Title: Sildenafil Treatment for Chronic Traumatic Microvascular Injuries

NCT number: NCT03598140

Protocol Date: 1 March 2018, Version Number: 2.0

STUDY PROTOCOL

Title: Sildenafil Treatment for Chronic Traumatic Microvascular Injuries

Protocol Identifying Number: UT-7529

Principal Investigator(s): Kan Ding, M.D.

Institution: UT Southwestern Medical Center

IND/IDE Sponsor: Food and Drug Administration (IND# 134596)

Version Number: 2.0

1 March 2018

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LIST OF ABBREVIATIONS

AE	Adverse Event
AIC	Akaike Information Criterion
ASL	Arterial Spin Labeling
BBB	Blood Brain Barrier
BHI	Breath Holding Index
BIC	Bayesian Information Criterion
BOLD (MRI sequence)	Blood Oxygen Level Dependent
BP	Blood Pressure
BSI	Brief Symptom Inventory
CBF	Cerebral Blood Flow
CC	Cubic Centimeter
CDE	Common Data Elements
CVR	Cerebrovascular reactivity
cGMP	cyclic guanosine monophosphate
CO_2	Carbon dioxide
CVLT	California Verbal Learning Test
DOD	Department of Defense
DSMP	Data Safety Monitoring Plan
DTI	Diffusion tensor imaging
DTI-SPGR	Diffusion Tensor Imaging - Spoiled Gradient Echo
DVI	Diffuse Vascular Injury
DWI	Diffusion Weighted Imaging
Et	End Tidal (CO2)
FDA	Food and Drug Administration
FLAIR	Fluid attenuated inversion recovery
fMRI	Functional Magnetic Resonance Imaging
GCP	Good Clinical Practices
GEE	Generalized Estimating Equations
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GRE	Gradient Echo
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
ML	Milliliters
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MPRAGE	Magnetization Prepared Rapid Gradient Echo
mTBI	Mild Traumatic Brain Injury
NAION	Non-arteritic Anterior Ischemic Optic Neuropathy
NIH	National Institutes of Health
NIH CC	National Institutes of Health Clinical Center
NINDS	National Institutes of Neurological Disease and Stroke
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
PDE5	Phosphodiesterase 5 (inhibitors)
PI	Principal Investigator
QC	Quality Control
REDCap	Research Electronic Data Capture

rfcMRI	Resting state functional connectivity MRI
SAE	Serious Adverse Event
Sildenafil	Viagra®
SWI	Succeptability Weighted Imaging
SWLS	Satisfaction with Life Scale
TBI	Traumatic Brain Injury
TCVI	Traumatic Cerebral Vascular Injury
TCD	Transcranial Doppler
TMT	Trail Making Tests
TTP	Time to Peak
UTSWMC	University of Texas Southwestern Medical Center
WAIS-IV	Wechsler Adult Intelligence Scale
WRAT	Wide Range Achievement Test

Table 1. List of Abbreviations.

STATEMENT OF COMPLIANCE

(1) The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the following*:*

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)
- ICH E6

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.]

