Protocol Title: Effect of Exercise Training on Physical, Cognitive and Behavioral Function in Patients with Traumatic Brain Injury
Abbreviated Title: Exercise and cognition
Protocol Number: 15-CC-0164
Date of This Submission/Version: Version 15 (2/7/2023)

Principal Investigator

Diane Damiano, PhD	FAB/RMD/ CC	10 CRC/1-1469	301.451.7544	damianod@cc.nih.gov

Lead Associate Investigator

Lisa Chin, PhD	RMD/CC	10 CRC/1-2341	301.443.9072	lisa.chin@nih.gov

Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff: None

Total requested accrual: 100 subjects (80 TBI Patients and 20 healthy volunteers)
N = 26 Wait-list control
N = 27 Aerobic Exercise Intervention
N = 27 Rapid-Resistive Exercise Intervention
N = 20 Healthy Volunteer Controls

Project Uses Ionizing Radiation: ☒ No ☐ Yes (*attach RSC/RDRC documentation)*
- ☐ Medically-indicated only
- ☐ Research-related only
- ☐ Both

IND/IDE ☒ No ☐Yes *(attach FDA documentation)*
Drug/Device/#_____
Sponsor: _____

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☐ No ☒ Yes
Institution#1: Center for Neuroscience and Regenerative Medicine
FWA #_ A00001628_____
Date of IRB approval _September 15, 2016____*(attach IRB documentation)*

Institution#2: Department of Veterans Affairs Medical Center, Washington DC
FWA # A00002475_ _____
Date of IRB approval _January 19, 2018_____*(attach IRB documentation)*

Data and Safety Monitoring Board ☐ No ☒ Yes - Monitor

John Leddy, MD
University Sports Medicine
State University of New York at Buffalo
160 Farber Hall, Buffalo, NY 14214
Office Phone: 716-829-5501
e-mail: leddy@buffalo.edu

Technology Transfer Agreement ☐ No ☒ Yes – see Appendix 12
Agreement type and number
Collaboration agreement with DCVAMC Expiration Date__4/19/2022_

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form: _10.0_____

Precis

Objective

The broad objective of this study is to examine the effects of moderate and more intense aerobic exercise as an intervention on cognitive performance, physical functioning and health-related quality of life in patients with chronic (more than 12 months post-injury) traumatic brain injury (TBI). Importantly, structural and biological brain changes will be measured to examine whether functional outcomes are related to exercise-induced adaptations. It is hypothesized that in the chronic phase of persons with TBI, there will be improved: 1) cognitive function, 2) physical fitness and fatigue severity, 3) motor performance and balance, and 4) mood and depressive symptoms, in those that performed the exercise intervention compared to a control group. It is also hypothesized that these functional improvements will be related to exercise intensity, improved cortical connectivity, dopamine transmission gene scores, and blood biomarkers related to neuro- and angio-genesis.

Study Population

80 ambulatory adults with non-penetrating TBI will be enrolled. We will also enroll up to 20 healthy volunteers as a comparison group for some of the outcome measures. Subjects will be recruited from NIH, affiliated hospitals/clinics and the community.

Design

Healthy volunteers will have a limited assessment that includes brain imaging, blood draw for genetic testing, and a subset of the cognitive and behavioral testing at a single time-point. All subjects with TBI will perform baseline assessments including cognitive and behavioral performance, brain imaging, fitness, motor and balance testing, and selected blood and genetic testing. Thereafter, subjects with TBI will be randomized to either a waitlist control, or one of two exercise conditions: 1) 30 minutes at a fast pace, moderate-intensity (rapid-resistive exercise; RET); 2) 30 minutes at higher-intensity (aerobic exercise; AET). Both exercise groups will perform the exercise on an elliptical trainer 3 times a week, for a session duration of 45 minutes including warm-up and cool-down. The RET group will focus on rapid reciprocal motion with minimal resistance, while the AET group will exercise at an elevated intensity known to produce an aerobic effect. After 12 weeks, all groups will repeat the baseline assessments (3 month follow-up). Following this assessment, the waitlist control group will be randomized to either RET or AET and the exercise groups will cease formal supervised exercise sessions. A third assessment visit will be performed after an additional 12 weeks (6 month follow-up).

Outcome Measures

Cognitive performance will be tested and interpreted compared to norms. Performance on motor and balance tasks will be assessed with the Smart Balance Measurement System and the GAITRite System. Physical fitness will be determined by peak oxygen consumption and aerobic threshold as measured by pulmonary gas exchange during an exercise tolerance test on the treadmill. Structural brain volumes will be determined by magnetic resonance imaging (MRI) and cortical connectivity will be quantified using resting state functional MRI and Diffusion Tensor Imaging (DTI) to evaluate integrity of and changes in white matter tracts in response to exercise and compared to healthy

volunteers. Blood will be collected to quantify the presence of biomarkers (such as VEGF, BDNF and IGF-1) and dopamine transmission, and compared to healthy volunteers. Other self-reported measures of quality of life, fatigue severity, depression and sleep quality would also be collected, and compared to healthy volunteers.

Table of Contents

List of Abbreviations

ACSM	American College of Sports Medicine
ADT	Adaptation Test
AE	Adverse Event
AET	Aerobic Exercise Training
AI	Associate Investigator
ANOVA	Analysis of Variance
AT	Anaerobic Threshold
BDI	Beck Depression Inventory
BDNF	Brain-Derived Neurotrophic Growth Factors
BOLD	Blood Oxygen Level Dependent
Bpm	Beats per minute
BSI	Brief Symptom Inventory
BVMT	Brief Visual Memory Test
CC	Clinical Center
CDE	Common Data Element
CNRM	Center for Neuroscience and Regenerative Medicine
COG	Center of Gravity
COMT	Catecholomethyltransferase
CPET	Cardiopulmonary Exercise Test
CRADA	Cooperative Research and Development Agreement
CRIS	Clinical Research Information System
CVLT	California Verbal Learning Test
DAT	Dopamine Transporter Protein
DCVAMC	Washington DC Veterans Affairs Medical Center
D-KEFS	Delis-Kaplan Executive Function System
DoD	Department of Defense
DT	Dual Task
DTI	Diffusion Tensor Imaging
EKG	Electrocardiogram
ETOH	Ethyl Alcohol
FSS	Fatigue Severity Scale
FTT	Finger Tapping Test
GCS	Glasgow Coma Scale
GMU	George Mason University
GUID	Global Unique Identifier
HR	Heart Rate
HRR	Heart Rate Reserve
HRQOL	Health-Related Quality of Life
HSPU	Human Subjects Protection Unit
IGF-1	Insulin-like Growth Factor-1
LOC	Loss of Consciousness

LOS	Limits of Stability
MCT	Motor Control Tests
MET	Metabolic Equivalent
MRI	Magnetic Resonance Imaging
MSVT	Medical Symptom Validity Scale
NBSI	Neurobehavioral Symptom Inventory
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
PASAT	Paced Auditory Serial Addition Test
PI	Principal Investigator
PSQI	Pittsburgh Sleep Quality Index
PTA	Post-Traumatic Amnesia
QOLIBRI	Quality of Life after Brain Injury
RET	Rapid-Resistive Exercise Training
RMD	Rehabilitation Medicine Department
RPM	Revolutions per Minute
SAE	Serious Adverse Event
SOT	Sensory Organization Test
SSRT	Seashore Rhythm Test
SWLS	Satisfaction with Life Scale
TBI	Traumatic Brain Injury
TBI-QOL	TBI Quality of Life
THR	Target Heart Rate
TMT	Trail Making Test
ToPF	Test of Premorbid Functioning
USU	Uniformed Services University
USUHS	Uniformed Services University of Health Sciences
VEGF	Vascular Endothelial Growth Factors
VO2	Oxygen Consumption
WR	Work Rate
WRIISC	War Related Illness and Injury Study Center
ZCG	Impedance Cardiogram

1. Introduction

Over 1.7 million people sustain a traumatic brain injury (TBI) each year, with more than 50,000 resulting in deaths [1]. Almost 80% are treated and released from emergency departments [1], however over 100,000 will further experience long-term disability [2]. TBI costs the United States approximately $76.5 billion in direct and indirect costs [3], which includes medical costs from acute care and rehabilitation, and loss of productivity due to an inability to work.

TBI may result in physical impairments, however neuropsychological problems affecting cognition, emotional functioning and behavior are often more problematic. Particularly in those with mild TBI, subtle functional and cognitive deficits may be present that often go undetected in the acute setting or by routine clinical examination. These patients can experience drastic changes in their quality of life [4], and have difficulties returning to daily activities and work [5].

This study was initially sponsored by the Center for Neuroscience and Regenerative Medicine (CNRM) and received CNRM funds directly for the conduct of the study. CNRM sponsorship for this study has ended. The study will continue in collaboration with CNRM. Under this collaboration, the study will continue to use CNRM Core resources including the CNRM Biospecimen Repository and CNRM Informatics Data Repository.

1.1 Statement of the Problem

The aim of rehabilitation of individuals with chronic TBI is to restore functioning, promote neurobehavioural and psychosocial skills for re-integration into the community [6] and to return to work [2]. However, the heterogeneous mechanism of injury that occurs with TBI presents a major challenge to researchers and clinicians for optimal rehabilitative strategies and management of those with TBI. Another problem is the difficulty in comparing post-acute interventional studies because of differences in the method of data collection, outcome measures and intervention period [6]. While progress has been made in this area in recent years, research studies are needed to further develop rehabilitation strategies and our understanding of the clinical management of TBI. Considering the large proportion of patients that would benefit from rehabilitation, the need for specific interventions that would improve their quality of life is imperative.

1.2 Importance of the Problem

It is well recognized that physical exercise offers many benefits in both general and clinical populations. Besides the obvious cardiovascular and metabolic adaptations that occur with exercise training, physical activity has also been related to diminished cerebral inflammation and enhanced brain plasticity [7]. Exercise increases the release and synthesis of neurotrophic factors related to cognitive functioning, neurogenesis, angiogenesis and plasticity. Among these include the brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1) and vascular endothelial growth factor (VEGF).

In the animal model, deficits associated with TBI such as neuronal loss in the hippocampus [8, 9], have been found to be attenuated by exercise. A well studied and reproducible finding in the animal model is exercise-related hippocampal neurogenesis [10], which is believed to be linked to the commonly observed improvements in learning and memory following a brain injury [11, 12]. BDNF is involved in hippocampal neuronal plasticity, thus plays a major role in exercise-induced cognitive improvements [11]. Exercise stimulates BDNF gene expression in the hippocampus of the rat [13-15] and is volume-dependent, such that greater BDNF is expressed with more exercise [13]. The presence of BDNF is of primary importance, as inactivation or even the absence of an increase in BDNF is related to attenuation of the exercise induced improvements in cognitive performance in TBI rats [11, 16].

Despite encouraging findings in the animal model with exercise interventions, there are very few studies that extend this research to the TBI population. Hippocampal volume has been observed to be reduced following TBI [17-20] with greater hippocampal atrophy related to reduced memory performance [18, 20, 21]. However, there are currently no studies in the literature that have examined the effect of exercise on hippocampal volume and BDNF levels in patients with TBI. Studies in other populations such as normal aging and schizophrenia have demonstrated exercise-induced increases in hippocampal volume [22, 23], while exercise training has been associated with increased resting BDNF levels in young adults [24, 25]. The combined body of evidence from TBI animal studies and those of other disease states in humans suggest that this line of research needs to be extended to the TBI population.

Review of the current literature on exercise interventions is also conflicting, with results being equivocal or the lack of studies on specific interventions making it unreasonable to draw conclusions. For instance, cognitive function in TBI patients was found to be either improved [26] or unchanged [27] following 4 weeks of exercise training, while fewer cognitive symptoms were reported by TBI patients that were considered to be exercisers based on self-reported physical activity [28]. A major problem in these studies is that the exercise was mostly unsupervised [27], or based on the subject's reported perceived exertion [26] or exercise habits [28].

The intensity of exercise that produces the greatest benefit in patients with TBI is also currently unknown. While aerobic exercise training is generally viewed as having the greatest effect from a cardiovascular and metabolic perspective, this level of intensity may not be appropriate for all individuals with TBI. Exercising at a higher level of intensity is related to greater cardiorespiratory fitness, however the effect of exercise on cognitive performance is not strictly dose-dependent. Improved cardiorespiratory fitness is not always associated with greater cognitive improvements [29]. Indeed, exercise in the light to moderate intensity domain has been suggested as being ideal for stimulating hippocampal neurogenesis [30]. Considering that individuals with TBI experience wide variability at the cellular, macro and outcome-level, the exercise prescription should take into account these concerns [7]. Currently, there is not enough research in this area to warrant recommendations for intensities of physical exercise for optimal rehabilitative outcomes for patients with TBI.

The existing data from the literature with regards to exercise training in chronic TBI patients are sparse. There is a need for clinical research with an integrative approach that examines neuroplastic changes that occur concurrently with objective behavioural measures across multiple functional domains.

1.2.1 Preliminary Results

Two recent pilot studies were performed in the Rehabilitation Medicine Department in patients with chronic TBI. The findings are discussed below:

11-CC-0088: Effect of aerobic exercise training on cardiorespiratory function in patients with TBI

In this study, results from 10 subjects (age, 33 ± 7 years; mean ± SD) suggest that patients with TBI are able to demonstrate improvements in cardiorespiratory function with a training regimen that is generally prescribed to the general population. Specifically, patients increased their time to volitional fatigue, attained higher peak O_2 consumption and demonstrated improved aerobic capacity (Table 1). Patients exercised on a treadmill for 30 minutes at an intensity considered to be "vigorous" (i.e., heart rate range of 70 - 80 % heart rate reserve), 3 times a week for 12 weeks. Despite the challenging training regimen, patients were able to adhere and maintain their target intensity throughout the session duration (Table 2). These findings have been published and are available ahead of print [31].

