

If so, please indicate below all family members with problems. Also, be sure to **indicate the age at which their heart problem began**:

_____

_____

Educational Background

Primary/First Language: _____ Secondary Language: _____

Years of Education: _____ Highest Academic Degree: _____

Occupation: _____

(if retired, please indicate your occupation before retirement)

If you were not born in the USA, please indicate what year you moved to the USA_____

Current Medications

Please list current medications and dosages, use the back of the page if necessary:

Medication Type	Name of Medication and Dose	Last time taken?
Heart medicine		
Blood pressure medicine		
Cholesterol medicine		
Thromboembolic disease medicine		
Hypercoaguability medicine		
Steroids		
Hormones/HRT		
Birth Control		
Medicine for breathing/lungs		
Insulin		
Other medicine for diabetes		
Arthritis medicine		
Medicine for depression		
Medicine for anxiety		
Thyroid medicine		
Medicine for ulcers		
Allergy medicine		
Pain killers (prescription or over-the-counter)		
Dietary supplements (herbs, vitamins, etc)		
Other (please specify)		

Medical Problems

Have you ever had any of the following **general medical problems?**

Medical Problem	Please Circle	Year of Onset	If your answer is yes…
Angina (Chest pain)	YES or NO		Does this limit your ability to exercise?
Arrhythmia	YES or NO		
Arthritis	YES or NO		What type?
Asthma	YES or NO		How is it treated?
Atrial fibrillation	YES or NO		
Autoimmune Disease	YES or NO		What type?
Blood Clots	YES or NO		Where? (e.g. lungs, legs)
Blood pressure	YES or NO		Was it too high or too low?
Cardiac Arrest/Heart Attack/Heart Failure	YES or NO		
Coronary Artery Disease	YES or NO		
Angioplasty/Bypass Surgery/Heart Valve Surgery	YES or NO		
Cancer	YES or NO		What type (e.g., prostate, colon)?
Cushing's Syndrome	YES or NO		
Diabetes	YES or NO		Circle: Type I (child onset) or Type II (adult onset) Circle: Controlled by diet, oral medication, or insulin
Glaucoma	YES or NO		
Hepatitis	YES or NO		Circle: Hepatitis A, B, or C
High Cholesterol	YES or NO		
Kidney Problems	YES or NO		What type?
Liver Problems	YES or NO		What type?
Thyroid Problems	YES or NO		Hypothyroidism (underactive) or hyperthyroidism (overactive)
Viral Infection	YES or NO		What type? Date of last infection:

Have you ever had any of the following **neurological problems**?

Neurological Problem	Please Circle	Year of Onset	If your answer is yes…
1. Head Injury	YES or NO		Was there a loss of consciousness? For how long?
2. Brain hemorrhage	YES or NO		Please explain:
3. Stroke	YES or NO		Please explain:
4.Transient Ischemic Attack (TIA or Mini-Stroke)	YES or NO		Please explain:
5. Brain infection/meningitis	YES or NO		
6. Multiple Sclerosis	YES or NO		
7. Parkinson's disease	YES or NO		

Have you ever had any of the following **additional problems**?

Problem	Please Circle	Year of Onset	If your answer is yes…
1. Anxiety	YES or NO		Have you undergone treatment?
2. Depression	YES or NO		Have you undergone treatment?
3. Schizophrenia	YES or NO		Have you undergone treatment?
4. Other Psychiatric Illness	YES or NO		Please specify: Have you undergone treatment?
5. Alcohol/Drug Abuse	YES or NO		Have you undergone treatment?
7. Smoking	YES or NO		How many packs per day: If you quit, please indicate when:

Diet Information

In an average day do you:	**Please Circle**
Add salt to your cooking?	YES or NO
Add salt at the table?	YES or NO
Add salt before you have tasted your food?	YES or NO
Try to reduce the amount of salt you use because of health reasons?	YES or NO
Eat 3 or more fruits and vegetables? Note: fruit juice counts as only 1 serving, no matter how much you drink. (Please do not count potatoes as vegetables).	YES or NO
Use whole milk or milk products instead of lowfat milk products?	YES or NO
Eat at least 1 meal or snack containing fried foods (deep fried foods, chips, French fries)?	YES or NO
Eat at least one meal containing red meat (beef, pork, or lamb)?	YES or NO
Consume 2 or more sugar containing soft drinks (e.g. soda) or fruit juice?	YES or NO
Consume 2 or more servings of sweets (e.g. desserts, candy, cookies, pastries, Pop Tarts, ice cream)?	YES or NO
Consume pre-prepared foods (e.g. canned soups, frozen dinners)?	YES or NO
Cook for yourself?	YES or NO
Drink 2 or more alcoholic beverages?	YES or NO
Skip breakfast?	YES or NO

Exercise Information

These questions are about your physical activity in the last 7 days. These can be activities you do at work, at home or in your yard, to get from place to place, and in your spare time for recreation, exercise, or sport.

vigorous activities refer to activities that take hard physical effort and make you breathe much harder that normal.

moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder that normal.

1. During the last 7 days, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ days per week

   ☐ No vigorous physical activities → Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

   _____hours _____ minutes per day

   ☐ None

3. During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Think only about those physical activities you did for at least 10 minutes at a time. Do not include walking.

   _____days per week

   ☐ No moderate physical activities → Skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?

   _____hours _____ minutes per day

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

_____ days per week

☐ No walking → Skip to question 7

6. How much time did you usually spend walking on one of those days?

_____ hours _____ minutes per day

7. During the last 7 days, how much time did you spend sitting on a week day? Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

_____ hours _____ minutes per day

Sleep Information

Sleep Scale from the Medical Outcomes Study

1. How long did it usually take for you to fall asleep during the past 4 weeks? (Circle One)

0-15 minutes……..…..1

16-30 minutes……..2

31-45 minutes……..3

46-60 minutes……..4

More than 60 minutes……...5

---

2. On the average, how many hours did you sleep each night during the past 4 weeks?

Write in number of hours per night: ☐ ☐

How often during the past 4 weeks did you…
(Circle One Number On Each Line)

	All of The Time ▼	Most of the Time ▼	A Good Bit of the Time ▼	Some of the Time ▼	A Little of the Time ▼	None of the Time ▼
3. feel that your sleep was not quiet (moving restlessly, feeling tense, speaking, etc., while sleeping)?	1	2	3	4	5	6
4. get enough sleep to feel rested upon waking in the morning?	1	2	3	4	5	6
5. awaken short of breath or with a headache?	1	2	3	4	5	6
6. feel drowsy or sleepy during the day?	1	2	3	4	5	6
7. have trouble falling asleep?	1	2	3	4	5	6
8. awaken during your sleep time and have trouble falling asleep again?	1	2	3	4	5	6
9. have trouble staying awake during the day?	1	2	3	4	5	6
10. snore during your sleep?	1	2	3	4	5	6
11. take naps (5 minutes or longer) during the day?	1	2	3	4	5	6
12. get the amount of sleep you needed?	1	2	3	4	5	6

Hays, R. D., & Stewart, A. L. (1992). Sleep measures. In A. L. Stewart & J. E. Ware (eds.), Measuring functioning and well-being: The Medical Outcomes Study approach (pp. 235-259), Durham, NC: Duke University Press.

Cognitive Difficulty Questionnaire

COGNITIVE DIFFICULTY SCALE
Adapted from: Gass & Apple (1997, JCEN) by Dr. D. J. Crockett, CCI, 2006

Subject ID: _____ Date: _____

Age: _____Gender: _____ Education: _____

Instructions: The following statements describe everyday inefficiency, lapses in attention on memory and related functions that people notice about themselves. Please answer the following questions using this scale:

(0) Never

(1) Rarely
(e.g., once a week)

(2) Sometimes
(e.g., 2-3 times a week)

(3) Often
(e.g., 4-5 times a week)

(4) Most of the time *or very often*
(e.g., 6-7 times a week)

ITEM	COGNITIVE DIFFICULTY SCALE	CIRCLE ONE				
1B	I had trouble recalling frequently used phone numbers.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
2B	I put down things (glasses, keys, wallet, purse, and papers) and had trouble finding them.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
3	When interrupted while reading, I had trouble finding my place again.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
4G	I needed a written list when I did errands to avoid forgetting things.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
5B	I forgot appointments, dates, or classes.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
ITEM	**COGNITIVE DIFFICULTY SCALE**	**CIRCLE ONE**				
6B	I forgot to return phone calls.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
7C	I had trouble putting my keys into a lock.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
8G	I forgot errands I planned to do on my way home.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
9G	I had trouble recalling the names of people I know.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
10A	I found it hard to keep my mind on a task or job.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
11A	I had trouble describing a program that I just watched on television.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
12A	I did not quite say what I meant.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
13E	I failed to recognize people I know.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
14A	I had trouble getting out information that was at the tip of my tongue.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time

ITEM	COGNITIVE DIFFICULTY SCALE	CIRCLE ONE				
1B	I had trouble recalling frequently used phone numbers.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
2B	I put down things (glasses, keys, wallet, purse, and papers) and had trouble finding them.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
3	When interrupted while reading, I had trouble finding my place again.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
4G	I needed a written list when I did errands to avoid forgetting things.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
5B	I forgot appointments, dates, or classes.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
ITEM	**COGNITIVE DIFFICULTY SCALE**	**CIRCLE ONE**				
6B	I forgot to return phone calls.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
7C	I had trouble putting my keys into a lock.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
8G	I forgot errands I planned to do on my way home.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
9G	I had trouble recalling the names of people I know.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
10A	I found it hard to keep my mind on a task or job.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
11A	I had trouble describing a program that I just watched on television.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
12A	I did not quite say what I meant.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
13E	I failed to recognize people I know.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
14A	I had trouble getting out information that was at the tip of my tongue.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time

30D	I had trouble sewing or mending.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
31A	I found it hard to keep my mind on what I'm reading.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
32A	I forgot right away what people say to me.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
33	When walking or riding, I forgot how I had gotten from one point to another.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
34 F	I had trouble deciding if I have received the correct change.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
35F	I forgot to pay bills, record checks, or mail letters.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
36A	I had to do things very slowly to be sure I was doing them right.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
37A	My mind went blank at times.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
38B	I forgot the date of the month.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time
39C	I had trouble using tools.	0.Never	1.Rarely	2.Sometimes	3.Often	4.Most of the time