Principal Investigator: Kan Ding, M.D.
Print/Type Name

Signed: Date: ___

PROTOCOL SUMMARY

Title: Sildenafil Treatment for Chronic Traumatic Microvascular Injuries

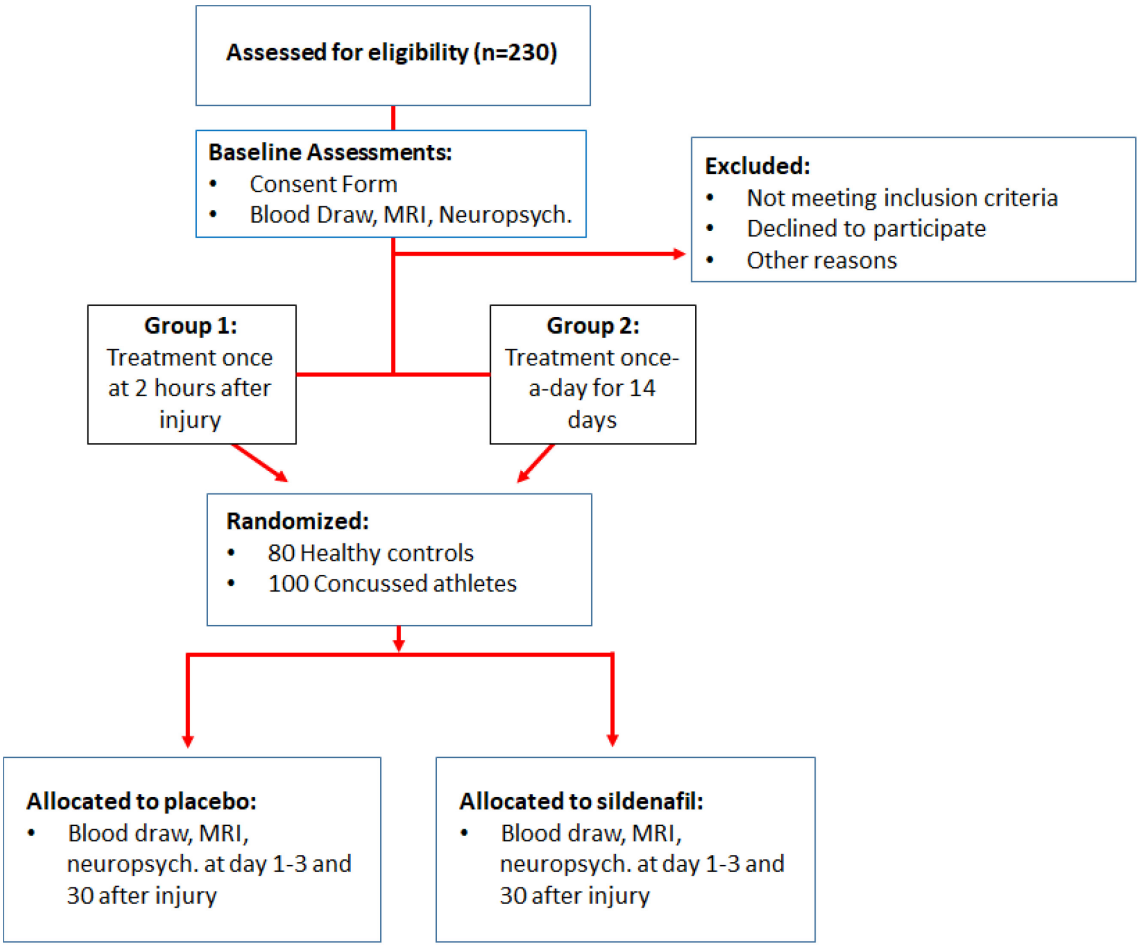
Précis: About 300,000 people are hospitalized for traumatic brain injury (TBI) each year (1, 2). After TBI, secondary brain injury escalates due in part to heightened levels of oxidant injury, inflammation, and vascular injury. Traumatic cerebral vascular injury (TCVI) may begin almost immediately after the primary injury and evolve into chronic neurodegenerative conditions. TCVI is a very complex TBI endophenotype and microvascular injuries have been described in a plethora of animal and human TBI studies. These injuries consist of endothelial injury, disruption of the blood brain barrier (BBB), a reduction of capillary density, intravascular microthrombi, and white-

	matter degeneration (3-13). Recently, use of magnetic resonance imaging (MRI)-Blood Oxygen Level Dependent (BOLD) combined with hypercapnia (high spatial and temporal resolution) by our research group has proven to be more sensitive at measuring alterations of cerebral blood flow (CBF) in TBI subjects. The goal of our proposed research is to test the efficacy of Viagra® (sildenafil) at normalizing CBF and improving cognitive outcomes in people that have experienced a TBI. Sildenafil is a phosphodiesterase-5 (PDE-5) inhibitor that has previously been administered as a therapy for high blood pressure and erectile dysfunction (14, 15). In people that have been affected by stroke-induce neurotrauma, sildenafil improved CBF and was found to be neuroprotective (16-21). With respect to chronic TBI, our preliminary data demonstrates that sildenafil therapy potentiates cardiovascular reactivity (CVR) in areas of the brain with damaged endothelium. In this proposal, we will test the hypothesis that sildenafil treatment in boxers/MMA fighters soon after concussion normalizes CBF, potentiates CVR, reduces post-concussion symptoms, and improves cognition.
Objectives:	**Objective 1:** To demonstrate that administration of the vasodilator, sildenafil, to concussed boxers/MMA fighters will improve/normalize various vascular properties. **Objective 2:** We will determine if sildenafil reduces symptoms and improves cognition in the athletes.
Endpoint	The primary outcome measures will be global CVR to hypercapnia, reduced symptoms, improved cognition in concussed athletes treated with a single or multiple doses of sildenafil (60mg). In the secondary outcome measure, we assess sildenafil (60mg) therapy reduces the levels of fluid-based biomarkers of injury.
Population:	The study population for this protocol consists of 100 boxers between the ages of 18 and 35 (both men and women) who have suffered a concussion. The athletes will be recruited from fitness centers within the North Texas area. Testing will occur at UT Southwestern Medical Center, Dallas, Texas. The study will be comprised of the experimental (boxers/MMA fighters) and age-matched non-boxer, healthy controls.
Phase:	2
Number of Sites enrolling participants:	1