Table 1. Results of the Cardiopulmonary Exercise Test

CPET Variables	Pre	Post	Δ	P-Value
Time to peak VO_2 (s)	982 (113)	1066 (142)	+84 (45)	**< 0.001**
Peak VO_2 (ml/kg/min)	37.1 (8.2)	40.2 (8.1)	+3.1 (2.4)	**0.003**
Peak WR (W)	324 (90)	383 (104)	+59 (48)	**0.005**
Time at AT (s)	556 (92)	662 (92)	+106 (63)	**< 0.001**
VO_2 at AT (ml/kg/min)	18.9 (4.1)	22.5 (3.5)	+3.6 (2.1)	**< 0.001**

Abbreviations: VO_2, oxygen consumption; WR, work rate; AT, anaerobic threshold

Table 2. Summary of Aerobic Exercise Training

	Group Mean
Adherence, %	93 (4)

Average training heart rate, bpm	155 (7)
% HRR	78 (2)
Total AET time, min	1005 (38)

Abbreviations: HRR, heart rate reserve; AET, aerobic exercise training

Neuropsychological testing was also performed on 7 consecutive subjects and these results are available ahead of print [32]. Significant improvements ($p < 0.05$) were observed for processing speed and executive functioning following AET, as measured with the Trail Making Test A and B (Figure 1). Overall cognitive function, including those specific to delayed memory and visuospatial/constructional was also improved after the exercise intervention (Figure 2).

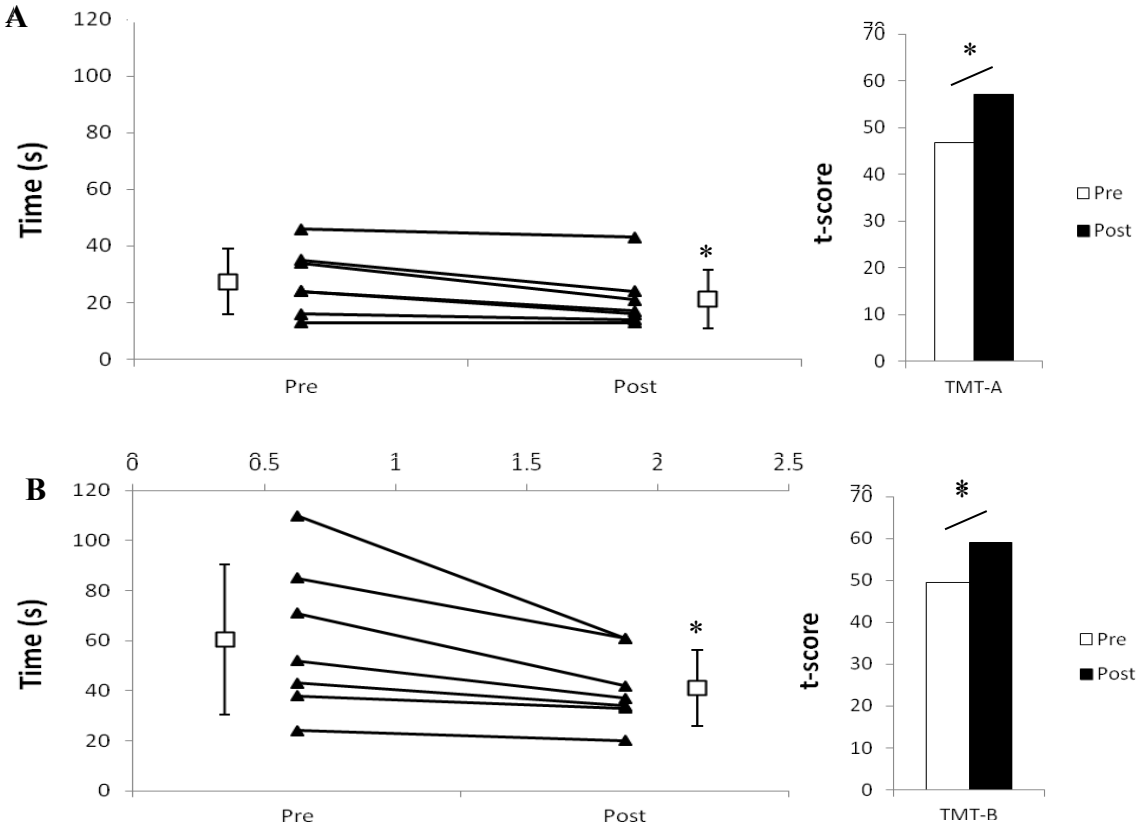

Figure 1. Time taken to complete the Trail Making Test (TMT) A and B (left), with standardized t-scores (right) shown before and after AET.

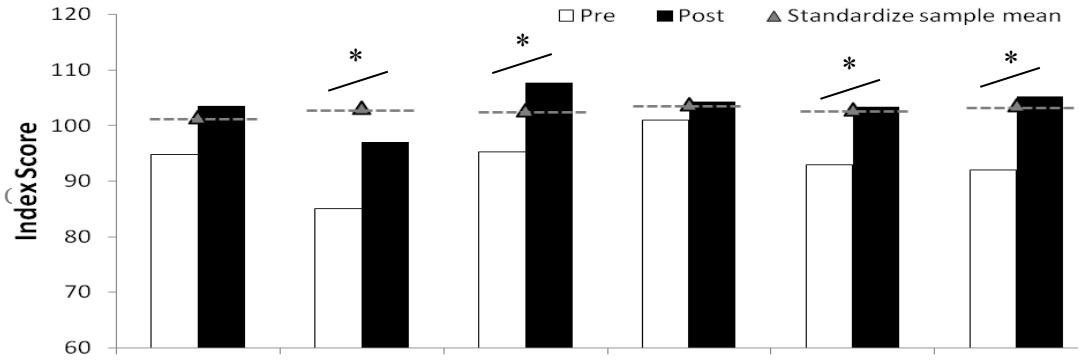

Figure 2. Averaged index scores for the Repeatable Battery for Assessment of Neuropsychological Status (R-BANS) at pre- and post-AET. Symbol with lines indicate the mean for a standardized sample based on age (20 – 49 years) and education level (> High school).

Results suggest that patients with TBI are able to safely perform intense aerobic exercise and gain not only physical benefits but likely mental improvements as well.

10-CC-0150: Effects of rapid-resistive exercise on ambulatory adults with traumatic brain injury

In this study, 10 patients (age, 32 ± 9 years) exercised on an elliptical trainer that focused on fast reciprocal motion of the arms and legs. Patients were instructed to perform the exercise 5 days per week for 30 minutes, over the course of 8 weeks. This was a non-aerobic exercise training program, where speed was gradually increased with slight resistance added as patients progressed.

Table 3. Compliance with exercise by patient-report and monitoring device on the pedal

Subject	# Training Sessions		% Completed	
	Patient Report	Monitor	Patient Report	Monitor
1	37	35	92.5%	87.5%
2	37	31	92.5%	77.5%
3	40	42	100.0%	105.0%
4	40		100.0%	-
5	40	22	100.0%	55.0%
6	40	40	100.0%	100.0%
7	40	40	100.0%	100.0%
8	no sheet	36	-	90.0%
9	37	37	92.5%	92.5%
10	40	26	100.0%	65.0%
11	39	32	97.5%	80.0%
12	40	7	100.0%	17.5%
AVERAGE	39	31.2	97.5%	78.0%

Preliminary results from this study showed significant improvement in the automatic and voluntary control of balance in TBI patients. The voluntary control of balance assessed with the Limits of Stability test measures the reaction time (Fig. 3A) and center of gravity (COG) displacement (Fig. 3B) of patients to respond to a command to lean as far and as

quickly as possible towards a target while standing without moving their feet. Reaction time was faster following rapid-resistive exercise training (RET) (Fig. 3A) and COG displacement was larger (Fig. 3B). The automatic control of balance assessed with the Motor Control Test measures the automatic reaction time (latency of response) between a translation movement in the forceplate and the torque at the ankle to regain balance during standing on a forceplate (Fig. 3C). Note that the reaction was faster following RET (Fig. 3C).

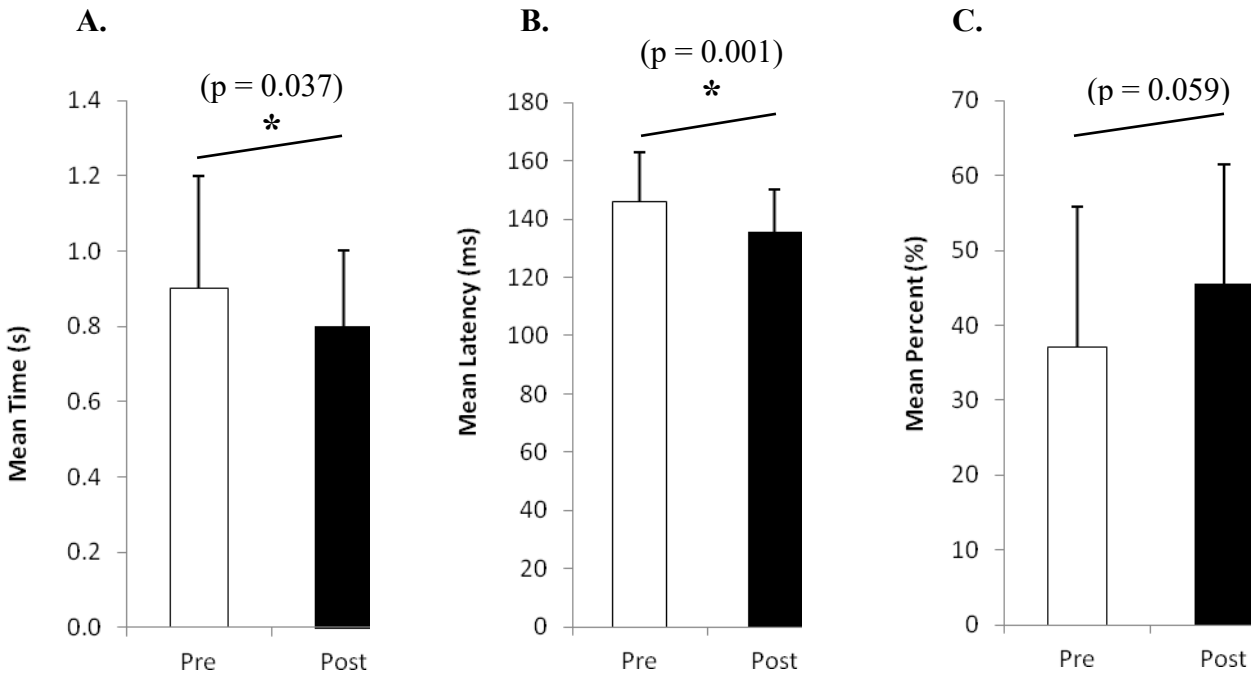

Figure 3. Balance measures performed prior to (Pre) and following (Post) RET. Measures of Limits of Stability test reaction time in seconds (A) and the Motor Control Test latency in milliseconds (B) improved at posttest. Measures of displacement of the COG expressed as a percentage of the patients theoretical limits of stability (C) also improved at post-test.

Furthermore, a significant correlation was found between a dual task word generation test and automatic balance changes, suggesting that patients with improved reaction time were able to walk faster while talking (generating words).

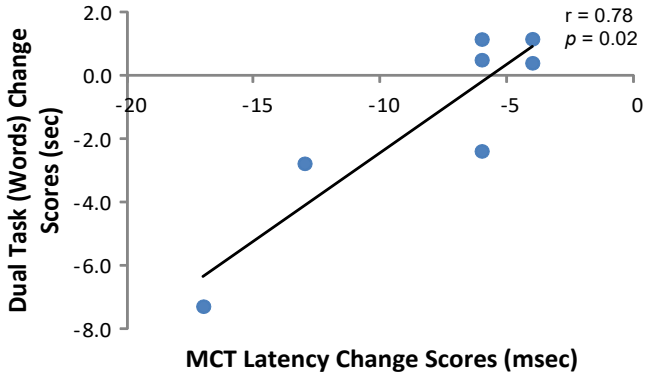

Figure 4. Changes in dual task word generation and mean latency of the Motor Control Task between pre and post RET.

While no significant changes in cognition were observed, 7 of the 10 patients scored within the normal range at baseline which demonstrates an absence of cognitive impairment in the majority of patients enrolled in this study. Therefore, little improvement would be expected from this subset of patients.

Both these pilot studies revealed improvements in different aspects of functioning in a TBI population using different exercise intensities. Cardiorespiratory fitness and cognitive function was improved with vigorous intensity exercise training, while balance and motor control was improved with moderate levels of exercise intensity. Likely activation of different mechanisms/pathways occurred to result in the observed improvements in the respective studies. However, specific outcomes were selected for each study and there was limited overlap in the measured outcomes. Whether similar improvements would be observed regardless of exercise intensity is therefore unknown. Considering the wide range of symptoms and deficits a patient with TBI may experience, the optimal type and intensity of exercise may also differ depending on the patient's level of impairment.

2. Study objectives

The broad objective of this study is to determine the effectiveness of two types of exercise training in patients with non-penetrating TBI for improving cognitive performance, balance, physical fitness, fatigue severity and other behavioral or quality of life outcomes. This study will also measure structural and functional changes in the brain and specific biomarkers known to be involved in brain plasticity as well as to examine genetic factors that may affect responsiveness to the training. This study may also identify the intensity of exercise training that is more highly associated with specific cognitive, functional and structural improvements.

The 2 exercise regimens to be used are as follows:

Aerobic Exercise Training (AET)

Aerobic exercise will be performed using an elliptical trainer. Exercise intensity would be vigorous (~70 – 80% of maximal VO_2) and similar to a previous pilot study (11-CC-0088). A target heart rate range will be used to ensure vigorous intensity is maintained throughout the training session. While treadmill walking was used previously (in 11-CC-0088), adjustment of the resistance on the elliptical trainer will allow for aerobic exercise to be safely achieved with this mode.

Rapid-Resistive Exercise Training (RET)

Rapid, reciprocal exercise will be performed using an elliptical trainer, which allows for safe fast speed training. Similar to the previous study (10-CC-0150), this type of training will involve rapid movement and coordination of all four extremities. Emphasis will be placed on increasing speed, however resistance can be increased to keep the level of exercise at a light to moderate intensity. Exercise intensity would not exceed ~ 50% of maximal VO_2 (moderate intensity).

The objectives of this study are:

- Primary - To evaluate the effects of exercise training intensity on cognitive function in patients with TBI
- Secondary - To evaluate the effects of exercise training intensity on balance, physical fitness and quality of life in patients with TBI
- Secondary - To evaluate the effects of exercise training intensity on structural and functional changes in the brain of patients with TBI
- Secondary - To examine the relationship between cognitive, behavioral, and motor outcomes and brain imaging, biomarker and genetic outcomes or variables
- Secondary – To examine structural/functional brain differences, blood biomarkers and a subset of cognitive and self-reported outcomes between patients with TBI and healthy volunteers within the same age range

Since the direct effects of physical exercise are well known, the primary focus here is on the effects of exercise on cognitive function and on brain connectivity. While we anticipate that exercise regardless of intensity will improve cognitive and behavioral outcomes, we anticipate that the effects will be greater for the aerobic training group such that when the results are analyzed separately for each exercise condition, only the aerobic group will demonstrate a significant change in the primary outcome measure. Study outcomes such as brain imaging, blood biomarkers, cognitive performance and self-reported questionnaires in patients with TBI will also be compared to a healthy volunteer sample matched to the TBI cohort for sex and age within 5 years.