Thank you for your cooperation. Any comments?_____

Handedness Questionnaire

Date: _____

Subject #: _____

This handedness questionnaire was adapted from:
Oldfield, R.C. ***The assessment and analysis of handedness: the Edinburgh inventory.*** **Neuropsychologia. 9(1):97-113. 1971.**

Edinburgh Handedness LQ
Using the rating method of Oldfield, Laterality quotients range from 100 (right handed) to 100 (left handed)

Which hand do you prefer when:	**Right or Left**			**Do you ever use the other hand?**	
Writing	R	L	No Preference	Yes	No
Drawing	R	L	No Preference	Yes	No
Throwing	R	L	No Preference	Yes	No
Using Scissors	R	L	No Preference	Yes	No
Using a Toothbrush	R	L	No Preference	Yes	No
Using a Knife	R	L	No Preference	Yes	No
Using a Spoon	R	L	No Preference	Yes	No
Using a Broom (upper hand)	R	L	No Preference	Yes	No
Striking a Match	R	L	No Preference	Yes	No
Opening a Box (lid)	R	L	No Preference	Yes	No

To score, go to:
http://airto.bmap.ucla.edu/BMCweb/Private/3TLab/LabNotes/edinburgh.html

Laterality Quotient:_____

Decile:_____

MRI Safety Questionnaire

University of Texas Imaging Research Center MRI Research Subject Screening Form

Date: ___ / ___ / ___ (Month / Day / Year) Exam Number: _____

Principal Investigator: _____ Level 2 User: _____

Name: _____ (Last / First / Middle Initial) Age: _____ Height: _____ Weight _____lbs

Date of Birth: ___ / ___ / ___ (Month / Day / Year) Gender: ❑ Male ❑ Female Body part to be scanned: _____

Address: _____ (Street) Phone number: _____

_____ (City) _____ (State) _____ (Zip)

1. Have you had prior surgery or an operation (e.g., arthroscopy, endoscopy, etc.) of any kind? ❑Yes ❑No
   If yes, please indicate the date and type of surgery:

   Date: ___ / ___ / ___ (Month / Day / Year) Type of surgery: _____

   Date: ___ / ___ / ___ (Month / Day / Year) Type of surgery: _____

2. Have you had a prior MRI imaging study or examination? ❑Yes ❑No
   If yes, please specify:

   Body Part: _____ Date: ___ / ___ (Month / Year) Facility: _____

   Body Part: _____ Date: ___ / ___ (Month / Year) Facility: _____

   Body Part: _____ Date: ___ / ___ (Month / Year) Facility: _____

3. Have you experienced any problem related to a previous MRI examination or MR procedure? ❑Yes ❑No
   If yes, please describe: _____
4. Have you had an injury to the eye involving a metallic object or fragment (e.g., metallic slivers ,shavings, foreign body, etc.)? ❑Yes ❑No
   If yes, please describe: _____
5. Have you ever been injured by a metallic object/foreign body (e.g., BB, bullet, shrapnel, etc.)? ❑Yes ❑No
   If yes, please describe: _____
6. Are you currently taking or have you recently taken any medication or drug? ❑Yes ❑No
   If yes, please list: _____
7. Are you allergic to any medication? ❑Yes ❑No
   If yes, please list: _____
8. Do you have a history of asthma, allergic reaction, respiratory disease, or reaction to a contrast medium or dye used for an MRI, CT, or X-ray examination? ❑Yes ❑No
9. Do you have anemia or any disease(s) that affects your blood, a history of renal (kidney) disease, renal (kidney) failure, renal (kidney) transplant, high blood pressure (hypertension), liver (hepatic) disease or seizures? ❑Yes ❑No
   If yes, please describe: _____

For female participants only: It is crucial that we find out whether there is any chance that you are pregnant.

10. Are you post menopausal? ❑Yes ❑No
11. Are you pregnant? ❑Yes ❑No
12. Do any of the following conditions apply:
    Has it been more than 28 days since your last menstrual period? ❑Yes ❑No
    Are you taking any type of fertility medication or are you having fertility treatments? ❑Yes ❑No
13. Are you currently breast feeding? ❑Yes ❑No

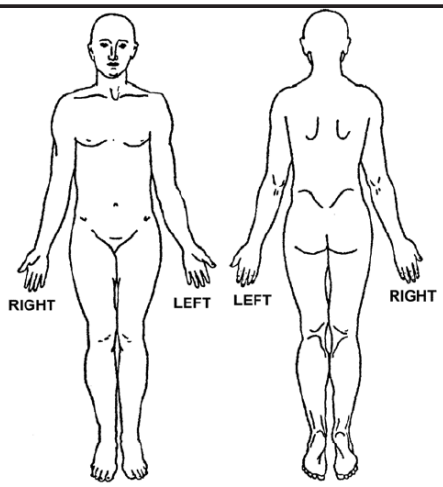
Subject Screening Form Version 090908/Luci

WARNING: Certain implants, devices, or objects may be hazardous to you and/or may interfere with the MR procedure (i.e., MRI, MR angiography, functional MRI, MR spectroscopy). Do not enter the MR system room or MR environment if you have any question or concern regarding an implant, device, or object. Consult the researcher BEFORE entering the MR system room. The MR system magnet is ALWAYS on.

Please indicate if you have any of the following:

❑Yes	❑No	Aneurysm clip(s)
❑Yes	❑No	Cardiac pacemaker
❑Yes	❑No	Implanted cardioverter defibrillator (ICD)
❑Yes	❑No	Electronic implant or device
❑Yes	❑No	Magnetically-activated implant or device
❑Yes	❑No	Neurostimulation system
❑Yes	❑No	Spinal cord stimulator
❑Yes	❑No	Internal electrodes or wires
❑Yes	❑No	Bone growth/bone fusion stimulator
❑Yes	❑No	Cochlear, otologic, or other ear implant
❑Yes	❑No	Insulin or other infusion pump
❑Yes	❑No	Implanted drug infusion device
❑Yes	❑No	Any type of prosthesis (eye, penile, etc.)
❑Yes	❑No	Heart valve prosthesis
❑Yes	❑No	Eyelid spring or wire
❑Yes	❑No	Artificial or prosthetic limb
❑Yes	❑No	Metallic stent, filter, or coil
❑Yes	❑No	Shunt (spinal or intraventricular)
❑Yes	❑No	Vascular access port and/or catheter
❑Yes	❑No	Radiation seeds or implants
❑Yes	❑No	Swan-Ganz or thermodilution catheter
❑Yes	❑No	Medication patch (Nicotine, Nitroglycerine)
❑Yes	❑No	Any metallic fragment or foreign body
❑Yes	❑No	Wire mesh implant
❑Yes	❑No	Tissue expander (e.g., breast)
❑Yes	❑No	Surgical staples, clips, or metallic sutures
❑Yes	❑No	Joint replacement (hip, knee, etc.)
❑Yes	❑No	Bone/joint pin, screw, nail, wire, plate, etc.
❑Yes	❑No	IUD, diaphragm, or pessary
❑Yes	❑No	Dentures or partial plates
❑Yes	❑No	Tattoo or permanent makeup
❑Yes	❑No	Body piercing jewelry
❑Yes	❑No	Hearing aid *(Remove before entering MR system room)*
❑Yes	❑No	Other implant _____
❑Yes	❑No	Breathing problem or motion disorder
❑Yes	❑No	Claustrophobia

Please mark on the figures below the location of any implant or metal inside of or on your body.



IMPORTANT INSTRUCTIONS

Before entering the MR environment or MR system room, you must remove all metallic objects including hearing aids, dentures, partial plates, keys, beeper, cell phone, eyeglasses, hair pins, barrettes, jewelry, body piercing jewelry, watch, safety pins, paperclips, money clip, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clipper, tools, clothing with metal fasteners, & clothing with metallic threads.

Please consult the experimenter if you have any questions or concerns BEFORE you enter the MR system room

NOTE: You are required to wear earplugs or other hearing protection during the MR procedure to prevent possible problems or hazards related to acoustic noise.

I attest that the above information is correct to the best of my knowledge. I read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signature of person completing form: _____ (Signature) Date: ___/___/___ (Month / Day / Year)

Form completed by: ❑Subject ❑Relative _____ (Printed Name) _____ (Relationship to subject)

Form reviewed by: _____ (Signature) _____ (Printed Name)

Appendix C: Neuropsychological Assessment

Mini-Mental Status Exam (MMSE): a 30-item test of general mental status including arithmetic, memory, and orientation. Scores below 24 indicate possible dementia.

Wechsler Test for Adult Reading (WTAR): provides an estimate of general intelligence since unlike many intellectural abilities, reading recognition is relatively stable in the presence of cognitive declines associated with normal aging or brain injury. The test consists of reading aloud from a list of 50 words that have atypical grapheme to phoneme translations. There is a maximum of 50 raw points, which can be transformed to age-normed T-scores and an IQ composite score with a mean of 100 and a standard deviation of 15.

California Verbal Learning Test-II (CVLT-II): a measure of verbal learning and memory. A 16-item word list is presented five times and participants must recall as many words as they can after each presentation. Following the presentation of a 16-word distracter list, participants are asked to recall the original list with and without semantic cuing. After a 20-minute delay, participants recall the original list with and without semantic cuing and complete a yes/no item recognition test. Free recall trials have a maximum of 16 points with 1-point awarded for each correctly recalled item from the list. A recognition discriminability index (d') is calculated from the yes/no item recognition section. The calculation of d' is based on the number of hits, number of possible total hits, number of false-positives, and number of total possible false-possible, yielding a maximum of 4.0.

Trail Making Test A & B: a measure of visual attention and processing speed. For part A, participants draw a line connecting 25 randomly dispersed numbers in numerical order. On part B, participants must alternate between connecting randomly dispersed numbers and letters in sequence (e.g., 1-A-2-B-3-C). Time to completion is recorded for both parts.

Controlled Oral Word Association Test (COWAT): a measure of verbal fluency. Participants must name as many words as they can, beginning with a specified letter within a 1-minute time frame.