Description of Study Agent :	Sildenafil is a phosphodiesterase-5 (PDE-5) inhibitor that has previously been administered as a therapy for high blood pressure and erectile dysfunction. Sildenafil (60mg) will be administered at various time-points to the athletes to assess efficacy and tolerability.
Study Duration:	3 Years
Participant Duration:	30 Days

SCHEMATIC OF STUDY DESIGN

Figure 1. Sildenafil Pilot Study.



1 KEY ROLES

Name, degree, title: Kan Ding, M.D.; Assistant Professor

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Role: co-I, Medical Monitor; **Institution Name:** UTSWMC
Phone Number: 214-648-0499; **Email:** Christopher.madden@utsouthwestern.edu

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Traumatic Brain Injury (TBI). Each year in the United States, about 235,000 persons are hospitalized for TBI, of which approximately 50,000 die. The cost of TBI each year is approximately $77 billion in the U.S. (Injury Prevention and Control: Traumatic Brain Injury; <www.cdc.gov/traumaticbraininjury/statistics.html>). Despite advances in neurosurgical interventions and acute monitoring, many survivors do not fully recover and are left with permanent disability (1, 2, 22, 23). Secondary brain damage which is caused by oxidant injury, inflammation, and vascular injury, may begin almost immediately after the primary injury. Even those with mild TBI may have prolonged neurological deficits, with about 15% having disabling persisting symptoms. 43% of those hospitalized for TBI have long-term disability (24-26). Consequently, chronic secondary brain injury is difficult to manage and the administration of neuroprotective agents in a chronic fashion is warranted to assist in the recovery process and decrease neuro-degeneration in this population. Multiple studies that aimed to treat mild TBI acutely with various interventions showed poor results (27). These limited findings may be due to insufficient treatment times, ineffective therapies, or lack of endophenotype data. Endophenotype guided therapies are ideal and may yield drug therapies that reduce secondary brain injury and improve outcomes within the TBI population.

Vascular Injury and TBI. Traumatic cerebral vascular injury (TCVI) is a debilitating TBI endophenotype. In both animal and human TBI models, TCVI results in vasodilatory deficits and other microcirculatory problems. In these models, the response to vasodilators such as nitric oxide (NO) was reduced significantly. Also, these deficits were a result of damage to the endothelium, a reduction of capillary density, intravascular microthrombi, and white-matter degeneration, resulting in motor and cognitive deficits (3-13, 28).

Following TCVI, cerebral blood flow (CBF) is affected in TBI subjects as indicated by hypo-perfusion in numerous brain structures. For example, CBF has been found to be reduced significantly in people who have experienced a severe TBI, resulting in poor long-term cognitive and functional outcomes (29-32). Also, CVR is decreased after TBI and correlates with reduced CBF, cognitive deficits, and heightened symptoms (33, 34). Other studies have demonstrated that at chronic time-points, the CBF remained abnormal in athletes even though they showed improvements with cognitive scoring and reduced symptoms at later time-points (35-38). Imaging diagnostics have proven to be vital in detecting TCVI in both symptomatic and

asymptomatic TBI subjects and should be utilized to monitor disease progression and efficacy of various treatments.

Imaging tools such as transcranial doppler (TCD) and near infra-red spectroscopy (NIRS) have been shown to measure vasospasms and CVR abnormalities in TBI survivors. A major limitation of TCD and NIRS is their lack of sensitivity, which has been demonstrated in a number of research studies (39-45), warranting the use of a more sensitive approach for identifying CBF and CVR abnormalities. Utilization of magnetic resonance imaging (MRI)-Blood Oxygen Level Dependent (BOLD) combined with hypercapnia (high spatial and temporal resolution) by our group and others has proven to be more effective in determining the hemodynamic response in TBI subjects (46-48). In control and injured people, MRI-BOLD measures the transition rate of oxygenated hemoglobin to deoxygenated hemoglobin and defines the regional/global CBF in the brain (49). Recently, preliminary data generated by our research group has proven that CBF and CVR are decreased in people with a history of TBI. The primary goal of this study is test whether sildenafil improves global CBF/CVR, reduces symptoms, and improves cognition in concussed athletes.