3. Subjects

3.1 Study populations

This study will recruit a maximum of 80 adults (aged between 18 and 79 years old) with non-penetrating TBI and a comparison group of up to 20 healthy volunteers matched to the TBI cohort for sex and age within 5 years. The healthy volunteers will only undergo brain imaging assessments, blood collection and a subset of questionnaires and neuropsychological testing. The healthy volunteers will not participate in the intervention phase. Withdrawals or dropouts will be replaced to have at least 20 subjects with TBI in each group to complete the second assessment visit. An eligibility checklist is included in Appendix 3A and B.

3.2 Inclusion criteria

Inclusion criteria for those with TBI:

1. Ages 18 to 79 inclusive
2. Diagnosis of non-penetrating TBI
3. Injury occurred at least 12 months prior to enrollment
4. Physically inactive as identified by a physician
5. Able to stand and walk independently and safely without any assistance
6. Able to follow the study protocol
7. Fluent in English and able to provide informed consent

Inclusion criteria for healthy volunteers:

1. Ages 18 to 79 inclusive
2. Physically inactive as identified by a physician
3. Fluent in English

3.3 Exclusion criteria

Exclusion criteria for those with TBI:

1. History of exercise intolerance
2. History of heart disease
3. History of pulmonary disease, other than controlled, non-exercise-induced asthma
4. History of uncontrolled diabetes
5. Uncontrolled hypertension, defined as a resting blood pressure > 140/90 mmHg
6. On medications that would influence aerobic capacity or treadmill performance such as beta blockers or antiretroviral therapy
7. Active substance abuse including ETOH
8. Presence of an injury to any extremity, or other medical condition that would affect motor function or the ability to perform the assessment or the exercise program, specifically balance problems due to vestibulopathy
9. Unable to refrain from smoking at least 4 hours prior to exercise testing sessions
10. Medical or psychological instability such that the subject could not reasonably be expected to fulfill the study requirements
11. Pregnancy
12. BMI >40 kg/m^2 due to the limits of the treadmill, elliptical machine and MRI scanner
13. Planning to make a change in medication or therapy during the enrollment period with the goal of improving mood, cognitive function or motor function
14. Have any of the following contraindications to having an MRI scan:
    a. A ventriculo-peritoneal shunt
    b. Have claustrophobia and not comfortable in small enclosed spaces
    c. Have metal that would make an MRI scan unsafe such as: cardiac pacemaker, insulin infusion pump, implanted drug infusion devise, cochlear or ear implant, transdermal medication patch (nitroglycerine),

any metallic implants or objects, body piercing that cannot be removed, bone or joint pin, screw, nail, plate, wire sutures or surgical staples, shunts, cerebral aneurysms clips, shrapnel or other metal embedded (such as from war wounds or accidents or previous work in metal fields or machines that may have left any metallic fragments in or near your eyes).

d. Excessive startle reaction to or fear of loud noises

Exclusion criteria for healthy volunteers:

1. History or presence of cardiopulmonary or respiratory disease
2. History or presence of other disease of the neurologic, metabolic, or renal systems
3. Active substance abuse including ETOH
4. Pregnancy
5. BMI >40 kg/m2
6. Medical or psychological instability such that the subject could not reasonably be expected to fulfill the study requirements
7. Have any contraindications to having an MRI scan

4. Study Design and Methods

4.1 Study Overview

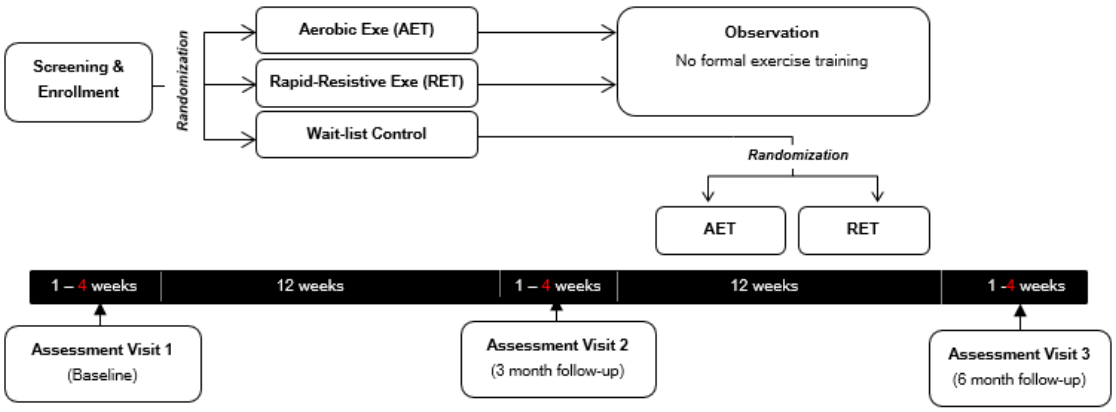

Figure 5. Overview and time point of assessment visits during the study for subjects with TBI.All subjects will be officially enrolled after informed consent, and physician assurance of meeting the inclusion criteria and absence of exclusion criteria. Once enrolled, subjects with TBI will perform the baseline assessment visit consisting of neuropsychological testing, brain imaging scans, physical performance test, motor testing and questionnaires (Figure 5). Subjects with TBI who have completed the same neuropsychological assessments or motor assessments within 3 months under another CNRM study will not be required to repeat those assessments. Instead, we will use the previously obtained assessments as the baseline measures. Healthy volunteers will complete only a single assessment visit consisting of blood collection, brain imaging scans and a subset of questionnaires and neuropsychological testing, which can be done over more than one day at the participants' convenience. Healthy volunteers who have completed the same neuropsychological assessments or within 3 months under another CNRM study may not have to repeat those assessments and we may use the previously obtained assessments instead. The estimated time to complete testing is approximately 6 hours for the healthy volunteers. Following completion of these assessments, study participation ends for the healthy volunteer.

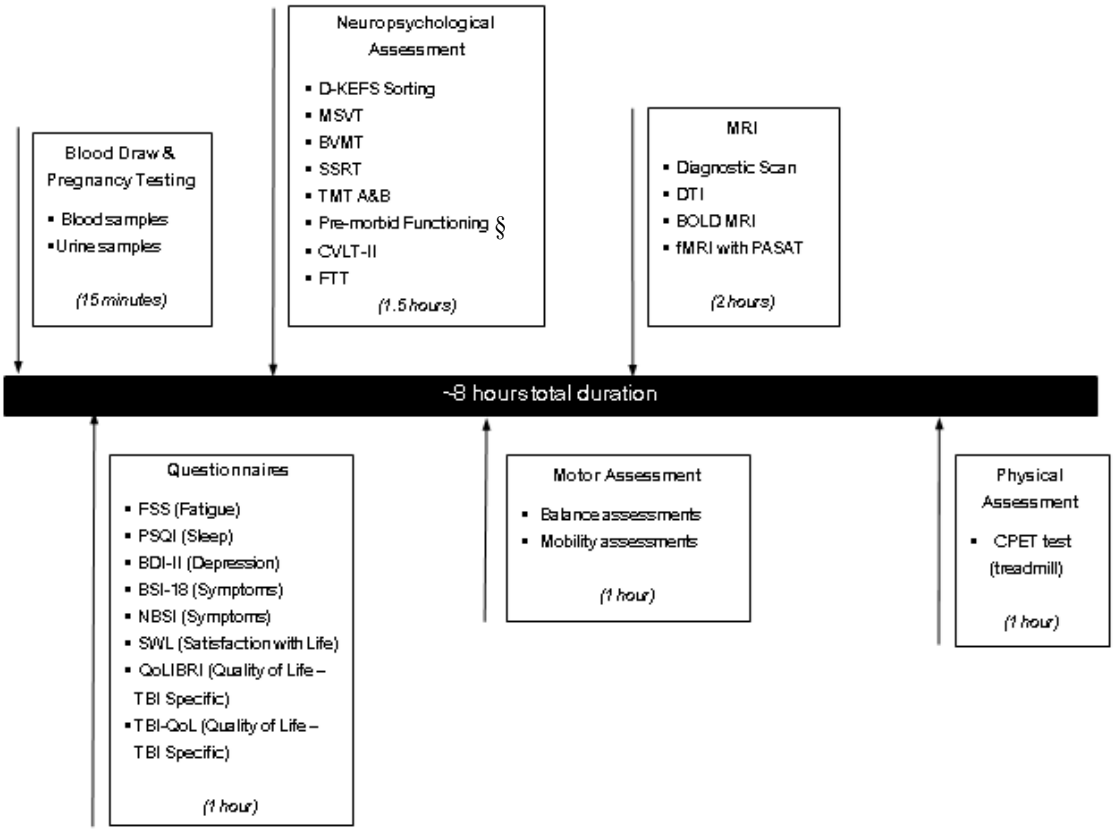

Figure 6. Outline of assessment visit performed within a day for subjects with TBI. Various assessments to be performed are shown with estimated session duration. Order of assessments may vary depending on availability of subject and equipment, however, the physical assessment will never precede another assessment. All assessments will be repeated at the post-intervention visit (3 and 6 months follow-up visits) unless noted otherwise. Assessments are described in detail in section 4.4 below. § indicates this test will be performed at the baseline visit only.

This assessment visit will last approximately 8 hours, including time for rest and meals, and may be performed within one day or over the course of several days within a 4 week period. Upon completion of all baseline assessments, patients will be block randomized to one of three groups: 1) a waitlist control, 2) an exercise intervention consisting of exercise at vigorous intensity (Aerobic exercise training group, AET), or 3) an exercise intervention consisting of exercise at moderate intensity (Rapid resistive exercise training group, RET). Both training interventions will be performed 3 times a week for 12 weeks, with a session duration of approximately 1 hour. After 12 weeks, all patients will repeat the same assessments performed at baseline (3 month follow-up). Patients in the control group will then be randomized to either AET or RET, while patients in the exercise training groups will cease their formal supervised exercise training regimen. All patients will return after 12 weeks to perform their final assessment visit (6 month follow-up).

All assessments and training interventions are outpatient visits and will occur at the NIH Clinical Center in the Rehabilitation Medicine Department.

4.2 Recruitment

Individuals with TBI and healthy volunteers may be recruited from participants that have previously participated in research protocols in the RMD that are led by other NIH investigators, including 10-CC-0188 "Long-term clinical correlates of TBI: Imaging, biomarkers and clinical phenotyping parameters". Recruitment will be limited to participants that had indicated a willingness to be contacted regarding other on-going clinical studies. These participants will be contacted by phone.

The recruitment strategy offered by the NIH/CC Office of Patient Recruitment, including sample recruitment messages and advertisements are attached as Appendix 1A and B.

We will also utilize NIH Clinical Center-sponsored digital media resources such as Twitter, Facebook, CC Radio, CC TV, Photo Gallery; databases such as NIH Search the Studies Clinical Trials.gov and Research Match. Other recruitment methods will involve advertisements in the form of flyers to be posted in healthcare facilities or in the local community with approval of the venue or in accord with their policy. Flyers may be sent using commercially-available mailing lists via direct mail. If used for direct mail, the flyer will identify the source of the mailing list. Flyers for patients with TBI may be posted electronically on websites for brain injury and Veteran support groups (such as Brain Injury Association of Maryland, National Military Family Association, TerpVets, etc), neighborhood blogs (FitnessNow) and publications' websites including the Washington Post, Washington Post Express, Montgomery County Sentinel and Montgomery Magazine. IRB-approved flyers for patients with TBI may also be placed in e-newsletters and community online boards of support or health care organizations for TBI and Veterans. Flyers may be sent electronically to those requesting study information. IRB-approved flyers for patients with TBI will also be shared with moderators of Facebook pages (related to brain injury and Veteran support groups) for posting (Appendix 1). These materials may also be shared with users that are authorized to post messages to the group. Permission for posting of these recruitment material will be made by the moderator, according to the groups' rules and regulations. Direct communication with the administrators or moderators of Facebook pages will not occur through Facebook, rather will be achieved through other approved means (e.g. contact made through recruitment events, interested queries, etc.). These postings are not paid sponsored ads and Facebook will not be paid for "pushing" recruitment material for this study. IRB-approved recruitment message for patients with TBI can also be posted on listservs with the permission of the moderator and IRB-required statement on how the receiver was identified (Appendix 1). Listservs may include schools such as University of Maryland and George Mason University and advocacy or profession groups, such as TerpVets. IRB-approved advertisements in the form of flyers may be placed in local print publications of newspapers, newsletters, magazines and support or health care organizations for TBI, including Washington Post, Express, Gazette, Washingtonian, Bethesda Magazine, Washington Examiner, Military papers, the NIH Record/CC News. The flyers for the advertisements will be used in color as submitted, or may be printed in black and white. The color of the ads may vary. Color changes will not be used to change the emphasis of an ad. The size of the ads may vary, but all parts of the ads, including

fonts and pictures, will be changed proportionately to the rest of that ad. Disproportionate changes in size will not be used to change the emphasis of an ad.

Approved recruitment materials for patients with TBI such as flyers may be left with clinics for interested treating physicians to refer subjects to this study with approval of the venue or in accord with their policy; self-referral is also permitted. Flyers may be given directly to those requesting study information. Recruitment efforts will also include an increased presence at local Brain Injury support groups, meetings, conferences and awareness events to broaden awareness of the research being performed on TBI at the NIH, and among the local community. Flyers may be made available at outreach exhibits, speaking engagements, support group meetings, parent groups, professional meetings or association/trade meetings with approval of the venue or in accord with their policy.