Wechsler Adult Intelligence Scale-III (WAIS-III) Digit Span Subtest: a measure of apprehension span and working memory. On Digits Forward, participants must repeat sequences of verbally presented numbers. On Digits Backwards, participants must recall the numbers in reverse sequence. 1-point is awarded for each correctly sequenced item on Digits Forward and Backwards with their composite sums creating a Digits Total Score.

*Neuropsychological measures were grouped into one of the three cognitive domains: 1) *global cognition*, 2) *memory*, and 3) *executive function*. The following test scores were included in each domain and raw total scores were utilized unless otherwise stated: 1) *global cognition*: MMSE and WTAR; 2) *memory*: CVLT-II immediate recall, delayed recall, and recognition discrimination; and 3) *executive functions*: Trail making A and B time to completion, COWAT, and WAIS-III Digit Span Subtest. Participants' raw test scores were converted to z-scores using the study sample mean and standard deviation. Timed test scores (i.e., Trail making A and B) were multiplied by -1 so that higher scores indicate better performance. Three composite cognitive domain z-scores were calculated

for each participant by averaging the z-scores of all tests within each domain. Total composite cognitive z-score was calculated for each participant by averaging the z-scores of all tests as whole.

Mini-Mental State Examination (MMSE)

"I will be asking you to do a number of things today, some will be easy, others more difficult. Please remember that not everyone answers every question, or solves every problem. All we ask is that you give us your best effort."

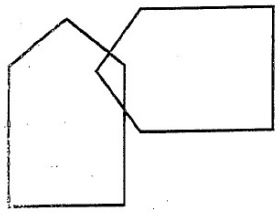
MINI-MENTAL STATE EXAMINATION

1. What day is today_____, month _____, day of the month_____, year_____, season_____ ( )5
2. What is the name of the building we are in_____, town_____, county_____, state_____, country_____ ( )5
3. Remember these words: peach, truth, chair. Repeat them now. (*Practice until subject is able to repeat them, recording number of repetitions required _____*) ( )3
4. Subtract 7 from 100 and continue subtracting 7 from the remainder until I say stop. _____(*give one point for each correct subtraction even if the starting number was incorrect*). *If unable, have the subject spell the word "WORLD" backwards*_____ (DLROW) ( )5
5. What were those words I asked you to remember?_____ ( )3
6. What is this? (*show the subject a pen/pencil and a watch*) _____ ( )2
7. Repeat after me: "NO IFS, ANDS, OR BUTS." _____ ( )1
8. Do this: (*show subject the phrase Close Your Eyes written in large print*) ( )1
9. Take this paper in your right hand, fold it in half, and put it on the floor. ( )3
10. Write a complete sentence here: ( )1

_____

_____

11. Copy this drawing: ( )1



_____/30

WTAR Word List

Say, **I will show you some words that I will ask you to pronounce.** Place the WTAR Word Card in front of the examinee. As you point to the card, say, **Beginning with the first word on the list, pronounce each word aloud. Start with this word** (point to Item 1), **and go down this column, one right after the other, without skipping any. When you finish this column, go to the next column** (point to the second column). **Pronounce each word even if you are unsure. Do you understand?** When you are sure that the examinee understands the task, say, **Ready? Begin.**

	Item	Pronunciation	Score (0, 1)		Item	Pronunciation	Score (0, 1)
1.	again	uh-GEHN *or* uh-GAIN		26.	conscientious	kon-chee-EN-shus *or* kon-chee-INCH-us	
2.	address	uh-DRESS *or* AD-dress		27.	homily	HAHM-uh-lee	
3.	cough	kawf *or* kof		28.	malady	MAL-uh-dee	
4.	preview	PREE-vyue		29.	subtle	SUH-tl	
5.	although	awl-THO		30.	fecund	FE-cund *or* FEE-cund	
6.	most	mohst		31.	palatable	PAL-uh-tuh-bul	
7.	excitement	eck-SITE-munt *or* ik-SITE-munt		32.	menagerie	muh-NAJ-uh-ree	
8.	know	noh *or* no		33.	obfuscate	OB-fuh-skate *or* ob-FUH-skate	
9.	plumb	plum		34.	liaison	lee-A-zahn *or* LAY-a-zahn *or* LEE-ah-zahn	
10.	decorate	DEK-uh-rate		35.	exigency	EKS-eh-jen-see *or* ek-ZEE-jen-see	
11.	fierce	firhrss		36.	xenophobia	zen-uh-FO-bee-uh *or* zeen-uh-FO-bee-uh	
12.	knead	need		37.	ogre	OH-gur	
13.	aisle	EYE-l		38.	scurrilous	SKUR-uh-lus *or* SKUH-ruh-lus	
14.	vengeance	VEN-junts *or* VIN-junts		39.	ethereal	ih-THEER-ee-uhl *or* ih-THIR-ee-uhl	
15.	prestigious	pre-STIJ-us *or* pre-STEEJ-us		40.	paradigm	PAIR-uh-dime *or* PAIR-uh-dim	
16.	wreathe	reeTH		41.	perspicuity	pur-spuh-KYEW-uh-tee	
17.	gnat	nat		42.	plethora	PLETH-er-aah	
18.	amphitheater	AM(p)-fuh-the-uh-ter		43.	lugubrious	loo-GOO-bree-us *or* luh-GOO-bree-us *or* loo-GYEW-bree-us	
19.	lieu	loo		44.	treatise	TREET-us	
20.	grotesque	gro-TESK		45.	dilettante	DILL-uh-tahnt	
21.	iridescent	ihr-uh-DESS-unt		46.	vertiginous	vur-TI-jin-us *or* vur-TIJ-uh-nus	
22.	ballet	BA-lay *or* ba-LAY		47.	ubiquitous	you-BIC-wuh-tus *or* you-BIH-kwah-tus	
23.	equestrian	ih-KWESS-tree-un		48.	hyperbole	hi-PUR-buh-lee	
24.	porpoise	POR-pus		49.	insouciant	in-SOO-see-yunt	
25.	aesthetic	ess-THET-ik *or* ees-THET-ik		50.	hegemony	heh-JEM-o-nee *or* he-je-MO-nee	

WTAR Raw Score	
WTAR Standard Score	

California Verbal Learning Test-II (CVLT-II)

List A Immediate Free Recall Trial 1

I'm going to read a list of words to you. Listen carefully, because when I'm through, I want you to tell me as many of the words as you can. You can say them in any order. Just say as many of them as you can. Are you ready?

Read List A at an even pace, taking slightly longer than one second per word, so the entire list takes 18 to 20 seconds. Then say: Go ahead.

	List A	Trial 1 Resp Type
1	truck	
2	spinach	
3	giraffe	
4	bookcase	
5	onion	
6	motorcycle	
7	cabinet	
8	zebra	
9	subway	
10	lamp	
11	celery	
12	cow	
13	desk	
14	boat	
15	squirrel	
16	cabbage	
17		
18		
19		
20		
	Total Correct C	
	Total Repetitions R	
	Total Intrusions I	

Trial 2

I'm going to read the same list again. Like before, tell me as many of the words as you can, in any order. Be sure to also say words from the list that you told me the first time.

Record all responses verbatim, in the order recalled. Prompt only once (e.g., Anything else?) at the end of each free and cued recall trial (i.e., after 15 seconds with no response or when the examinee says he/she cannot remember more words).

	Trial 2 Resp Type	Trial 3 Resp Type	Trial 4 Resp Type	Trial 5 Resp Type
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
	Total Correct C	Total Correct C	Total Correct C	Total Correct C
	Total Repetitions R	Total Repetitions R	Total Repetitions R	Total Repetitions R
	Total Intrusions I	Total Intrusions I	Total Intrusions I	Total Intrusions I

Trials 3 and 4

I'm going to read the same list again. Like before, tell me as many of the words as you can, in any order, including words from the list you've said before.

Trial 5

I'm going to read the same list one more time. Like before, tell me as many of the words as you can, in any order, including words from the list you've said before.

List B Immediate Free Recall

Now I'm going to read a second list of words to you. When I'm through, I want you to tell me as many words from this second list as you can, in any order. Don't tell me words from the first list, just this second list.

Read List B at an even pace, taking slightly longer than one second per word, so the entire list takes 18 to 20 seconds. Then say: Go ahead.

List B
violin
cucumber
elephant
closet
turnip
guitar
basement
sheep
clarinet
garage
corn
rabbit
patio
saxophone
tiger
radishes

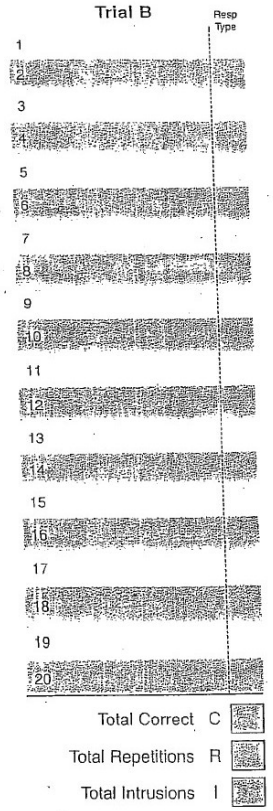
List A Short-Delay Free Recall

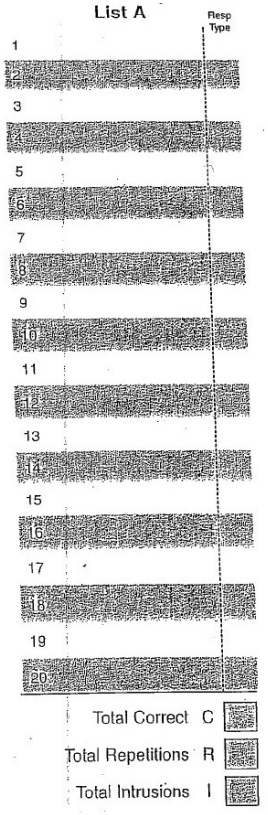
Now I want you to tell me all the words you can from the *first* list, the one I read to you several times. Don't tell me words from the second list, just the first list. Go ahead.