We will also work with the Washington DC Department of Veterans Affairs Medical Center (DCVAMC), specifically the War Related Illness and Injury Study Center (WRIISC) and Polytrauma Unit, to specifically target recruitment of Veterans that may be interested in participating in this study. Dr. Reinhard will lead the DCVAMC site specific recruitment protocol, and approved DCVAMC personnel will use the inclusion/exclusion criteria to identify Veterans who appear to qualify for participation through the Computerized Patient Record System and Clinician Referrals. Identified potential Veterans will be mailed an introductory recruitment letter (see Appendix 13 – DCVAMC Recruitment Letter) and followed up approximately 2 weeks later with a phone call inviting them to participate. As soon as a Veteran indicates that they are interested in participation, DCVAMC study staff will provide the patient with the contact information of the NIH study team for further pre-screening. If the Veteran does not answer the phone call, study staff will leave a message, and then call back a maximum 2 more times—no more than once per day—before ceasing outreach with the veteran. The DCVAMC staff will not provide the consent and HIPAA authorization since Veterans will be acting on their own agency and reaching out to the NIH staff directly for study participation.

4.3 Screening

4.3.1 Pre-Screening

All interested participants will be pre-screened by protocol assistants and other members of the research team for general inclusion/exclusion criteria, by telephone or in person before consent is obtained (Prescreen Questionnaire (inclusion/exclusion criteria and interest); Appendix 2A and B). If the participant meets the inclusion criteria based on the Prescreen Questionnaire, an appointment will be scheduled for the participant to come to NIH CC RMD for a study eligibility evaluation. To prepare for this visit we will ask them for their full legal name, contact information (address, phone number, e-mail address), and date of birth to be entered into the NIH Admissions, Travel and Vouchers program. The medical records of those who appear to meet the study criteria may be requested (such as copies of recent medical evaluations, TBI-related medical history, diagnostic testing/imaging, rehabilitation evaluation and treatment) to further evaluate their inclusion into the study. Should the participant not qualify for the study, copies of these records will be destroyed (i.e., shredded) or returned to the participant.

4.3.2 Screening

Consent form will be obtained before any study procedures, including screening procedures, are done. Screening of healthy volunteers will include a brief history and physical by a credentialed RMD medical staff member to determine study eligibility. The standard MRI screening questionnaire (Appendix 4) will also be used to check for MRI exclusions. For women of child-bearing potential, a urine pregnancy test will be done within 24 hours before the MRI scan with the results available prior to the start of the scan. Formal screening for those with TBI includes a history and physical examination, resting electrocardiogram (EKG), blood sample collection and urine pregnancy testing (in women of childbearing potential). The history and physical examination will be performed by a credentialed RMD medical staff member. All participants with TBI will have a baseline resting EKG performed. Study eligibility will be documented using the subject enrollment checklist (Appendix 3). A MRI screen will be done for all participants using the standard MRI screening questionnaire to check for MRI exclusions (Appendix 4). Should the participant choose not to participate in the study and had not completed the consent process, copies of their medical records will be returned to the participant or destroyed (i.e., shredded). Additional clinical consults and procedures (e.g. repeat EKG, echocardiogram, lab work, etc) may be performed as deemed necessary by medical staff to establish risk for exercise participation, inclusion and safe participation in the study by the subject. An in-person cardiology consult may also be performed for subjects with a family history of coronary artery disease and/or presence of other risk factors for cardiovascular disease. Subjects with TBI will also be screened for vestibular-related balance problems. If the medical staff member suspects vestibulopathy, the subject will be referred for a clinical evaluation, specifically the Sensory Organization Test (SOT) which is performed using the Neurocom Balance Master Equitest System, prior to enrollment. The test involves standing quietly under different conditions that challenge the 3 sensory systems involved in the control of balance: somatosensory, visual and vestibular systems. This test identifies how patients integrate normal and altered inputs from each system for the control of balance. There is a slight risk of falls especially in those with TBI since we are challenging their motor capabilities, but this risk should be no greater than those encountered in everyday life. A harness can be used during balance testing and the examiner will closely guard the patient. If the SOT confirms a deficit in the use of the vestibular system to control balance the subject will not be participating in the exercise program, and we will recommend that they discuss further follow-up for this with their own personal physician. If the test is normal, the subject will be allowed to do the rest of the study and will not need to repeat this test during the balance assessment of the baseline visit. This test will be performed by one of the trained physical therapists in RMD.

4.4 Study procedures

All healthy volunteers will perform biospecimen collection (section 4.4.1), brain imaging assessments (section 4.4.6) and a subset of questionnaires and neuropsychological testing as indicated in section 4.4.2 and 4.4.3 below. All subjects with TBI will perform the following assessments for research purposes at three time points (at baseline, 3 month

and 6 month follow-up; Participant's flowchart, Appendix 5). The general order and duration of the assessments to be performed during the testing visit is as shown and described below. Assessments that are considered part of the TBI common data elements (CDE) are noted with an asterisk (*), while assessments that are similar to the CDE are hash-marked (#):

4.4.1 Biospecimen Collection

Urine sample: Prior to each MRI, urine specimens will be collected from women of child bearing potential for pregnancy tests. A urine pregnancy test must be done within 24 hours prior to each MRI. Subjects who have a positive pregnancy test will not undergo MRI and will be excluded from the study. Monthly pregnancy testing will also be performed in subjects undergoing exercise. Urine will not be stored.

Blood samples: Venous blood samples will be collected from study participants by a trained phlebotomist or nurse at the Clinical Center. During this study, up to 40 ml of blood may be collected from participants at each study visit. Part of the blood sample collected for participants with TBI may be used to examine their safety risk for exercise participation. Specimens will be used to study DNA and multiple biological markers* (e.g. BDNF, VEGF, IGF-1, tau proteins, dopamine transmission genes, epigenetic mechanisms through DNA methylation) that may be related to brain injury and plasticity, or in response to motor training and exercise. Some of the samples will be immediately analyzed. For samples not immediately analyzed, they will be saved in a secured freezer for batch analysis or transferred to a biorepository as outlined in section 5. Results of genetic tests obtained at NIH are often preliminary and difficult to interpret because the testing is being done for research purposes only and the laboratories are not clinically certified. Results will not be shared with participants, their families, or insurance companies.

4.4.2 Questionnaires

Participants will complete self-reported questionnaires on quality of life, depression, symptoms, sleep quality and fatigue (Appendix 8). Total time for completion is approximately 1 hour. The questionnaires to be completed by the healthy volunteers are indicated by a symbol (‡).

A. Fatigue Severity Scale (FSS)*‡

An assessment used to monitor change in fatigue in response to therapeutic interventions. This scale is composed of nine items with a seven-point response format. A response of one indicates strong disagreement and a response of seven indicate strong agreement with statements regarding how fatigue affects physical and social functioning. Clinical improvement in fatigue is associated with reductions in scores on the FSS. The FSS is also a practical measure due to its brevity and ease of administration and scoring. The FSS takes less than 5 minutes to complete.

B. Pittsburgh Sleep Quality Index (PSQI)* ‡

Assesses the duration, depth, number of arousals, latency, and restfulness of sleep. The PSQI has been used to assess sleep quality in apparently healthy individuals and those with a number of disorders including TBI. It takes 5 to 10 minutes to complete.

C. Beck's Depression Inventory-II (BDI-II)* ‡

Measures the severity of depression and its interaction with other mood states. The BDI is a frequently used tool in clinical studies of depression and mood and in clinical evaluation. This assessment takes less than 5 minutes to complete.

D. Brief Symptom Inventory 18 (BSI-18)* ‡

Used to identify self-reported clinically relevant psychological symptoms in adolescents and adults. The shortened form of the BSI instrument provides a highly sensitive assessment of psychological factors. The BSI-18 takes approximately 10 minutes to administer.

E. Neurobehavioral Symptom Inventory (NBSI)* ‡

A questionnaire that can be administered to someone who sustains a concussion or other form of traumatic brain injury to measure the presence and severity of symptoms. The NBSI takes approximately 10 minutes.

F. Satisfaction With Life Scale (SWLS)* ‡

A short 5-item instrument designed to measure global cognitive judgments of satisfaction with ones' life. The scale usually requires only about 1 minute of a respondent's time.

G. Quality of Life after Brain Injury (QOLIBRI)*

This questionnaire was developed to assess health-related quality of life in individuals after traumatic brain injury. There are 37 items that cover 6 dimensions related to quality of life: cognition, self, daily life & autonomy, social relationships, emotions and physical problems. The QOLIBRI takes approximately 10 minutes to complete.

H. TBI Quality of Life (TBI-QOL)

A comprehensive set of items (i.e. physical, emotional, cognitive and social) that specifically affect quality of life in person with TBI [33] will be administered using the computer adapted versions. Total time for completion will be less than 20 minutes.

4.4.3 Neuropsychological Assessments

The following battery of neuropsychological assessments would be administered to assess executive functioning, list learning, attention, motor speed and visual memory (Appendix 7). Total time is approximately 1.5 hours. The assessments to be completed by the healthy volunteers are indicated by a symbol (‡).

A. Delis-Kaplan Executive Function System (D-KEFS) Sorting Test (Free Sort Condition Only)*‡

An executive functioning test of concept-formation, reasoning skills and problem-solving abilities. The examinee is asked to sort six mixed-up cards into two groups, three cards per group, according to as many different categorization rules or concepts as possible, and to describe the concepts used to generate each sort. The Free Sort Condition of the D-KEFS Sorting Test takes approximately 10-15 minutes to administer.

B. Medical Symptom Validity Scale (MSVT)* ‡

A brief computerized verbal memory-screening test to help distinguish malingering from genuine impairments in memory. The MSVT takes approximately 5-10 minutes to complete.

C. Brief Visual Memory Test-Revised (BVMT)*

A test of visual memory with recall and recognition trials. Administration time is 10 - 15 minutes.

D. Trail Making Test A & B (TMT)#‡

An assessment of visual conceptual and visual motor tracking (involves motor speed and attention functions). The test requires visual scanning, numeric sequencing and visual motor speed. The TMT takes approximately 5 minutes to administer. This test is similar to the D-KEFS Trail Making Test included as a CDE, however is not dependent on other baseline assessments.

E. Seashore Rhythm Test (SSRT)‡

An assessment of auditory attention and concentration. This test evaluates an individuals ability to discriminate between non-verbal sounds. Administration time is 10 minutes. This assessment is not part of the TBI CDE, however it is a sensitive measure of attention in patients with TBI.

F. Test of Premorbid Functioning (ToPF)#‡

An assessment that estimates an individual's pre-injury IQ and memory abilities and takes less than 10 minutes to complete. This is an update to the Wechsler Test of Adult Reading that is part of the TBI CDE. This test is only performed once at the baseline visit.

G. California Verbal Learning Test (CVLT-II)*

A list-learning task to evaluate verbal learning/encoding, effects of interference, immediate and delayed recall, cued recall and delayed recognition. The CVLT-II takes approximately 20 minutes to administer.

H. Finger Tapping Test (FTT)‡

This is an assessment of motor speed to assist in motor lateralization. This task will take about 10 minutes to complete and is not part of the TBI CDE.

4.4.4 Motor Assessments

These motor assessments[#] use specialized equipment that produces more detailed and likely more sensitive outcome measures than the recommended CDE for mobility and balance. Healthy volunteers will not perform this assessment.

A. Mobility assessment

Mobility will be assessed with the GAITRite System (CIR Systems Inc., Clifton, NJ, USA) to evaluate gait speed, stability and variability during various walking tasks as follows:

1. Free and fast walking: Walking will be evaluated along a hallway. Participants will be asked to walk across a 4.6 meter long electronic walkway (GAITRite) at their freely chosen speed and as fast as possible without running. This will be repeated each 2 times.
2. Performance during a dual task (DT) paradigm: This assessment will use a "walking while talking" paradigm. Subjects will be tested under 3 conditions:
   i. Single task cognitive: e.g. generating words that start with the letter "s" and counting backwards by 3s starting at number 150 for 30 sec, while seated
   ii. Single task gait: walking quietly across the electronic walkway at a comfortable speed. This is performed as described above.
   iii. Dual tasking: walking at their comfortable speed and talking at the same time. Subjects will perform 2 trials: 1) e.g. walking across the walkway while generating words that start with the letter "f"; 2) e.g. walking across the walkway while counting backwards by 3 starting at number 148.
3. Reciprocal Coordination Test: this test will be done in the motion analysis lab. Reflective markers will be placed on the subjects' feet to record elliptical stepping performance during the freely chosen speed and a speed as fast as possible. The subject will grip the handrails and move the arms and legs smoothly in a forward elliptical motion. The subject will move at free and fast speeds for 20-30 seconds at each speed after an accommodation/warm up period of 1-2 minutes.

B. Balance assessment

Participants will perform the balance assessment using the Balance Master and SMART Balance Master System® (NeuroCom International, Inc; Clakamas, OR). Tests are performed on a long force plate and inside a booth, as described in Appendix 6. Center of gravity is measured during different tasks that are described below.

1. The Limits of Stability (LOS) Test: This test is performed on the long force plate. Participants are required to actively shift their center of balance while standing (lean without taking a step) towards 8 targets that are presented individually. Targets are arranged in a circular pattern, and light up one by one. The system then computes changes in the person's center of gravity (COG) and outputs postural reaction time, movement velocity, excursion and directional control. During this test, subjects are in a comfortable standing position at the start and initiate all movements by themselves. One trial is collected for each target. They are encouraged to keep good posture and stay within their own postural limits but will have both standby assistance on the left side and a wall on their right for

safety should they lose their balance. Outcome variables include reaction time, maximum and endpoint excursion and directionality.

2. Sensory Organization Test (SOT): This test is performed inside the booth. The task is to stand quietly under different conditions that challenge the 3 sensory systems involved in the control of balance: somatosensory, visual and vestibular systems. There are 6 conditions: 1) normal vision + fixed support surface, 2) no vision + fixed support surface, 3) vision sway (the booth moves with the movement of the subject's COG), 4) normal vision + surface sway (the surface moves with the movement of the subject's COG, 5) no vision + surface sway, 6) vision sway + surface sway. Three trials are collected for each condition. This test identifies how patients integrate normal and altered inputs from each system for the control of balance. The outcome variable measured in this test is called the equilibrium score. This score tells how well the patient's sway remains within the expected angular limits of stability (12.5 degrees) during each condition. Scores close to 100 indicate the patient was stable, there is very little sway. Scores that reach "0" indicate there was a fall.
3. Motor Control Tests (MCT): This test is performed inside the booth. The subject is instructed to maintain balance after sudden translations of the support surface. This test measures the latency of automatic postural response mediated by long loop pathways connecting the peripheral sensory and motor nerves to the brain. There are six conditions (translations):
    i. Small forward
    ii. Medium forward
    iii. Large forward
    iv. Small backward
    v. Medium backward
    vi. Large backward

    Three trials are collected for each condition.
4. Adaptation Test (ADT): This test is performed inside the booth. The subject is instructed to maintain balance after repeated rotations of the support surface. This test measures the participant's ability to minimize sway over time when exposed to repeated perturbations in support surface. There are 2 conditions (rotations): toes up and toes down. Each condition is repeated 5 times in a row. This test reflects the patients' automatic ability to adapt to the perturbations by swaying less each time.

4.4.5 Magnetic Resonance Imaging

Brain imaging* is considered a TBI CDE and subjects must meet the criteria for MRI (repeated at each assessment time point). The MRI scans will be performed in the Department of Radiology in the NIH Clinical Center. Scanning will be performed on a 3 Tesla scanner. There will be no radiation exposure and no gadolinium will be administered. MRI scans may use research sequences that adhere to Specific Absorption Rate (SAR) safety guidelines and/or use research coils approved by the NMR Center Safety Committee. During the MRI, participants will be asked to lie on a table that can slide in and out of the cylinder. The MRI scan session will typically last 90 minutes and is unlikely to be more than 2 hours.

1. All women of childbearing potential will provide a urine sample for a pregnancy test performed no more than 24 hours before each MRI scan. Women who are discovered to be pregnant on their follow-up visit will no longer be eligible to participate in this study and will not perform any further imaging or study procedures.

2. A physician's order for a research brain MRI will be written in the subject's medical chart or entered into the electronic medical record by a member of the research team with clinical privileges as per study site policy. The research coordinator or assistant will escort the participant to the MR scanner in the CNRM Neuroimaging Core, housed at the NIH Clinical Center and will be in attendance with the participant throughout the procedure.

3. Subjects will be screened for MRI safety using the Screening Questionnaire used by the Radiology and Imaging Sciences Program of the Clinical Center (Appendix 4).

4. Subjects may be asked to lie still for up to 12 minutes at a time. While in the scanner they will hear loud knocking noises, and they will be fitted with earplugs or headphones to muffle the sound. They will be able to communicate with the MRI staff at all times during their scan, and they may ask to be moved out of the machine at any time.

5. Participants will first have a structural MRI scan. The structural MRI sequences we will use do not require contrast. They are not required to do any tasks during the structural MRI. Sequences used for the structural MRI may include T1 Magnetization Prepared Rapid Gradient Echo (MPRAGE), Fluid Attenuated Inversion Recovery (FLAIR), Diffusion Tensor Imaging (DTI), resting state functional connectivity (rfMRI), diffusion weighted imaging (DWI), Arterial Spin Labeling (ASL) to assess resting CBF, gradient echo (GRE) and/or T2* susceptibility weighted imaging (SWI). If image quality of a given sequence is inadequate due to patient motion for example, these may be repeated at the discretion of the MRI technologist or radiologist. The structural scan takes approximately 45 minutes.

6. For the fMRI, the following cognitive test will be administered:
   Paced Auditory Serial Addition Test (PASAT): a measure of cognitive function that specifically assesses auditory information processing speed and flexibility, calculation ability, and working memory. Single digits are presented every 3 seconds and the patient must add each new digit to the one immediately prior to it. The PASAT takes approximately 10-15 minutes to complete.

7. The BOLD sequence with hypercapnia challenge will have a BOLD MRI scan with cerebral vascular reactivity (CVR) hypercapnia measurements. Region-specific CVR will be assessed using hypercapnia induced by breathing 5% carbon dioxide (CO_2) mixed with 21% O_2 and 74% N_2. Study participants will be taken out of the scanner and fitted with a breathing mask that covers the mouth or a mouthpiece and nose clip to keep the patient from breathing through his/her nose. After the mask or mouthpiece

and nose clip is in place, the patient is placed back into the magnet. Hypercapnia will be induced via a Douglas bag fitted with a valve to switch between room air and 5% CO_2

All or parts of the planned MRI procedures may be omitted for participants with non-ferromagnetic metal or other substances in the head that do not constitute a safety hazard but may create an artifact that will interfere with analysis. We may evaluate the quality of previous MRIs on the participant if available or consult a radiologist to help in making this determination.

All MRI scans will be performed with dedicated radiofrequency coils that have received approval from the FDA or the NIH NMR Center Safety Committee. Research MRI coils that are not approved by the FDA may be used in this study. The NMR Center Safety Committee will approve all research coils for human use. A list of research MRI coils used will be submitted to the IRB at the time of continuing review. The CNS IRB has previously determined that research coils approved by the NMR Center Safety Committee are non-significant-risk devices and that no Investigational Device Exemption is needed.

The IRB has determined that the 32-channel head coil that may be used in this study does not meet the definition of significant risk (SR) device in accordance with FDA regulations (21 CFR 812), and therefore is considered an NSR device.

A SR device means an investigational device that:

- Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject;
- Is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject;
- Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject; or
- Otherwise presents a potential for serious risk to the health, safety, or welfare of a subject. (21 CFR 812.3(m))

4.4.6 Physical Fitness Assessment

These physical fitness assessments use specialized equipment that produces more detailed and likely more sensitive outcome measures than the recommended CDE for physical function. Patients will perform a cardiopulmonary exercise tolerance test on the treadmill with measures of pulmonary gas exchange to determine their maximal exercise tolerance. Healthy volunteers will not perform this assessment.

A. Cardiopulmonary exercise test (CPET) and EKG[#]

A computerized treadmill with motor-driven speed and grade adjustments will be used for the graded exercise test. The treadmill was chosen as the method of testing because it is well known to evoke higher values of O_2 consumption (VO_2) at maximum exercise, compared to other standard methods such as the leg cycle ergometer. Also, treadmill walking is a functional performance measure and the RMD

exercise laboratory personnel are well experienced in clinical treadmill exercise testing.

Subject preparation will consist of placing 10 electrodes on the chest at rest in the Mason-Likar 12-lead EKG modification for treadmill testing. The purpose of the EKG during the exercise test is to obtain digital HR information over the course of the CPET. After the electrodes have been placed, either a facemask placed over the lower portion of the subject's face (mouth and nose) or a mouthpiece and nose-clip will house the subject interface for pulmonary gas exchange. After collection of seated and standing resting data, the subject will begin the treadmill test. The test protocol begins with subjects walking at a slow speed on a flat treadmill. The speed and grade will then gradually increase at a rate that is supposed to bring the subject to exertional intolerance within 8 to 12 minutes. The target endpoint is exertional intolerance defined as the subjects expressed desire to stop walking despite strong verbal encouragement from the testing staff. A standard approach will be used to gain feedback from the subject regarding their current state of exhaustion during the test by frequently asking how they feel during the test. When they indicate they are becoming fatigued, the testing staff asks if they can continue for another minute. If the answer is "yes," after 30-45 additional seconds the subject is again asked if they can continue for an additional minute. If the answer is "yes," the process is repeated. When the subject's answer is "no," the subject is asked if they can continue for another 30 seconds. If the answer is "yes," then after an additional 15 seconds the subject is again asked if they can continue for an additional 30 seconds. If the answer is "yes," the process continues. When the answer is "no" or when the answer is "I must stop now," the test is stopped and the treadmill speed and grade are decreased to cool down levels. The test may also be stopped immediately at the subject's request, therefore the subject determines the stopping point and is never pushed beyond their expressed ability to continue. No expressed disappointment is ever mentioned to the subject in an attempt to force continuation. It is recognized that this stopping point is subjective and may occur at a level that is potentially lower than their actual metabolic capacity. To determine if the metabolic capacity has been reached there are two physiologic indices used. One is the attainment of 90% of the subjects maximum predicted heart rate (220 beats per minute minus one beat per minute per year of life). The other is attainment of an expiratory exchange ratio (total body oxygen consumption / carbon dioxide expiration) of 1.10 or greater as measured by pulmonary gas exchange analysis. One of these criteria must be met to substantiate attainment of maximal cardiorespiratory capacity.

B. Pulmonary gas exchange

Pulmonary gas analyses will be performed using a computerized metabolic cart including rapid response oxygen and carbon dioxide analyzers, and a pneumotachometer interfaced with a microprocessor. Subjects will interface the system using a fitted facemask or mouthpiece and nose-clip attached to a plastic air collection pneumotachometer. The pneumotachometer is in turn attached to a plastic tube connected to a flow pump, which regulates the volume of air passing into the oxygen and carbon dioxide analyzers. The total volume of air expired and inspired

(passing through the plastic tube) is measured by a digital, bidirectional pneumotachometer. Gas exchange variables measured during the tests will be measured continuously, breath-by-breath. The main variable of interest obtained from pulmonary gas analyses is VO_2. VO_2 will be determined electronically by the Haldane equation for every breath and the highest value recorded at volitional exhaustion will be recorded as the score for the measurement. Other pulmonary gas exchange variables (such as anaerobic threshold) may be analyzed to assist in explaining subjects' exercise response. These supporting variables will not require further data collection.

4.4.7 Exercise Training Intervention

TBI participants will be block randomized to one of 3 exercise training regimen for 12 weeks. All exercise training interventions will be supervised and performed 3 times per week, for 45 minutes each session at the NIH Clinical Center. All women of childbearing potential randomized to either exercise group will perform monthly pregnancy testing. The specific exercise intervention is as described below:

A. Aerobic exercise training (AET)

Subjects will perform physical exercise on an elliptical trainer, with emphasis on aerobic exercise being performed at a target heart rate (HR) range for 30 minutes. The target HR will be calculated as a range of between 70% and 80% of the heart rate reserve (HRR); where HRR = 0.70 to 0.80 * (peak HR – resting HR) + resting HR. The session will also include 15 minutes of warm-up and cool-down at sub-training intensity. Aerobic exercise will be continuous over the 30 minutes unless the patient cannot tolerate the regimen continuously. If the training regimen cannot be tolerated, exercise bouts will be alternated with rest intervals until the total time of 30 minutes of exercise at the target HR is achieved. The primary goal of this type of exercise is to increase the intensity at which the target HR is achieved. This would be achieved by preferentially increasing the resistance on the elliptical trainer, rather than speed. Subjects in AET would be exercising at ~ 70 to 80% of maximal VO_2. A paper-based log sheet will be used to record the heart rate, resistance and speed used in each session (Appendix 9).

B. Rapid-resistive exercise training (RET)

Subjects will perform physical exercise on an elliptical trainer, with emphasis on maintaining a near maximal speed of movement for 30 minutes. The session will also include 15 minutes of warm-up and cool-down at a slower speed. Subjects will be encouraged to progressively increase their maximal speed over time, while maintaining a smooth rhythm throughout the session. Once the subject has reached a speed of 80 RPM, resistance will be added. It will be increased incrementally as long as adding resistance does not decrease their speed below 80 RPM and does not increase their heart rate beyond the target set for moderate exercise as described below. Rapid-resistive exercise will be continuous over the 30 minutes unless the patient cannot tolerate the regimen continuously. If the training regimen cannot be tolerated, exercise bouts will be alternated with rest intervals until the total time of 30 minutes of exercise at near maximal speed of movement is achieved. The primary goal of this type of exercise is to increase movement speed and reciprocal coordination, while being within moderate intensity. Subjects in

RET would be exercising below their anaerobic threshold (determined from the CPET), which is typically about 50% HRR and maximal VO_2. Therefore, the heart rate associated with ~85% of each subjects' anaerobic threshold would be determined and this target heart rate would not be exceeded during their exercise sessions to ensure exercise is of moderate intensity. A paper-based log sheet will be used to record the heart rate, resistance and speed used in each session (Appendix 9).

Subjects in the exercise training group will complete at least 30 of the 36 training sessions in twelve weeks to remain eligible for the study. Subjects will wear a heart rate monitor during the sessions such that beat by beat heart rate is displayed continuously. The frequency of the sessions will be three times per week, unless make up sessions are needed to adhere to the minimum sessions required. Sessions will be supervised by credentialed RMD personnel.

Subjects randomized to the wait-list control will be further randomized to either AET or RET after Assessment Visit 2. In the first 3 months, subjects in the wait-list control will receive no exercise training; however they will be contacted monthly to ensure compliance and to maintain contact (Appendix 10). For subjects initially randomized to either AET or RET, they will no longer follow a formal exercise intervention at NIH following Assessment Visit 2. However, they will be contacted monthly for the next 3 months and asked about their weekly physical activities (Appendix 10).

4.4.8 Randomization

Randomization of subjects will be performed using randomized blocks to keep groups as equal in numbers as possible and to avoid early filling of one of the groups. An independent randomizer that is not affiliated to the protocol will perform the randomization of subjects.

4.5 Relationship to other protocols

Potential participants do not have to enroll or participate in any other protocol in order to participate in this study.

This study is related to a number of other protocols and projects. The Center for Neuroscience and Regenerative Medicine (CNRM) is a collaborative intramural federal program involving the United States Department of Defense (DoD) and the National Institutes of Health (NIH). It joins clinicians and scientists across disciplines to catalyze innovative approaches to traumatic brain injury (TBI) research.

The Uniformed Services University of Health Sciences (USUHS) heads the operation of the CNRM (www.usuhs.mil/cnrm).

CNRM initially provided Henry Jackson Foundation staff and equipment to NIH through a CRADA. These staff included several providers who perform the assessment instruments. All providers are credentialed at the Clinical Center for work at NIH.

The CNRM Informatics Core was created to store all imaging and clinical data from CNRM-related protocols. All clinical data will be coded and have personally identifying material removed prior to transfer to CNRM.
Research data from neuropsychological assessments, balancing and imaging performed within the past 3 months under other CNRM studies may be included as baseline assessments in this study. This is to avoid patient burden and practice effects. Research data from the same subject may also be shared directly with CNRM investigators in protocol 10-CC-0118 (Long-term Clinical Correlates of TBI: Imaging, Biomarkers and Clinical Phenotyping Parameters) and 11-N-0084 (Evaluation and Diagnosis of Potential Research Subjects with Traumatic Brain Injury).

4.6 End of participation

Subjects will remain under the care of their own providers for the duration of the study. Any incidental findings on brain images, laboratory or other assessment that may require further evaluation or may impact the subjects medical care will be recorded in the medical record and shared with the subject and their health care providers at subjects' request. However, this study does not provide treatment and does not replace any therapy that a subject may be receiving as part of standard medical care for TBI.

There is one questionnaire that indicates the presence of depression. If any question about suicidal ideation is answered in the affirmative, the study physician will talk with the patient and call in the NIH staff psychiatrist if there is a concern about the patient's stability and safety. NIH policy will be followed concerning a patient who may be a danger to him/her self.