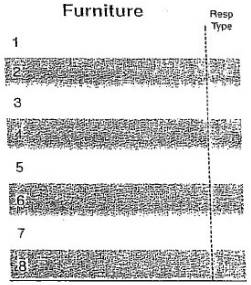
List A Short-Delay Cued Recall

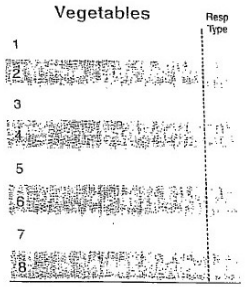
Tell me all the words from the first list that are furniture.
Tell me all the words from the first list that are vegetables.
Tell me all the words from the first list that are ways of traveling.
Tell me all the words from the first list that are animals.

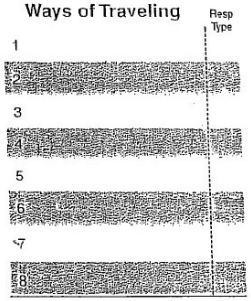
Record all responses verbatim, in the order recalled. Prompt only once (e.g., Anything else?) at the end of each free and cued recall trial (i.e., after 15 seconds with no response or when the examinee says he/she cannot remember more words).

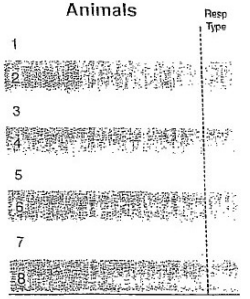












Total Correct C
Total Repetitions R
Total Intrusions I

There should be approximately a 20-minute delay between the completion of Short-Delay Cued Recall and the start of Long-Delay Free Recall. Do not inform the examinee that there will be later CVLT–II trials.

I read two different lists of words to you earlier: a first list that I read to you several times, and a second list that I read to you once. Tell me all the words you can that were from the *first* list. Don't tell me words from the second list, just the first list. Go ahead.

List A — Resp Type

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Total Correct C
Total Repetitions R
Total Intrusions I

Tell me all the words from the first list that are furniture.
Tell me all the words from the first list that are vegetables.
Tell me all the words from the first list that are ways of traveling.
Tell me all the words from the first list that are animals.

Furniture	Resp Type	Vegetables	Resp Type	Ways of Traveling	Resp Type	Animals	Resp Type
1		1		1		1	
2		2		2		2	
3		3		3		3	
4		4		4		4	
5		5		5		5	
6		6		6		6	
7		7		7		7	
8		8		8		8	

Total Correct C
Total Repetitions R
Total Intrusions I

List A Long-Delay Yes/No Recognition

Now I'm going to read more words to you. After I read each one, say "Yes" if that word was from the first list, or say "No" if it was not from the first list.

If the examinee responds "I don't know" during Yes/No Recognition, say, "Tell me whether you think _____ was on the first list."

	Response Y	Response N	Item Type
wallet			UN
boat			T
saxophone			BN
cucumber			BS
giraffe			T
carrot			PR
patio			BN
cabbage			T
desk			T
bracelet			UN
car			PR
elephant			BS
violin			BN
cow			T
fork			UN
bus			PR
celery			T
lamp			T
radishes			BS
table			PR
rose			UN
motorcycle			T
sheep			BS
basement			BN
dog			PR
bookcase			T
matches			UN
spinach			T
clarinet			BN
truck			T
rabbit			BS
chair			PR
corn			BS
seashell			UN
garage			BN
squirrel			T
turnip			BS
cabinet			T
onion			T
lion			PR
camera			UN
guitar			BN
subway			T
tiger			BS
coffee			UN
zebra			T
lettuce			PR
closet			BN

T = Target
Distractor Types: BS = List B Shared; BN = List B Non-Shared; PR = Prototypical; UN = Unrelated

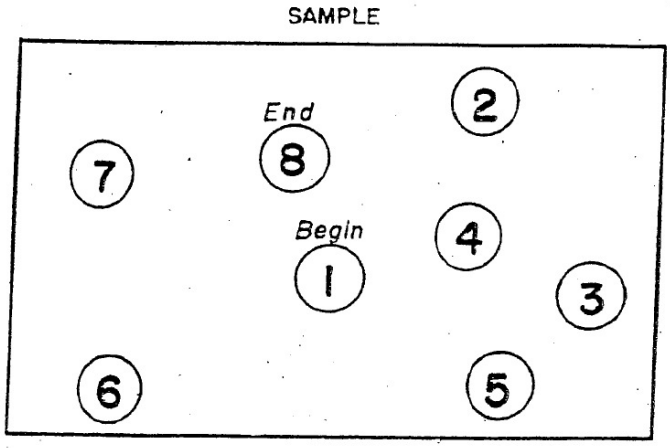
Total Hits
Total False-Positives

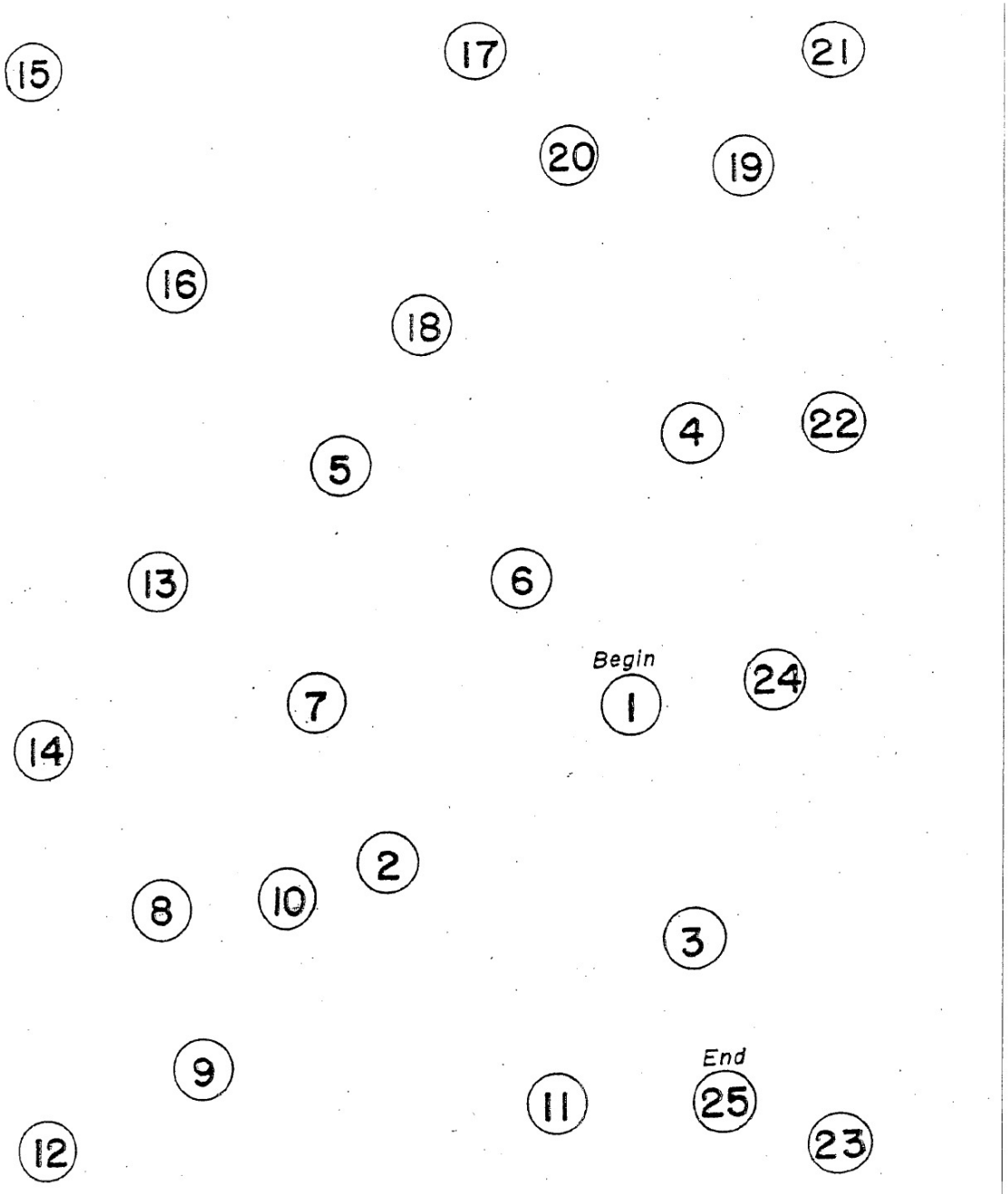
*There should be approximately a **10-minute delay** between the completion of Yes/No Recognition and the start of Forced-Choice Recognition. Do not inform the examinee that there will be a later CVLT–II trial.*