5. Management of Data and Samples

5.1 Storage

The CNRM GUID is a number assigned by the CNRM Informatics Core. The Informatics Core has established an encrypted system and will provide access to the site for generation of a GUID, developed locally at each site, from personal health identifiers (PHI) data. Only the local site will have access to PHI. Local sites will maintain Master Keys matching GUIDs to PHI. Electronic/computer Master Keys will be kept on password protected terminal(s) in locked rooms with access limited to designated study personnel.

Electronic Master Key records will be backed up electronically at each site at least monthly. Physical print outs/copies of Master Keys will be kept in a locked cabinet in the office of a designated study investigator, and will be updated monthly or at more frequent intervals. The mapping from PHI to GUID will not be stored by or known to the CNRM Informatics Core or NIH CIT personnel, but the central registration of issued GUIDs will help ensure uniformity of identifiers across sites and the ability to identify the enrolling site.

Subjects receiving follow up visits will retain initially-assigned GUIDs throughout their participation and all data will be stored linked to this GUID. Requirements or requests for

subject future contact (re-identification) must pass through the enrolling site for GUID-PHI Master Key deciphering. CNRM Master Keys will contain the following information: GUID, last name, last 4 digits SSN, date of birth, and/or medical record number.

All subjects' data will be coded and kept on password-protected computers or files in locked offices with access only by study personnel. Clinical data collected while at NIH will be coded and kept in locked cabinets. Data will also be stored using an electronic data capture system such as the NICHD Clinical Trials Database (CTDB), which is backed-up and password protected for acccess by pertinent research members. The CNRM Informatics Data Repository will provide for the CTDB data to be periodically transferred to NIH CIT, where the data repository is to be housed. Only study investigators and those associated with this protocol will have access to the samples and data. Any loss or destruction of saved data or samples will be reported to the IRB. The CNRM Informatics Core was created to store all imaging and clinical data from CNRM-related protocols. All clinical data will be coded and have personally identifying material removed prior to transfer to CNRM.

Blood samples will be transported and immediately analyzed or stored in NINR secure freezers at the NIH Clinical Center until they are ready for batch analysis. Participant names and identifying information will be removed and the samples will be assigned codes. The key to the code will be kept in a separate, secure area. NINR will be responsible for the samples at NIH.

Any samples not analyzed will be stored in the CNRM Biospecimen Repository which is operated under USUHS Protocol No. CNRM-004 "Biorepository and Informatics Warehousing". The samples will be stored at the CNRM Biospecimen Repository and de-identified and stored there for the future measurements. Blood samples will be stored in secured freezers at the CNRM Biospecimen Repository. The subject's name and identifying information will not be on the samples; they will be assigned a code. The samples will be stored for up to 20 years. The purpose of the repository is to store a large number of samples and related data so that we can learn more about traumatic brain injury and how to rehabilitate people who may have it. The CNRM is a federal medical research program of the U.S. Department of Defense (DOD), and DOD is the custodian of the samples. The Uniformed Services University operates the CNRM and the USUHS IRB reviews the repository for patient protection and receives reports from a Biorepository Steering Committee on the operations of the repository.

Before sending blood samples, we will remove the subject's name and identifying information; we will assign them a code. The key to the code will be kept in a separate, secure area. Once the samples and the code arrive at the CNRM Biospecimen Repository, a repository manager will be responsible for maintaining the samples and sending them to others who may use the samples for research. Subject's blood samples will only be accessible by current and future CNRM Investigators after approval by all relevant IRBs.

5.2 Data and Sample Sharing Plan

Results of genetic tests obtained at NIH are often preliminary and difficult to interpret because the testing is being done for research purposes only and the laboratories are not clinically certified. Results will not be shared with participants, their families, or insurance companies.

Blood samples that are not analyzed at the NIH Clinical Center will be transferred to the CNRM Biospecimen Repository (USUHS Protocol No. CNRM-004 "Biorepository and Informatics Warehousing") for storage and materials will be shipped in accordance with NIH and federal regulations. Data will be transferred to CNRM Informatics Data Repository (CNRMIDR-001). No personally identifiable data will be transferred to CNRM. A global unique identifier (GUID) that is not linked to any personal identifiers will be generated for each participant and be used to share samples with CNRM. A materials transfer agreement will be put in place before any transfers are made.

6. Risks and discomforts

6.1 Risks of Exercise Training

The risks of this study are those associated with participation in vigorous aerobic exercise. The general risks of exercise include myocardial infarction, angina, shortness of breath, dizziness, early onset of fatigue and exhaustion, joint or muscle pain and numbness. Many of these symptoms may occur during the immediate post-exercise recovery phase, which is generally thought to be the first six minutes following strenuous exercise.

Many subjects will experience delayed onset muscle soreness after the exercise tolerance tests or after the exercise-training phase. This soreness will be mild to moderate in intensity and could begin immediately after exercise or up to 48 hours following the exercise session. It will typically subside within a seven-day period. Continued participation in exercise training does not appear to increase or prolong delayed muscle soreness. Many subjects will also experience increased levels of fatigue that are sustained for up to 24 hours following the exercise sessions. This fatigue will typically subside after one to three weeks.

The most serious risks for those participating in exercise are cardiovascular abnormalities. These risks are slight even in individuals with known severe coronary atherosclerosis during exercise tolerance testing. In studies that include patients with known heart disease as well as patients in whom the presence of heart disease is unknown, morbidity and mortality during maximal exercise testing remains low at 0.0 to 8.3 per 100,000 and 0.0 to 1.0 per 100,000 respectively [33-42]. Morbidity and mortality remain at similar lows for maximal exercise testing environments that are attended by a physician and those supervised by a physician but conducted by qualified physician extenders [34, 37, 40, 43, 44]. Exercising at submaximal intensities, such as during AET, carries with it even lower risk of adversity.

To minimize risk and maximize safety, all exercise tests will be carried out in laboratories that are in the NIH Clinical Center with immediate response backup. All subjects will be screened by medical history and medical examination to exclude those

with contraindications to exercise in whom testing and training might be unsafe. Subjects will be monitored during the treadmill exercise tests by 12-lead EKG. Subjects will be informed of the signs and symptoms of heart disease and questioned frequently during the test regarding the presence of symptoms of heart, neurological, or musculoskeletal conditions. An infographic (Appendix 14) for the common warning signs of a heart attack will also be available for subjects. As recommended by the American Heart Association and the American College of Sports Medicine, exercise will be overseen by personnel appropriately trained to extend physician services in the form of exercise testing and training in the clinical setting [45]. People with TBI comorbid with severe systemic illnesses will not be participants. The investigators and clinical exercise training and testing staff in our laboratory are highly experienced in treadmill cardiorespiratory testing, even in high-risk patients such as those with congestive heart failure and pulmonary artery hypertension.

TBI is not thought to increase cardiovascular risk or the risk of life-threatening adverse events during treadmill testing. According to the American College of Sports Medicine, subjects in this study will be classified as low or moderate risk for adverse events [45]. Recommendations for this classification are that physical examination is not necessary for low risk subjects prior to participation and physician supervision of a peak or submaximal exercise test is not necessary. In contrast, for moderate risk participants, a pre-training physical examination and physician supervision of a maximum exercise test is recommended, although physician supervision of a submaximal test is not necessary in the moderate risk group according to ACSM recommendations. However, RMD operating procedures are that a physician is in close proximity (in the department) at all times during an exercise test. This policy meets the definition of physician supervision in the ACSM guidelines [45]. In addition, all subjects will receive a physical examination, including the pre-participation history and physical exam parameters recommended by the ACSM [45]. All of the investigators and laboratory personnel are clinical exercise professionals certified by the ACSM, physical therapists, laboratory personnel certified and credentialed in the RMD exercise laboratory, or rehabilitation medicine physicians. All are trained in basic life support. Rapid code team response is readily available for the RMD Exercise Testing Laboratory and Fitness Center. All treadmill testing and elliptical training procedures will rigorously conform to ACSM guidelines and stopping points [45] (Appendix 11).

6.2 Risk of Treadmill or Elliptical Use

Risk of injury also includes those related to falling on a moving treadmill belt, such as abrasions, contusions, cuts, sprains, and fractures. The likelihood that any of these injuries would occur is small and preventive measures will be enforced to insure that the risks remain at a minimum.

The treadmill is equipped with handrails for aiding balance and providing increased stability. The treadmill also is equipped with a “hot” switch that is easily within the reach of the subject while walking on the treadmill. When the switch is activated, the treadmill stops and ends the test immediately. Under this circumstance the treadmill cannot be restarted unless both the treadmill itself and the computer control are reset. Subjects are instructed in treadmill safety and the use of the hot switch before beginning

the test protocol. Subject response to the test is monitored visually, electronically, and by verbal interaction between the subject and the testing staff.

Risk of injury with the use of an elliptical machine includes falling off or tripping caused by slippage off the pedals. Moving parts also create potential pinch hazards. There may also be feet, ankle and back discomfort if subjects are unused to the motion. The likelihood of these injuries occurring is minimal and proper form and familiarity with the device would reduce these risks further. Also sessions will be closely monitored by credentialed personnel and subjects will be asked for feedback during the exercise session.

6.3 Risk of Motor and Balance Assessments

There is a slight risk of falls especially in those with TBI since we are challenging their motor capabilities, but should be no greater than those encountered in everyday life. A harness will be used during balance testing and the examiner will closely guard the patient. The adhesive tape that will be placed on the skin may cause mild skin irritation or discomfort upon removal.

Stand-by assistance by a physical therapist or a safety belt or overhead harness will be used whenever there is a potential for unsteadiness that could lead to a fall during the motor and balance tests, or if the patient is concerned about falling.

6.4 Risk of Neuropsychological Testing and Questionnaires

The neuropsychological tests are not harmful, but may be frustrating or stressful. Filling out the questionnaires may be perceived as boring.

If the participant experiences distress, anger or any other emotional or behavioral symptoms during the neuropsychological tests, Dr. Chan will assess the situation and make recommendations or referrals as needed. All subjects will be informed prior to consent that they will be asked multiple questions that relate to their emotional and psychological health, and they will be told that they can refuse to answer any or all questions that they are not comfortable with. They may also stop the testing at any time for any reason.

6.5 Risk of MRI

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. It is not known if MRI is completely safe for a developing fetus and therefore will not be done if the subject is pregnant. People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Another risk is boredom in the MRI since

subjects will be asked to lie quiet and still for several minutes at a time. There are no known long-term risks of MRI scans.

All subjects will be screened for MRI contraindications before each scan, and if any are present, will not receive an MRI scan. In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room. The scan will not be done on anyone who is pregnant. A pregnancy test will be done on all female subjects who have the possibility of being pregnant within 24 hours of having each MRI. Everyone having a research MRI scan will be fitted with hearing protection.

6.6 Risk of Blood Draw

Needle punctures for drawing blood usually do not cause any serious problems. However, drawing blood may cause pain and carries a small risk of bleeding, localized bruising, discomfort and/or infection at the injection site, or temporary dizziness. If any of these occurs, they will be treated. The study will draw no more than 120 mL of blood for the entire study, which is below the maximum amount of blood that can be drawn as required by NIH guidelines which is no more than 450 mL in any 6 month period.

6.7 Risk of EKG

There is minimal medical risk or discomfort from the EKG.

6.8 Risk Associated with Hypercapnic Challenge

Some of the side effects of early hypercapnia include flushed skin, increased heart rate, muscle twitches, hand flaps, and possibly elevated blood pressure. Severe hypercapnia can also cause disorientation, headaches, panic, hyperventilation, and unconsciousness. Symptoms disappear with the resumption of breathing regular room air. The hypercapnic challenge used is of short duration and very mild, and the subject will be monitored closely during this challenge for discomfort and symptoms. The subject will be immediately switched back to room air at the subjects request or if staff detect excessive subject discomfort.

6.9 Risks of Sharing Research Data

Samples and data provided to the CNRM Biospecimen Repository (CNRM-004) and CNRM Informatics Data Repository (CNRMIDR-001) will be coded using a Global Unique Identifier (GUID) derived from PII and generated locally. Re-identification of data and samples collected at the NIH would be possible if repository data and samples are released with the GUID to other CNRM investigators who have access to the same subjects PII through participation in other IRB approved studies as they will generate the same GUID at their site. Re-identification is unlikely, however, as the CNRM Repository intends in most cases to release data and samples without the GUID.

The following measures will be made to minimize breach of confidentiality of records or data:

• All research procedures will be conducted by staff qualified to perform the procedure and with monitoring as defined in sections 7 and 10.
• Confidentiality will be protected as described in Section 19.
• Use of subject data that is re-identified through PHI used to generate a GUID is permitted only under IRB approved protocols with pre-defined research questions.

7. Subject Safety Monitoring

7.1 Parameters to be Monitored

Motor Assessment: All motor and balance assessments will be performed in the Functional & Applied Biomechanics Section under closely monitored conditions. A clinically credentialed staff member will remain with the subject at all times to ensure their safety and to assist them as needed. Subjects will be instructed to tell us if any of the tests are uncomfortable or concern them in any way. The primary parameters we will be monitoring are dizziness, unsteadiness, fatigue, or any indication that they are becoming distressed during the testing.

MRI: The subject will be in voice contact with MRI staff at all times during the scans and we will respond to their questions or concerns as needed. On site medical personnel and emergency medical assistance will be available for all MRI studies.

Treadmill CPET: All of the CPET tests will be performed in the RMD exercise testing laboratory in the Hatfield Clinical Research Center under closely monitored conditions. Clinically credentialed staff members will perform and monitor the tests and recovery period to ensure safety and assist the subjects as needed. Accepted clinical credentials are ACSM certification as a Clinical Program Director, Registered Clinical Exercise Physiologist, or Clinical Exercise Specialist, Licensed Physical Therapist, Licensed Physician, or individuals declared competent for administering exercise training and testing by the RMD. As part of their credentialing, staff members are knowledgeable in exercise test EKG monitoring and endpoints and will monitor EKG and blood pressure responses during the tests. The treadmill is equipped with handrails for aiding balance and providing increased stability to avoid risk of falling.

Exercise Training: Exercise training will be conducted on elliptical machines located in the RMD's Fitness Center, Exercise Testing Lab or Functional & Applied Biomechanics Lab. A clinically credentialed individual will monitor and record adherence to the target heart rate and session duration guidelines, changes in medications should they occur, exertional symptoms should they occur, and attendance. Supervision by the clinically credentialed individual will take place in the same room as the participant.

Severe Depressive Symptoms and/or Suicidal Ideation: Physicians and study staff in this study will monitor subjects throughout the protocol procedures. Physicians and study coordinator or other research staff member will be with the participant and will monitor for any anxiety and will assess any issues that arise such as suicide ideation. Procedures are done at the NIH Clinical Center and medical emergency services are available to participants in this study, if needed.

7.2 Criteria for Stopping Procedures

Treadmill CPET: Subjects will be reminded of test stopping points and exertional symptoms and will be queried by the staff frequently during the tests regarding their presence. While volitional exhaustion is the target test endpoint on the CPET, other stopping points are those recommended by the ACSM [45] (Appendix 11).

7.3 Criteria for Individual Subject Withdrawal

Federal regulations require that subjects may withdraw from participation in the study at any point without prior notice and without adversely affecting their medical management, educational or employment status. At the time of withdrawal, subjects will also be able to withdraw their data from the study. The study investigators may also withdraw subjects from the study for medical, administrative or issues of non-compliance. If the subject experiences a clinically significant worsening of mood or behavioral symptoms (not an emergency), she/he will be removed from the study and the physician and/or neuropsychologist will ask the subjects if they would like them to make a referral for further outside evaluation and treatment. Any psychiatric emergency would also be cause for withdrawal from the study and will prompt immediate psychiatric evaluation at NIH.

During the course of the study, subjects will be instructed to continue with their current care. Alterations of care must not include changes in therapy, exercise regimen or medication that would potentially alter cardiorespiratory capacity, mood, or cognitive or motor abilities, or the ability to achieve a training adaptation measurable by the methods used in this study. If the subject does make these types of changes, they will be administratively withdrawn from the study. If a subject misses an assessment and is unable to reschedule it within four weeks, they will be withdrawn from the study. We will replace all who withdraw or were withdrawn from the study for the above reasons.

8. Outcome measures

8.1 Primary Outcome

The primary outcome measure for this study is the change in cognitive function as measured by Trail Making Test Part B (TMT-B). This is measured as the difference in the time taken to complete the test between pre and post-exercise intervention and compared to the control group. Standardized t-scores will be used to interpret the results of this test.

8.2 Secondary Outcomes

Secondary outcome measures included here are those which have been shown previously or that we hypothesize to improve as results of exercise. Analysis will include comparisons of both exercise groups both combined and separately to the control group, and pre- to post-intervention changes within and across exercise groups in the following:

- Changes in other measures of cognitive function as measured by the

neuropsychological assessments

- Changes in motor assessments including balance and reaction time to perturbations
- Changes in cardiorespiratory fitness as measured by peak VO_2 achieved and time on the treadmill
- Change in fatigue severity as measured in the FSS; other quality of life, physical activity, and sleep quality scales will be used to enhance interpretation of the FSS findings
- Changes in MRI findings, both structural and functional measures; e.g., increases in hippocampal brain volume, or increases in the degree of functional connectivity or fractional anisotropy between brain regions that sub serve motor, cognitive and behavioral functioning.
- Changes in biomarkers

We are also interested in examining relationships among various outcomes (functional, behavioral, structural, mechanistic, etc.) in persons with TBI. Therefore, secondary analyses may include correlations or comparisons of baseline measures within and across the groups. Comparisons of outcome measures including blood biomarkers, brain imaging, neuropsychological and questionnaire data will also be made to a reference healthy volunteer sample that is matched for sex and age within 5 years.

Table 4. Summary of the Relevant Outcome Variables for the Secondary Outcome Measures

Motor Assessment

Test/Construct Measured	Relevant outcome variable
Gait Speed	Free and fast walking velocity
Dual Task Performance	Cognitive and motor cost (difference between DT and ST performance)
LOS/Dynamic Balance	Reaction time, maximal excursion
Elliptical Speed (reciprocal coordination)	Free and fast cadence on the elliptical
SOT/3 sensory systems	Equilibrium score for each sensory system
MCT/involuntary reaction time	Mean latency of responses
Adaptation/stability when perturbed	Sway velocity in 2 conditions

Neuropsychological Assessment
Test scores (and standardized scores) as indicated by the instrument

Questionnaires
Composite and domain scores as indicated by the instrument.

Physical Performance

Test/Construct Measured	Relevant outcome variable
CPET	Peak WR, peak HR, time to volitional exhaustion
Pulmonary gas exchange	VO_2 at peak exercise and AT

Imaging

Test/Construct Measured	Relevant outcome variable
Diagnostic MRI Scan	Volume of brain structures
DTI	Tracking of white matter tracts and brain connectivity
BOLD	Regional differences in cerebral blood flow at rest and during hypercapnic challenge indicating cerebral vascular reactivity
fMRI with PASAT	Comparison of active brain regions during a cognitive task

9. Statistical Analysis

9.1 Analysis of data

The primary analysis is a mixed model (with time as the within-subject factor and exercise group as the between subject factor) analysis of variance (ANOVA) which will be used to determine significant differences in the primary (ANOVA) and secondary outcome variables (MANOVA by outcome category – i.e. motor, cognitive, behavioral, etc – to help control for multiple comparisons) as a result of exercise. Therefore, the data will include the time points immediately pre and post exercise for all subjects. We are interested in both main effects for time and exercise as well as potential interaction effects between exercise group and time.

All subjects completing the protocol and not withdrawing or presenting off study criteria will be included in the analyses. Secondary analyses related to protocol adherence and other aspects of feasibility are anticipated. Estimation of the necessary and sufficient sample size was calculated for a large effect change in the main outcome variable, pre- to post-training change in executive functioning. Significance will be set at $p < 0.05$.

While we do not anticipate that subjects will improve over time in the absence of exercise, we have included a waitlist control to evaluate this, and we will compare the amount of change over time in the control group versus the exercise group that were not waitlisted using an independent t-test. Also for the exercise group that was not waitlisted, there will be a three month follow up period evaluated to determine whether there is any change compared to the end of the exercise period using paired t-tests.

The change in aerobic capacity and fast elliptical speed will be correlated with changes in the other outcomes measures. Baseline dopamine transmission scores will also be correlated with all motor outcomes using linear regression.

9.2 Power analysis

Based on findings in the pilot study (11-CC-0088) [32], calculations indicate that 20 subjects would be needed to detect a significant difference in the main outcome variable of executive functioning with Trail Making Test B for an $\alpha = 0.05$ and $\beta=0.05$. Therefore, 20 subjects in each of the conditions are expected (total 60 subjects). Attrition is approximately one-third for an exercise training protocol, so we predict recruitment of 80 subjects with non-penetrating TBI to have at least 20 subjects complete the protocol in each condition. Recruitment will remain open until at least 20 subjects in each condition have completed the second assessment visit.

10. Human Subjects Protection

10.1 Subject selection

Subject demographics will be dependent upon the clinical population affected with TBI. Since the majority of TBI occur in men, it is likely that there will be a higher percentage of males in our cohort. However, no preference or exclusion will be granted according to gender or ethnic/racial background, and efforts will be made to recruit under-represented minorities and women.

10.2 Justification for exclusion of children

Individuals younger than 18 years of age will not be included in the protocol because reference ranges for normative aerobic capacity and aerobic fitness have not been established for these age ranges. The objectives of this project do not include establishing these normative reference ranges. This lack of information could introduce critical levels of bias into the interpretation and cause the interpretation of our results to be misleading. Thus the exercise responses and adaptations in children and adolescents with TBI should be studied in separate and specific protocols.

10.3 Justification for exclusion of other vulnerable population

10.3.1 Justification for exclusion of older adults

Individuals 80 years and older will not be included in the protocol as reference ranges for exercise, motor and balance tests for these individuals are not available or well established.

10.3.2 Justification for exclusion of pregnant women

While women will be included in this study, pregnant women will be excluded. This is because of the unknown risk of MRI to an unborn fetus, and the effects of pregnancy on aerobic capacity and the risk that may be associated with beginning a new exercise program while pregnant. Subjects will not be able to participate if the pregnancy test is positive. Subjects that become pregnant during study participation will be administratively withdrawn from the study.

10.3.3 Justification for exclusion of mentally ill and those unable to consent themselves

Mentally ill subjects and individuals who cannot give consent themselves will not be included in this study as the research procedures require subjects to follow specific instructions (e.g. hypercapnia challenge in the MRI, neurocognitive measures, exercise and balance testing) and in some cases, provide reliable feedback related to their safety (e.g., indicating when they have approached their limit of tolerance during an exercise test).

10.3.4 Justification for exclusion of non-English speaking subjects

Non-English speakers will be excluded because the neuropsychological assessment instruments we are using are not validated or normed in other languages. Furthermore, many of the tests we are performing would require a native speaking psychometrist to administer theses test, who is not available.

10.4 Safeguards for vulnerable populations

Prior to any MRI scans, urine pregnancy tests will be performed on women of child bearing potential. Monthly pregnancy tests will also be performed in those undergoing the exercise intervention.

10.5 Qualifications of investigators

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP Policy 201 (Education Program) have completed all required training.

11. Anticipated Benefit

11.1 Direct benefits

TBI subjects in this protocol have the potential to receive direct benefit from participating in this research. TBI subjects may directly benefit from aerobic exercise training, where improvements in cardiorespiratory fitness enables individuals to accomplish a given amount of activity or work at a lower percentage of maximum capacity and with less fatigue. TBI subjects may also directly benefit from rapid-resistive exercise, with increased strength, flexibility, movement speed and balance. Exercise participation has been shown to be beneficial in the reduction of all-cause mortality and morbidity in some subsets of the population and is recommended for all who are physically able to

participate. Healthy volunteers are not expected to receive any direct benefits from participation in this study.

12. Classification of risk

This protocol is classified as a greater than minimal risk study. The risks are reasonable in relation to anticipated benefits.

13. Consent documents and process

13.1 Designation of those obtaining consent

Study investigators designated as able to obtain consent, will obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU "Elements of Successful Informed Consent" training.

13.2 Consent procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to review the written consent form carefully and ask questions regarding this study prior to signing.

13.3 Consent documents

There is one consent form for the individuals with TBI that contain all the required elements for participating in this study. There is a separate consent form for healthy individuals.

14. Data and Safety monitoring

14.1 Data and safety monitor

Data and safety will be monitored by the Principal Investigator, Dr. Damiano at least once a year. In addition, a data and safety monitor, Dr. Leddy, will review all adverse events at least every 6 months (see section on Data and Safety Monitoring Plan).

14.2 Data and safety monitoring plan

Data and safety monitoring for this protocol is an ongoing process. The PI will meet weekly with the study team to discuss the protocol progress and any relevant activities or occurrences. However, research staff have been instructed to report any issues related to protocol safety and data as soon as possible after occurrence or when they first became aware of it to the PI. The PI is then responsible for evaluating these, proposing and implementing corrective action as needed, and reporting these to the IRB in a timely manner.

As the principle investigator, Dr. Damiano will be contacted after every serious adverse event that is related to the protocol and notified of all deaths. These will also be reported to the NIH and USUHS IRB's with the continuing review.

Dr. John Leddy is a physician with cardiology experience and specific expertise in rehabilitation and management of persons with concussion/brain injury. He has been enlisted to conduct an independent data and safety monitoring review no less than every 6 months for this study. Dr. Leddy is board certified in Internal Medicine with a subspecialty in Sports Medicine, and is a Professor of Clinical Orthopaedics and Rehabilitation Sciences at the State University of New York at Buffalo. He is also the Medical Director of the Concussion Management Clinic in the Jacobs School of Medicine and Biomedical Sciences, and Division I Team Physician at the University at Buffalo. He will be apprised of all adverse events and will have access to study related data to make his assessment and review as deemed necessary.

14.3 Criteria for stopping the study or suspending enrollment or procedures

The PI, data and safety monitor (Dr. Leddy), and IRBs will assess the measures taken by the study team in response to safety issues that caused the study to be stopped or suspended. Revised safety measures taken by the study team will be in a written report submitted to the IRBs of record. Only when these entities agree in writing that the problems have been satisfactorily addressed can the protocol may be re-activated.

Annually there will be a continuing review of this study at the NIH and USUHS IRBs.

15. Quality Assurance

15.1 Quality Assurance monitor

This study will be monitored for quality assurance through the NIH Clinical Center's Quality Assurance Program.

15.2 Quality Assurance Plan

Accrual and safety data will be monitored by the principal investigator, who will provide oversight to the conduct of this study. The PI will continuously evaluate implementation of the protocol for any unusual or unpredicted complications that occur and will review the data for accuracy and completeness.

The NIH Clinical Center's Quality Assurance Program will conduct study monitoring at least annually. Monitoring visits will include a review of patient consent documents, primary outcome and safety laboratory results and diagnostic test results will be monitored for accuracy, correct dating, and agreement between case report forms and source documents. All regulatory reports, reviews and amendments, adverse events and problem reports related to study, along with investigator credentials, training records, and the delegation of responsibility log will also be reviewed during monitoring visits. Any major findings will be summarized in writing and reported to the study PI who will be responsible for submitting the monitoring report to the IRB.

All study staff will maintain current training in good clinical practice and protection of human subjects, and will complete other protocol- and role-specific training, as needed.

16. NIH Reporting Requirements

16.1 Adverse Event Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events.

16.2 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

The data and safety monitor (Dr. Leddy) will also be notified of all adverse events at least every 6 months.

It is anticipated that participants in this study may fail to complete the entire list of assessments or procedures, such as questionnaires or neuropsychological tests. Omissions such as these will be considered expected events and not protocol deviations provided they are infrequent, not the primary outcome, not related to determination of the exercise training range and do not include data needed to assess safety for a procedure. Cumulative proportions of these missed events in the study population will be presented to the IRB at the time of Continuing Review. In addition, the rate of omissions will be monitored by the Investigators. If an individual misses more than 15% of the required assessments/procedures or if more than 15% of the participants miss completion of the same assessment or procedure, it will be considered a deviation and reported according to Policy 801.

16.3 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

16.4 Clinical Director Reporting

Problems expeditiously reported to the IRB in iRIS will also be reported to the Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

17. Alternatives to participation

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

18. Privacy

All research activities will be conducted in as private a setting as possible.

19. Confidentiality

Confidentiality of patient files will be maintained at all times. Samples and data will be de-identified, using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked freezers in locked rooms. Paper records and case report forms will be maintained in locked rooms, or in computer files protected by computer passwords. Only study investigators will have access to the samples and data. In the presentation of research results, no information will be given that may reveal the identity of the research subjects. The consent form will discuss patient confidentiality protection.