Trail Making Test A & B

TRAIL MAKING

Part A




15
17
21
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19
16
18
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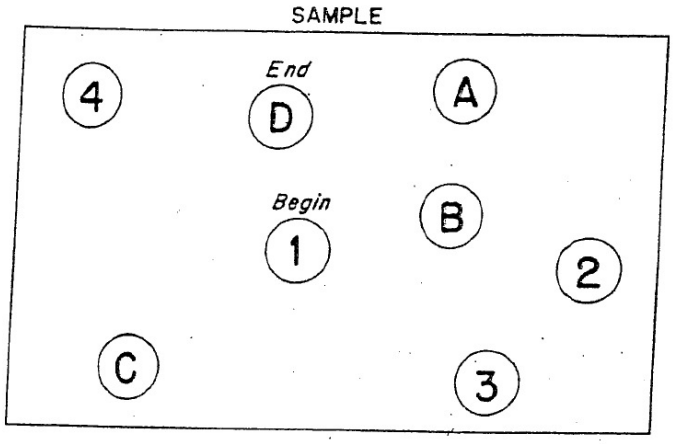
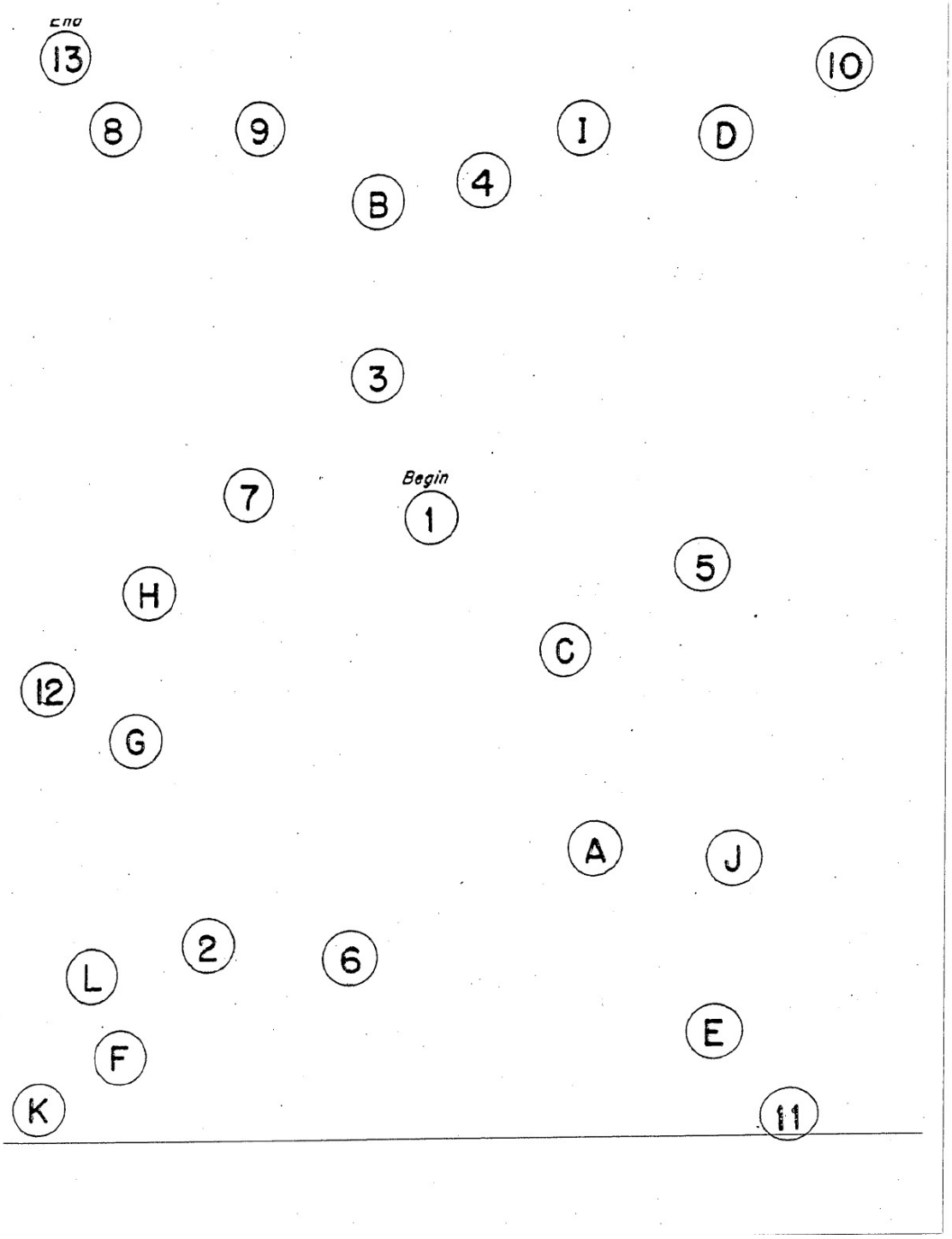
22
5
13
6
Begin
24
7
1
14
2
8
10
3
9
End
11
25
12
23

TRAIL MAKING

Part B




End
13
10
8
9
I
D
4
B
3
Begin
7
1
5
H
C
12
G
A
J
2
6
L
E
F
K
11

Controlled Oral Word Association Test (COWAT)

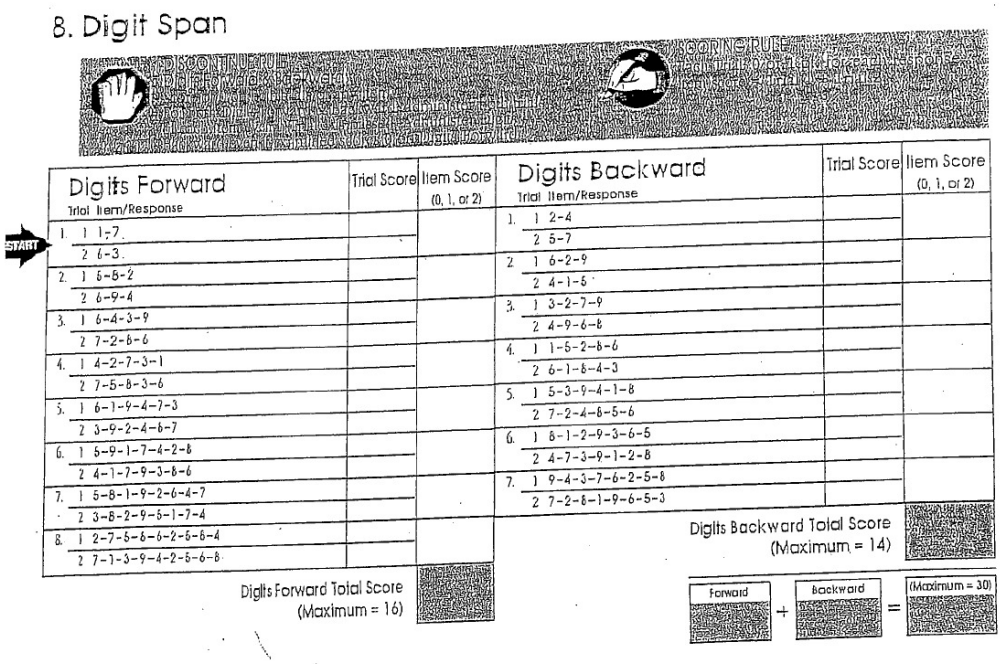
Controlled Oral Word Fluency Test (Need Stopwatch: TIME = 1 minute per letter)

- Say, **"I am going to say a letter of the alphabet, and what I would like you to do is then tell me as many words as you can thing of that begin with that letter as quickly as you can.**
- **"For instance, if the letter was 'B', you might give me words like, 'bad', 'battle', and 'bed'. I do not want you to use words which are proper names such as 'Bob', 'Boston', or 'Buick'.**
- **"Also, do not use the same word again with a different ending such as 'eat' and 'eating'. Finally, do not give me any numbers. Any questions?...** [Pause]**…Begin when I say the letter. The first letter is C….Go ahead."**
- TIME participant for 1 minute
- Record all answers verbatim, even if a word is repeated
- Repeat instructions for letters A and S. Stop each letter trial after 60 seconds.

	C	F	L
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			
21.			
22.			
23.			
24.			
25.			

Total Çorrect:	C_____	F_____	L_____	Total_____
# Perseverations:	C_____	F_____	L_____	Total_____
# Incorrect:	C_____	F_____	L_____	Total_____

Wechsler Adult Intelligence Scale-III (WAIS-III) Digit Span Subtest



8. Digit Span

Digits Forward Trial Item/Response	Trial Score	Item Score (0, 1, or 2)
1. 1 1-7 2 6-3		
2. 1 5-8-2 2 6-9-4		
3. 1 6-4-3-9 2 7-2-8-6		
4. 1 4-2-7-3-1 2 7-5-8-3-6		
5. 1 6-1-9-4-7-3 2 3-9-2-4-8-7		
6. 1 5-9-1-7-4-2-8 2 4-1-7-9-3-8-6		
7. 1 5-8-1-9-2-6-4-7 2 3-8-2-9-5-1-7-4		
8. 1 2-7-5-8-6-2-5-8-4 2 7-1-3-9-4-2-5-6-8		
Digits Forward Total Score (Maximum = 16)		

Digits Backward Trial Item/Response	Trial Score	Item Score (0, 1, or 2)
1. 1 2-4 2 5-7		
2. 1 6-2-9 2 4-1-5		
3. 1 3-2-7-9 2 4-9-6-8		
4. 1 1-5-2-8-6 2 6-1-8-4-3		
5. 1 5-3-9-4-1-8 2 7-2-4-8-5-6		
6. 1 8-1-2-9-3-6-5 2 4-7-3-9-1-2-8		
7. 1 9-4-3-7-6-2-5-8 2 7-2-8-1-9-6-5-3		
Digits Backward Total Score (Maximum = 14)		

Forward	+	Backward	=	(Maximum = 30)

Digit Span (Forward)

- Say, "I am going to say some numbers. Listen carefully, and when I am through, say them right after me. Just say what I say."
- Say one number per second and record participant's answers
- Try to avoid chunking the numbers or using a "sing song" voice
- Discontinue only when participant has failed two items in a row with the same amount of numbers

Digit Span (Backward)

- Say, " Now I'm going to say some more numbers, but this time when I stop, say them backwards. For example, if I say 7-1-9, what would you say?"
  - If patient responds correctly say… "That's right."
  - If patient responds incorrectly say… "No, you would say 9-1-7. I said 7-1-9 so to say it backwards you would say 9-1-7. Now try these numbers. Remember, you are to say them backwards; 3-4-8"
    - Whether or not patient responds correctly proceed to the first test item
  - "Now say these numbers backwards from the way I say them."
- Say one number per second and record participant's answers
- Discontinue rule is the same as Digit Forward, when participant has failed two items in a row with the same amount of numbers.

Appendix D: Vascular Function Measurements

Cerebral Carbon Dioxide (CO_2) Reactivity (CVRi): Blood flow velocity (BFV) of the middle cerebral artery (MCA) was measured by transcranial color-coded duplex ultrasonography (iE 33 Ultrasound System, Philips, Bothell, WA) during normocapnic, hypocapnic, and hypercapnic steady states. MCA was insonated from the left posterior temporal window using a 1.6 MHz transcranial Doppler probe which was mounted on a custom-made probe fixation device attached to commercially available headgear (Dia Mon, DWL Compumedics, Charlotte, NC)[43]. Subjects wore nose clips and breathed only through a mouthpiece with a Y-way valve (Hans-Rudolph, Shawnee, KS), one end connected to a 5-liter air reservoir containing a mixture of 5 % CO_2 and 21 % O_2 balanced with nitrogen and another end open to room air. End-tidal CO_2, an estimate of arterial CO_2 level, was measured from expired air and analyzed by a capnograph (Capnocheck Plus, Smiths Medical, Waukesha, WI). Non-invasive beat-by-beat blood pressure was measured by Portapres (Finapres Medical, Amsterdam, Netherlands). Image 1D shows the equipment setup for cerebral CO2 reactivity test.

After at least15 minutes of rest in the supine position, 3 minutes of baseline recordings were taken during spontaneous breathing of room air. Next, subjects underwent 1 minute of maximal voluntary hyperventilation with a duty cycle of 1 second. This short period of hyperventilation was intended to reduce end-tidal CO_2 level to ~25 mmHg without causing respiratory muscle fatigue or central hypoxia possibly associated with a prolonged hyperventilation. The MCA-BFV was recorded during the last 20

seconds of hyperventilation. From the pilot study conducted prior to data collection, 30-40 seconds of maximal hyperventilation effectively decreased end-tidal CO_2 to near minimal levels (~25 mmHg). After the MCA-BFV returned to the baseline following hyperventilation, a respiratory valve was switched to an air reservoir containing 5% CO_2 and 21% O_2 and the subjects were asked to breathe spontaneously for 3 minutes. The air reservoir was continuously filled from a cylinder whose air pressure was manually adjusted to subject's respiratory volume. The MCA-BFV was recorded during the last minute of hypercapnia.

The MCA-BFV waveform was manually traced by a single investigator who was blinded to subject characteristics and study design (Image 2D). Time-averaged peak velocity (i.e., area under curve of BFV waveform) was recorded from at least 10 cardiac cycles in normocapnic, hypocapnic, and hypercapnic steady states. Because transcranial Doppler does not measure blood flow per se, cerebral CO_2 reactivity index (CVRi) was calculated as a percent change in MCA-BFV over an absolute change in end-tidal CO_2[43]. The percent change in MCA-BFV has been reported to have a strong correlation with an absolute change in cerebral blood flow measured by intravenous Xenon dilution technique[44]. The change in CVRi was calculated from the three different ranges of end-tidal CO_2 levels (Image 3D): normocapnia to hypocapnia (NORM-HYPO), normocapnia to hypercapnia (NORM-HYPER), and hypocapnia to hypercapnia (HYPO-HYPER). The CVRi (HYPO-HYPER) was intended to represent cerebrovascular responsiveness to a wider range of end-tidal CO_2 fluctuation and to eliminate the potential effects of baseline neuronal activity and MCA-BFV on CVRi. In addition to CVRi, cerebrovascular

conductance index (CVCi) was calculated in order to account for the effect of blood pressure on MCA-BFV. The equations used to calculate the cerebrovascular reactivity and conductance indices are shown below:

$$\text{Cerebrovascular reactivity index } (\% * \text{mmHg}^{-1}) = \frac{\%\Delta\text{middle cerebral artery blood flow velocity}}{\Delta\text{End tidal carbon dioxide}}$$

$$\text{Cerebrovascular conductance } (\text{cm} * [\text{sec} * \text{mmHg}]^{-1}) = \frac{\text{Middle cerebral artery blood flow velocity}}{\text{Mean arterial pressure}}$$

$$\text{Cerebrovascular conductance index } (\% * \text{mmHg}^{-1}) = \frac{\%\Delta\text{middle cerebral artery vascular conductance}}{\Delta\text{End tidal carbon dioxide}}$$

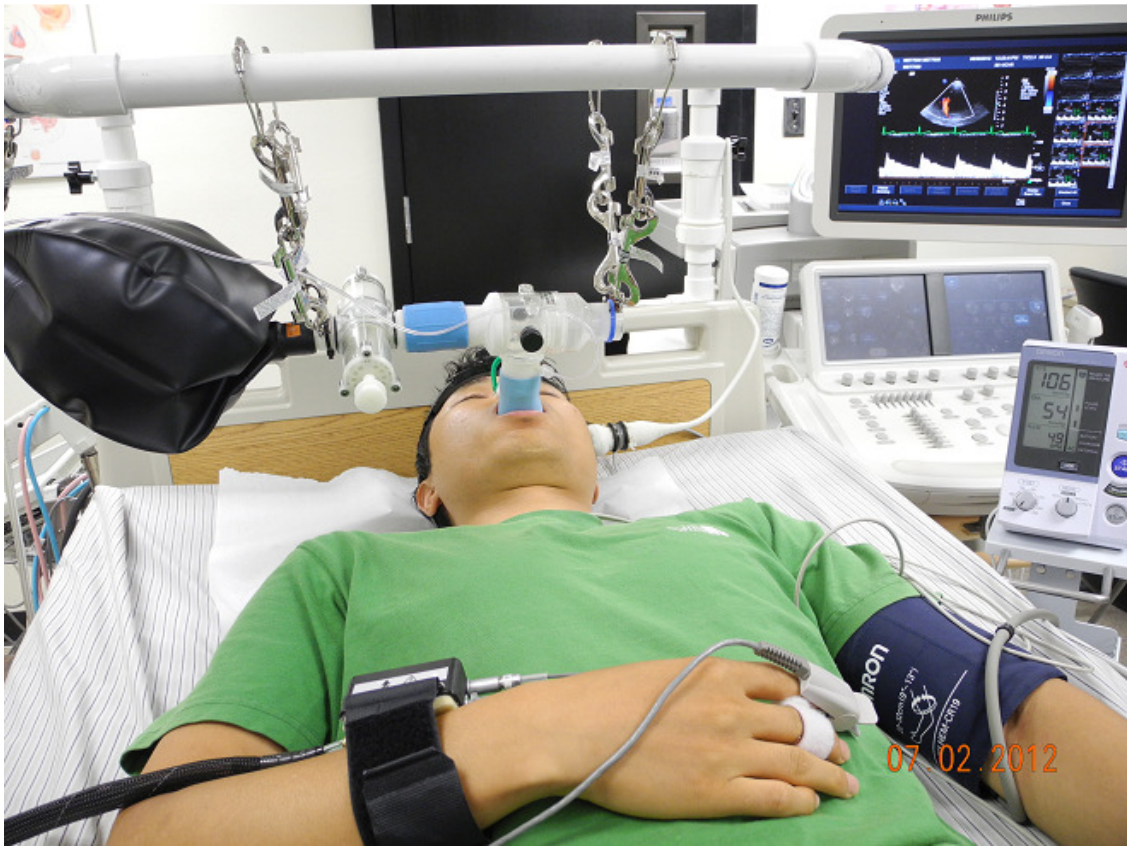


Image 1D: Equipment setup for cerebral CO2 reactivity test

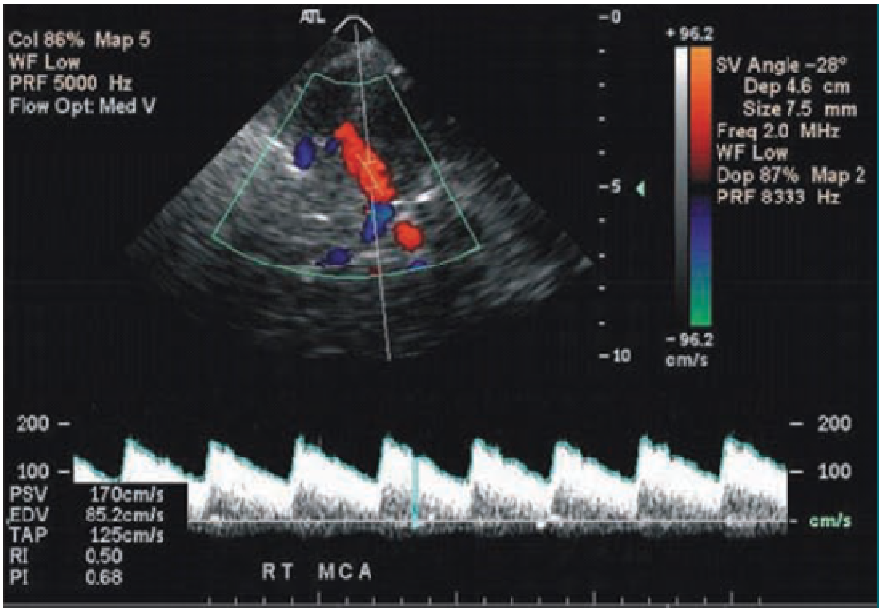


Image 2D: A screen shot of transcranial color-coded duplex ultrasonography. The waveform represents middle cerebral artery blood flow velocity.

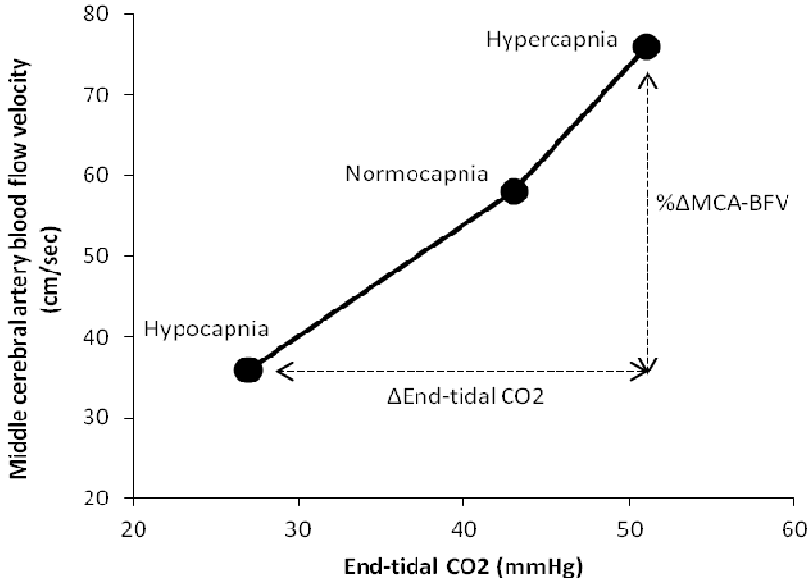


Image 3D: Typical responses of middle cerebral artery blood flow velocity during hypocapnic, normocapnic, and hypercapnic steady states.

Regional Cerebral Blood Flow: Regional cerebral blood flow was measured by arterial spin labeling (ASL) technique using magnetic resonance imaging (MRI)[67-69]. In ASL, arterial blood water is magnetically labeled below the region of interest and used as a tracer. After a period of time (i.e., transit time), this 'paramagnetic tracer' flows into region of interest, exchanges with tissue water, and alters the MR signal and image intensity during which an image is taken (tag image). Then, image acquisition is repeated without labeling arterial blood (control image). During analysis, the control image and the tag image are subtracted to produce a perfusion image. This image will reflect the amount of arterial blood delivered to each voxel within the slice within the transit time, measured in the unit of "ml/100 g/min."