De-identified results from clinical trials will be posted on clinicaltrials.gov

Confidentiality of the patient records will be protected to the extent possible under existing regulations and laws but cannot be guaranteed. Complete confidentiality cannot be promised, especially to military personnel, because information bearing on their health may be required to be reported to appropriate medical or command authorities. Members of CNRM, Uniformed Services University, Henry M Jackson Foundation, US Department of Defense (DoD), and NIH, may have access to the study data for auditing purposes.

Each study participant will be assigned a study number that will be used on study data collection forms and samples. The link between the study number and participants' identity will be kept on a password-protected computer. Only study researchers and members of the IRB will have access to identified information. Data transmitted to the Data Coordinating Center will be coded. Investigators at the clinical site where consent was obtained will have access to identifying information for the patients enrolled in the study at their site. Only coded de-identified information will be shared with persons outside of the enrolling site, except for audits.

NIH will assign a Global Unique Identifier (GUID) to all data submitted to the CNRM Repository. The purpose of the GUID is to allow data on the same subject, from multiple protocols and from different institutions, to be identified as coming from a single individual and to be combined in the repository. PII will be entered on a local server and a one-way encryption using a keyed-hash algorithm will be used to assign the GUID. CNRM will not receive PII. The one-way encryption assures that the GUID cannot be used to back-generate PII. Data that were delinked may then be sent to other non-CNRM Biorepositories.

Blood will be processed and frozen according to CNRM Biospecimen Repository guidelines for confidentiality. Samples will be assigned a unique identifier at time of blood draw. The key for the re-identification of samples will be maintained on a master list in the PI's office and accessible only to his designees. Some information will be on a password-secured server in one of the Investigator's offices on the NIH campus.

20. Conflict of Interest

NIH guidelines on conflict of interest have been distributed to all investigators. There are no conflicts-of-interest to report for NIH investigators. Non-NIH investigators will abide by the conflict-of-interest policies of their own institutions.

21. Technology Transfer

A materials transfer agreement will be in place before any samples leave the NIH and are placed in the CNRM Biospecimen Repository. A collaborative agreement for the transfer of human de-identified data between NIH and investigators at DCVAMC will be in place before any data are shared. Investigators at DCVAMC shall not make any effort to re-identify individuals who are the source of the shared de-identified data. A spreadsheet containing details and status of the technology transfer agreements for this study is included as Appendix 12.

22. Research and Travel Compensation

<u>Assessment Visits (1, 2 and 3)</u>
Participants will be compensated for time and research-related inconveniences as follows:
$20 for first hour (or part)
$10 for each additional hour thereafter for assessments performed in RMD for up to 5 hours
$20 for blood draws

In addition, compensation for the MRI:
$80 (includes $60 for the procedures and $10 for up to 2 hours of time)

<u>Exercise Intervention</u>
Participants will be compensated for completing the exercise training intervention (either AET or RET):
$150 for completion of the 12-week exercise training (assuming 80% attendance)

If participants are unable to finish the study, they will be paid for parts completed.

Payment will be processed after completion of the assessment visits. The maximum compensation for participants with TBI is $660.00 for all 3 assessment visits and training intervention. The maximum compensation for healthy volunteers is $170.

Travel will not be provided.

23. References

1. Faul M, Xu L, Wald M, Coronado V: **Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations, and Deaths**. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control; 2010.
2. Saltychev M, Eskola M, Tenovuo O, Laimi K: **Return to work after traumatic brain injury: Systematic review**. *Brain Inj* 2013, **27**(13-14):1516-1527.
3. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control: **Report to Congress on mild traumatic brain injury in the United States: steps to prevent a serious public health problem**. In. Atlanta, GA; 2003.
4. Pierce CA, Hanks RA: **Life satisfaction after traumatic brain injury and the World Health Organization model of disability**. *Am J Phys Med Rehabil* 2006, **85**(11):889-898.
5. Wagner AK, Hammond FM, Sasser HC, Wiercisiewski D: **Return to productive activity after traumatic brain injury: relationship with measures of disability, handicap, and community integration**. *Arch Phys Med Rehabil* 2002, **83**(1):107-114.
6. Lu J, Gary KW, Neimeier JP, Ward J, Lapane KL: **Randomized controlled trials in adult traumatic brain injury**. *Brain Inj* 2012, **26**(13-14):1523-1548.
7. Archer T: **Influence of Physical Exercise on Traumatic Brain Injury Deficits: Scaffolding Effect**. *Neurotox Res* 2012, **21**(4):418-434.
8. Hicks RR, Smith DH, Lowenstein DH, Saint Marie R, McIntosh TK: **Mild experimental brain injury in the rat induces cognitive deficits associated with regional neuronal loss in the hippocampus**. *J Neurotrauma* 1993, **10**(4):405-414.
9. Kotapka MJ, Gennarelli TA, Graham DI, Adams JH, Thibault LE, Ross DT, Ford I: **Selective vulnerability of hippocampal neurons in acceleration-induced experimental head injury**. *J Neurotrauma* 1991, **8**(4):247-258.
10. Cotman CW, Berchtold NC, Christie L-A: **Exercise builds brain health: key roles of growth factor cascades and inflammation**. *Trends Neurosci* 2007, **30**(9):464-472.
11. Griesbach GS, Hovda DA, Gomez-Pinilla F: **Exercise-induced improvement in cognitive performance after traumatic brain injury in rats is dependent on BDNF activation**. *Brain Res* 2009, **1288**:105-115.
12. Kim D-H, Ko I-G, Kim B-K, Kim T-W, Kim S-E, Shin M-S, Kim C-J, Kim H, Kim K-M, Baek S-S: **Treadmill exercise inhibits traumatic brain injury-induced hippocampal apoptosis**. *Physiol Behav* 2010, **101**(5):660-665.
13. Neeper SA, Gómez-Pinilla F, Choi J, Cotman CW: **Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain**. *Brain Res* 1996, **726**(1–2):49-56.
14. Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW: **Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus**. *Neuroscience* 2005, **133**(3):853-861.

15. Adlard PA, Perreau VM, Engesser-Cesar C, Cotman CW: **The timecourse of induction of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus following voluntary exercise**. *Neurosci Lett* 2004, **363**(1):43-48.
16. Griesbach GS, Hovda DA, Molteni R, Wu A, Gomez-Pinilla F: **Voluntary exercise following traumatic brain injury: Brain-derived neurotrophic factor upregulation and recovery of function**. *Neuroscience* 2004, **125**(1):129-139.
17. Bigler ED, Blatter DD, Anderson CV, Johnson SC, Gale SD, Hopkins RO, Burnett B: **Hippocampal volume in normal aging and traumatic brain injury**. *Am J Neuroradiol* 1997, **18**(1):11-23.
18. Tate DF, Bigler ED: **Fornix and Hippocampal Atrophy in Traumatic Brain Injury**. *Learn Mem* 2000, **7**(6):442-446.
19. Tomaiuolo F, Carlesimo GA, Di Paola M, Petrides M, Fera F, Bonanni R, Formisano R, Pasqualetti P, Caltagirone C: **Gross morphology and morphometric sequelae in the hippocampus, fornix, and corpus callosum of patients with severe non-missile traumatic brain injury without macroscopically detectable lesions: a T1 weighted MRI study**. *J Neurol Neurosurg Psychiatry* 2004, **75**(9):1314-1322.
20. Ariza M, Serra-Grabulosa JM, Junqué C, Ramírez B, Mataró M, Poca A, Bargalló N, Sahuquillo J: **Hippocampal head atrophy after traumatic brain injury**. *Neuropsychologia* 2006, **44**(10):1956-1961.
21. Bigler ED, Johnson SC, Anderson CV, Blatter DD, Gale SD, Russo AA, Ryser DK, Macnamara SE, Bailey BJ, Hopkins RO *et al*: **Traumatic brain injury and memory: The role of hippocampal atrophy**. *Neuropsychology* 1996, **10**(3):333-342.
22. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, Kim JS, Heo S, Alves H, White SM *et al*: **Exercise training increases size of hippocampus and improves memory**. *Proc Natl Acad Sci USA* 2011, **108**:3017-3022.
23. Pajonk F, Wobrock T, Gruber O, et al.: **Hippocampal plasticity in response to exercise in schizophrenia**. *Arch Gen Psychiatry* 2010, **67**(2):133-143.
24. Seifert T, Brassard P, Wissenberg M, Rasmussen P, Nordby P, Stallknecht B, Adser H, Jakobsen AH, Pilegaard H, Nielsen HB *et al*: **Endurance training enhances BDNF release from the human brain**. *Am J Physiol* 2010, **298**(2):R372-R377.
25. Zoladz JA, Pilc A, Majerczak J, Grandys M, Zapart-Bukowska J, Duda K: **Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men**. *J Physiol Pharmacol* 2008, **59**(4):119-132.
26. Grealy MA, Johnson DA, Rushton SK: **Improving cognitive function after brain injury: The use of exercise and virtual reality**. *Arch Phys Med Rehabil* 1999, **80**(6):661-667.
27. McMillan T, Robertson IH, Brock D, Chorlton L: **Brief mindfulness training for attentional problems after traumatic brain injury: A randomised control treatment trial**. *Neuropsychol Rehabil* 2002, **12**(2):117-125.
28. Gordon WA, Sliwinski M, Echo J, McLoughlin M, Sheerer MS, Meili TE: **The benefits of exercise in individuals with traumatic brain injury: a retrospective study**. *J Head Trauma Rehabil* 1998, **13**(4):58-67.

29. Ploughman M: **Exercise is brain food: The effects of physical activity on cognitive function**. *Dev Neurorehabil* 2008, **11**(3):236-240.
30. Kiraly MA, Kiraly SJ: **The effect of exercise on hippocampal integrity: review of recent research**. *Int J Psychiatry Med* 2005, **35**(1):75-89.
31. Chin LMK, Chan L, Woolstenhulme JG, Christensen EJ, Shenouda CN, Keyser RE: **Improved cardiorespiratory fitness with aerobic exercise training in individuals with traumatic brain injury**. In: *J Head Trauma Rehabil.* vol. 30; 2015: 382-390.
32. Chin LMK, Keyser RE, Dsurney J, Chan L: **Improved Cognitive Performance Following Aerobic Exercise Training in People with Traumatic Brain Injury**. *Arch Phys Med Rehabil* 2015, **96**(4):754-759.
33. Tulsky DS, Kisala PA, Victorson D, Carlozzi N, Bushnik T, Sherer M, Choi SW, Heinemann AW, Chiaravalloti N, Sander AM *et al*: **TBI-QOL: Development and Calibration of Item Banks to Measure Patient Reported Outcomes Following Traumatic Brain Injury**. *J Head Trauma Rehabil* 2016, **31**(1):40-51.
34. Atterhog JH, Jonsson B, Samuelsson R: **Exercise testing: a prospective study of complication rates**. *Am Heart J* 1979, **98**(5):572-579.
35. Franklin B, Hellerstein H, Gordon S, Timmis G (eds.): **Exercise Testing: Basic Principles**. New York, NY: Churchill Livingstone; 1992.
36. Gibbons L, Blair SN, Kohl HW, Cooper K: **The safety of maximal exercise testing**. *Circulation* 1989, **80**(4):846-852.
37. Gibbons LW, Mitchell TL, Gonzalez V: **The safety of exercise testing**. *Prim Care* 1994, **21**(3):611-629.
38. Hamm LF, Crow RS, Stull GA, Hannan P: **Safety and characteristics of exercise testing early after acute myocardial infarction**. *Am J Cardiol* 1989, **63**(17):1193-1197.
39. Modesto KM, Møller JE, Freeman WK, Shub C, Bailey KR, Pellikka PA: **Safety of Exercise Stress Testing in Patients With Abnormal Concentrations of Serum Potassium**. *Am J Cardiol* 2006, **97**(8):1247-1249.
40. Squires RW, Allison TG, Johnson BD, Gau GT: **Non-Physician Supervision of Cardiopulmonary Exercise Testing in Chronic Heart Failure: Safety and Results of a Preliminary Investigation**. *J Cardiopulm Rehabil Prev* 1999, **19**(4):249-253.
41. Stuart JRJ, Ellestad MH: **National survey of exercise stress testing facilities**. *Chest* 1980, **77**(1):94-97.
42. Vacanti LJ, Sespedes LB, Sarpi Mde O: **Exercise stress testing is useful, safe, and efficient even in patients aged 75 years or older**. *Arq Bras Cardiol* 2004, **82**(2):151-154, 147-150.
43. Young DZ, Lampert S, Graboys TB, Lown B: **Safety of maximal exercise testing in patients at high risk for ventricular arrhythmia**. *Circulation* 1984, **70**(2):184-191.
44. Irwin S, Blessry R: **Patient Evaluation**. In: *Cardiopulmonary Physical Therapy.* 2nd edn. Edited by Irwin S, Tecklin J. St. Louis, MO: Mosby Inc.; 1990: 139-140.
45. Rochmis P, Blackburn H: **Exercise tests: A survey of procedures, safety, and litigation experience in approximately 170,000 tests**. *JAMA* 1971, **217**(8):1061-1066.

46. Thompson WR (ed.): **ACSM's Guidelines for Exercise Testing and Prescription**, 8th edn. Baltimore, MD: Lippincott Williams & Wilkins; 2009.

24. Appendices

- Appendix 1A – Recruitment Material and Flyer for TBI
- Appendix 1B – Recruitment Material and Flyer for Healthy Volunteers
- Appendix 2A – Pre-screen questionnaire for TBI
- Appendix 2B – Pre-screen questionnaire for Healthy Volunteers
- Appendix 3A – Eligibility Checklist for TBI
- Appendix 3B – Eligibility Checklist for Healthy Volunteers
- Appendix 4 – MRI Safety Questionnaire
- Appendix 5 – Participant Flowchart
- Appendix 6 – Balance Description
- Appendix 7 – TBI Neuropsychological Assessment List
- Appendix 8 – TBI Questionnaire
- Appendix 9 – Exercise Data Collection Form
- Appendix 10 – Monthly Phone Call Form
- Appendix 11 – Exercise Safety Stopping Points
- Appendix 12 – Data Sharing spreadsheet
- Appendix 13 - DCVAMC Recruitment Letter
- Appendix 14 – Infographic of the Warning Signs of a Heart Attack