MRI data was acquired using 3T GE Signa Excite scanner. Whole-brain T1-weighted images were collected for anatomical reference (spoiled gradient echo sequence, 256 × 256 matrix, FOV = 24 x 24 cm², 1 mm slice thickness, 0 gap). Perfusion imaging included an ASL sequence with a single-shot spiral readout, cerebrospinal fluid reference scan, and a minimum contrast scan[70, 71]. Cerebral blood flow was computed by subtracting the tag/control image series as described above (CBFv3.2, Function Biomedical Informatics Research Network). These images were corrected for field inhomogeneities using the minimum contrast scan and converted to physiological units (ml/100 ml/min) using the reference signals[70, 71]. Average cerebral perfusion was calculated for bilateral *a priori* regions of interest chosen for their documented susceptibility to cerebrovascular disease[72, 73]. Spherical regions of interest, 5 mm in diameter, were automatically created around the central coordinate for the chosen regions

according to the Talairach and Tournoux atlas[74]. using the Analysis of Functional NeuroImages (AFNI) software[75].

Pulse Wave Velocity: Carotid-femoral, brachial-ankle, and femoral-ankle pulse wave velocities (cfPWV, baPWV, and faPWV respectively) were measured by the Colin VP-2000 (Colin Medical Instruments; San Antonio, Texas). Subjects rested quietly for 10 minutes in the supine position while laboratory personnel placed bilateral brachial and ankle blood pressure cuffs, electrocardiogram and phonocardiogram electrodes, and arterial pressure tonometers in the correct anatomical locations. The Colin VP-2000 instrument also records heart rate, heart sound, and blood pressures. Applanation tonometry incorporates an array of 12 micropiezoresistive transducers to detect pressure waveforms. The time it takes for the wave to travel between the 2 tonometers, and the distance between the tonometers was used to calculate PWV (see Image 4D)[265]. Heart rate, bilateral brachial and ankle blood pressures, carotid and femoral pulse waves were measured for 30-seconds at least 3 times per testing period[62].

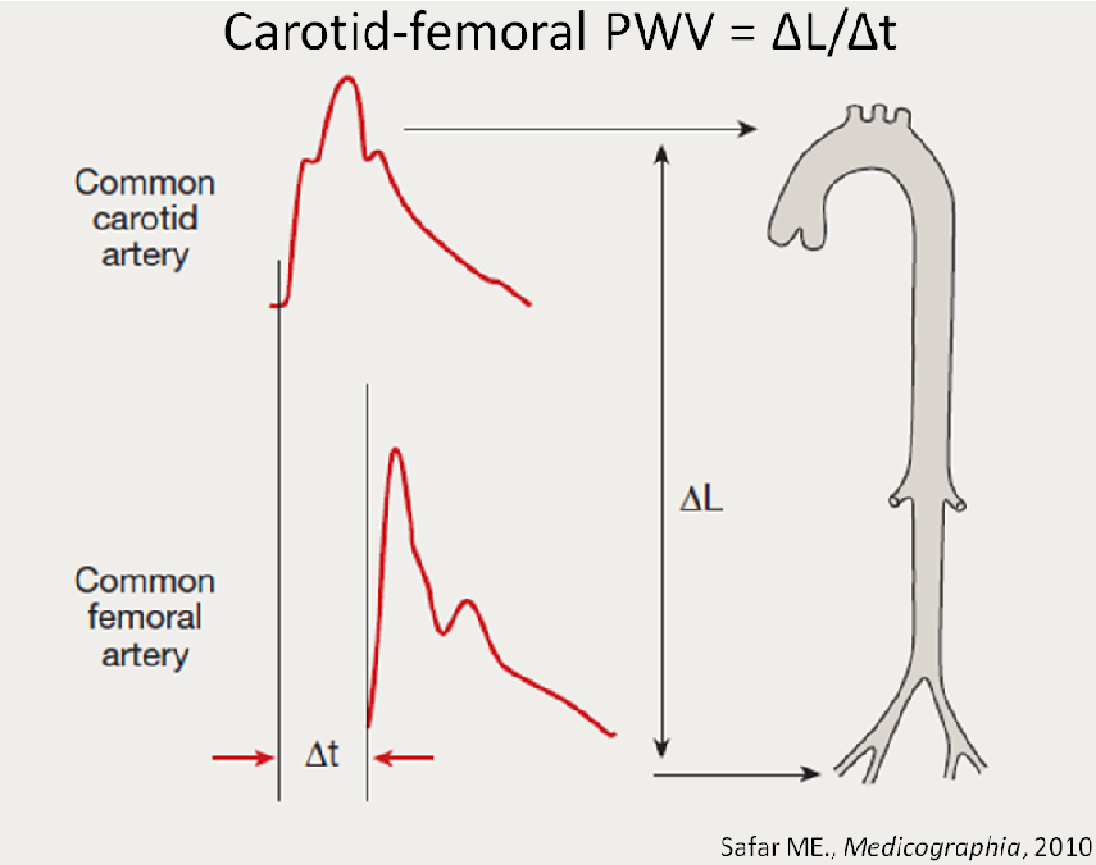


Image 4D: The methodological concept describing the pulse wave velocity measurement.

Carotid Arterial Distensibility, β-stiffness index, and Young's modulus: Arterial distensibility, β-stiffness index, and Young's modulus, parameters of local arterial stiffness, were non-invasively measured from common carotid artery in the supine position[66]. The common carotid artery was first located from a cross-sectional image of the neck, using an ultrasound machine equipped with a high-resolution linear array transducer (Phillips iE33 Ultrasound System, Bothel, WA). Once the bifurcation of the common carotid artery is determined, the transducer was rotated to display a longitudinal

image of the cephalic portion of the common carotid artery where the bifurcation and a linear segment of the artery are both displayed on the screen (See Image 5D). Once the ultrasound image of the carotid artery is optimized for diameter detection, a second investigator located the contralateral carotid artery for assessment of blood pressure using a high-fidelity applanation tonometer (Colin VP-2000, Colin Medical Instruments, San Antonio, Texas). To correct for investigator hold-down pressure, carotid pressure waveforms were calibrated to brachial mean and diastolic arterial blood pressures. Simultaneous measurement of carotid diameters and carotid blood pressure were obtained. If the image or the pressure waveforms were not satisfactory, the process was repeated. Images were captured for at least 20 heart cycles and transferred to an offline computer for analysis (Vascular Tools 5-Carotid Analyzer, Medical Imaging Applications, Coralville, IA). Diameter measurements of the carotid artery were taken approximately 1-2 cm proximal to the carotid bulb from the media-adventitia of the far wall to the media-adventitia of the near wall by one investigator. Ten to thirty heart cycles were analyzed. The equations used to calculate arterial distensibility, β-stiffness index, and Young's modulus are shown below:

Arterial distensibility ($mmHg^{-1}$) =

$$\frac{(\text{Systolic arterial lumen area}) - (\text{Diastolic arterial lumen area})}{(\text{Local pulse pressure}) * (\text{Diastolic arterial lumen area})}$$

$$\text{B-stiffness index (U)} = \frac{\ln\left(\frac{\text{Local systolic blood pressure}}{\text{Local distolic blood pressure}}\right)}{\frac{\text{Systolic arterial diameter} - \text{Diastolic arterial diameter}}{\text{Diastolic arterial diameter}}}$$

Young's modulus

$$= \frac{\text{Local pulse pressure} \times \text{Mean arterial diameter}}{(\text{Systolic arterial diameter} - \text{Diastolic diameter}) \times \text{Local arterial wall thickness}}$$

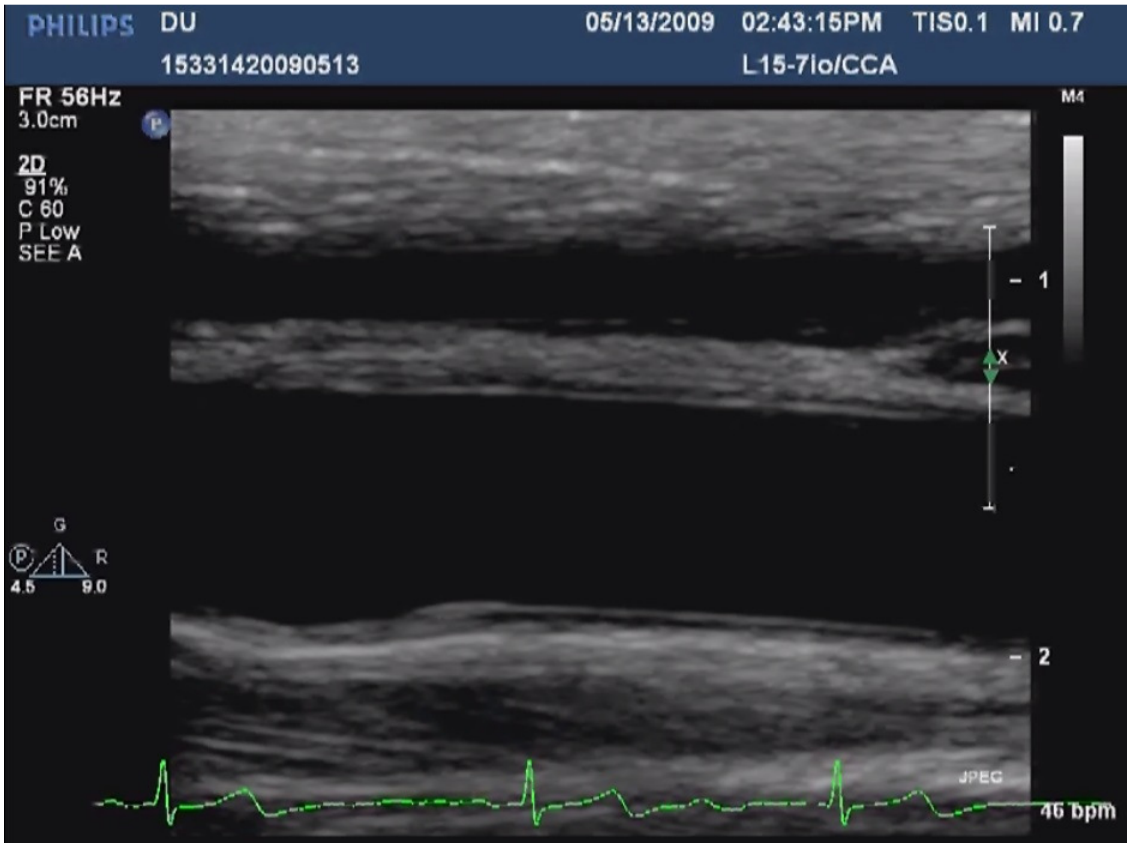


Image 5D: Linear segment of the common carotid artery.

Flow-Mediated Dilation (FMD): Endothelium-dependent vasodilation was assessed by FMD using noninvasive, standardized procedures[42]. This non-invasive test measures vascular endothelial function of the brachial artery by quantifying the amount of arterial vasodilation in response to reactive hyperemia. The subject rested in the supine position

for at least 10 minutes before setting up to measure FMD. The right arm was extended and placed in a customized arm support system to prevent movement of the arm and to standardize the position of the ultrasound transducer (See Image 6D). Brachial artery diameters and blood flow velocity were measured from images derived from a Doppler ultrasound machine equipped with a high-resolution linear array transducer (Philips iE33 Ultrasound System, Bothel, WA). Once the subject was resting in a comfortable position, the pneumatic arm cuff was placed on the forearm, 3-5 cm distal to the antecubital fossa and connected to a rapid cuff inflator (E20 Rapid Cuff Inflator, D.E. Hokanson; Bellevue, WA Hokanson). Once a longitudinal image of the brachial artery, 5-10 cm proximal to the antecubital fossa was obtained, the arm stabilizer was secured. One minute of baseline brachial artery diameter and blood flow velocity were recorded prior to cuff inflation. The arm cuff was then inflated to 100 mmHg above resting systolic blood pressure (measured prior to baseline image capture) for 5 minutes. Blood flow velocity was recorded 10 seconds prior to cuff deflation, and 20 seconds after cuff deflation. At 20 seconds after release of the cuff, the ultrasound was switched to 2D mode to optimize the image for brachial artery diameter measurements for the next 160 seconds. The image files were transferred to an offline computer and stored for later data analysis using commercially available image analysis software (Brachial Analyzer, Medical Imaging Applications; Coralville, IA). Brachial arterial diameter during end-diastole, as determined from the ECG trace, was taken from the media-adventitia interface on the near wall to the media-adventitia far wall boundary. Brachial image analysis was performed by the same investigator. The magnitude of change in end-diastolic diameter

was expressed as an absolute percentage of flow-mediated dilation (see equation below). To calculate baseline diastolic diameter, at least 10 cardiac cycles with clear media adventitia boundaries were averaged (See image 7D). Peak diastolic diameter was taken from the average of 3 consecutive cardiac cycles demonstrating the largest brachial artery dilation (See image 8D).

Flow-mediated dilation (%) =

$$\frac{\text{Peak diastolic diameter} - \text{Baseline diastolic diameter}}{\text{Baseline diastolic diameter}}$$

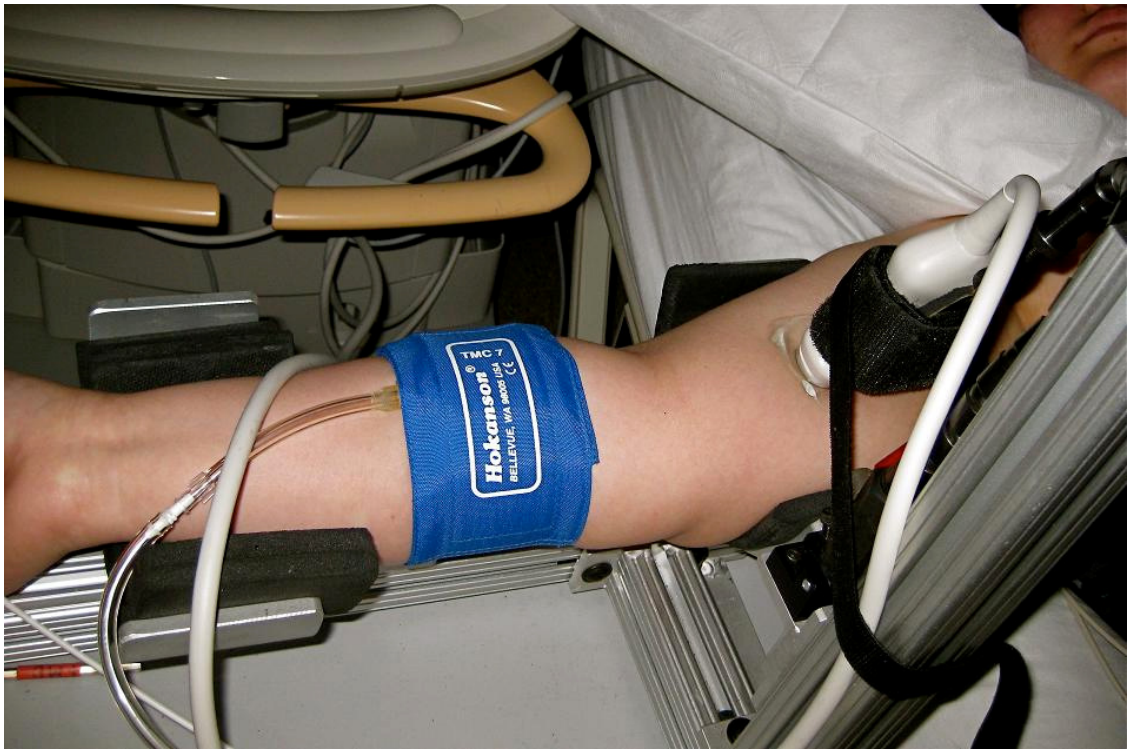


Image 6D: Arm support system used for FMD data collection.

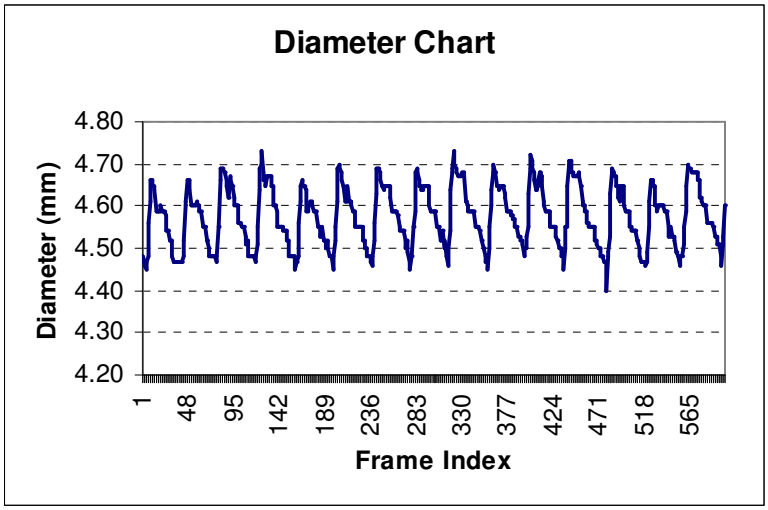


Image 7D. Brachial analyzer software baseline diameter output.

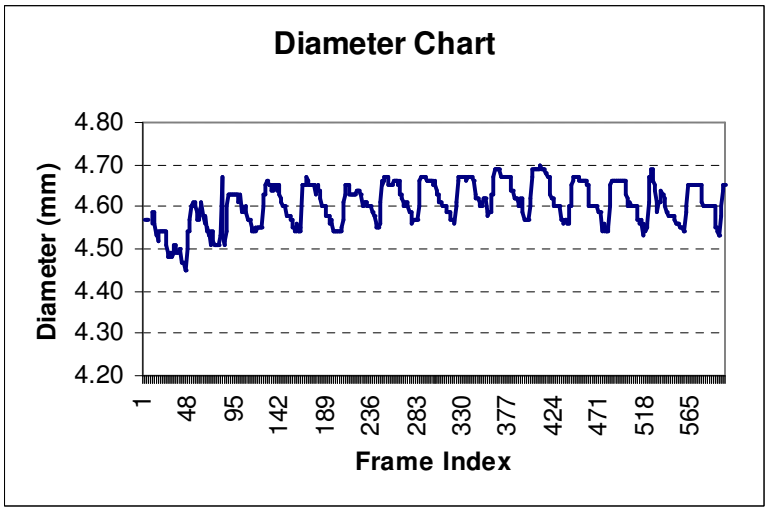


Image 8D. Brachial analyzer software FMD diameter output.

Appendix E: Analysis of Blood, Plasma, and Serum

Blood Sample Collection and Storage: 16-20 mL of whole blood sample was drawn from the antecubital vein using venapuncture from each subject. Whole blood was drawn into serum separator tubes (BD Vacutainer 4mL, Fisher Scientific # 02-683-93A) and EDTA plasma tubes (BD Vacutainer 4mL, Fisher Scientific catalog # 02-689-4). EDTA plasma tubes were centrifuged at 3500 rpm (Eppendorf 5702R, Westbury, NY) for 10 minutes at 4°C before distributing into microcentrifuge tubes for storage at -80°C. Serum separator tubes were allowed to clot at room temperature for at least 30 minutes before centrifuging for 20 minutes at 4400 rpm at 4°C. After separation, serum aliquots were dispersed into microcentrifuge tubes and stored at -80°C for later analysis.

Metabolic Risk Factors: Glucose, triglyceride, and cholesterols (i.e., total, HDL, and LDL) were measured from 35 uL of whole blood using standard enzymatic technique (Cholestech LDX system, Cholestech Corporation, Hayward, CA).

Hemoglobin A1c (HbA1c), or glycosylated hemoglobin, is a measurement of long-term (90-120 days) glucose control. HbA1c was measured from 10 μL of whole blood using a Micromat II Hemoglobin A1c Instrument (BioRad Laboratories, Hercules, CA). Insulin was measured in duplicate from plasma samples using commercially available RIA (radioimmunoassay) kit (MP Biomedicals, Orangeburn, NY).

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