Sildenafil Therapy to Improve CBF and CVR. Sildenafil is a vasodilator that inhibits the cGMP-specific phosphodiesterase type 5 enzyme, resulting in decreased degradation of cGMP. The original use of this drug was to treat hypertension and later for erectile dysfunction (50-52). This drug is relatively safe and has been shown to increase CVR in a number of studies (53, 54). For example, in 28 patients randomized to either placebo or sildenafil, an increase in CVR was observed as early as 1 hour after administration (54). In another study, utilization of TCD showed that a single 50mg dose of sildenafil in patients with vertebra-basilar insufficiency displayed a significant increase in the diameter, area, and flow volume of the vertebral artery (55). In contrast, in healthy individuals, no effect on CBF was observed when treated with sildenafil (56, 57). Preliminary data generated by our research group demonstrates that sildenafil is efficacious at normalizing CBF and increasing CVR in TBI patients. Given the paucity of studies testing the effect of sildenafil on CBF/CVR, in this novel study, we plan to test the hypothesis that sildenafil normalizes global CBF and potentiates CVR in concussed professional boxers.

2.2 RATIONALE

Since we have the capability to obtain baseline (prefight) data, this population is an ideal group to study the efficacy of sildenafil at normalizing CBF, potentiating CVR, improving cognition and symptoms. The use of sildenafil is based on published reports in stroke models and preliminary data generated from our research group in TBI subjects. After suffering a concussion, we believe that this compound will effectively diminish the secondary injury in the brain and mitigate symptoms and cognitive deficits. Also, this therapy has proven to be safe in people suffering from TBI.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

Sildenafil: There is a serious risk in subjects with pre-existing cardiovascular disease, hypotension (BP <90/50)/hypertension (BP> 170/110), coronary heart disease, subjects with retinitis pigmentosa there is cardiac risk.

MRI: Subjects are at risk for injury from the MRI magnet (3 Tesla) 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. Volunteers will be screened for these conditions before having an MRI, and if any are present, the MRI scan will not be performed. Volunteers will be asked specifically about metal objects being present in their body. If it is unknown whether metal is present and or if there is any question, MRI will not be performed.
Subjects 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.

Neuropsychological Testing: There are no expected risks for the participants answering the questions. Possible adverse events may include psychological stress.

Blood/Saliva collection: The risk of bruising or infection are the main risks involved with the blood collection procedures. There are no known risks of providing a saliva specimen.

2.3.2 KNOWN POTENTIAL BENEFITS

The information collected from this study will further our knowledge of secondary brain injury after TBI. Also, we will assess whether sildenafil therapy alters blood flow, which is hypothesized to be protective after TBI. Even though this study does not offer direct benefit to participants, the data collected will help shape how TBIs are managed in the future. The MR images will be collected for research purposes only. If any abnormalities are observed we will contact the medical monitors. The benefits outweigh the risks associated with this study.

3 OBJECTIVES AND PURPOSE

The main objective of this clinical trial is to test the hypothesis that sildenafil treatment in active professional boxers/MMA fighteres soon after concussion normalizes CBF, potentiates CVR, reduces symptoms, improves cognition, and is a tolerable therapy. If our hypothesis is supported, sildenafil will shape how secondary brain injury is treated and will improve long-term outcomes after TBI.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

In this placebo-controlled, double-blinded clinical trial, approximately 100 boxers/MMA fighters that have experienced a concussion or a significant number of sub-concussive blows will be treated with a placebo pill or Viagra® (sildenafil; 60mg) once (single-dose therapy) or once-a-day for 14 days. This is a phase 2 study that will be conducted at a single-site (UTSWMC, Dallas, Texas).

Prior to competition, the medical/concussion history and a blood (8ml) and saliva sample will be collected at the baseline (pre-fight) visit. Also, a battery of neuropsychological exams (NIH Common Data Elements) and a magnetic resonance imaging (MRI) scan will be performed at UT Southwestern Medical Center Advanced Imaging Research Center to obtain cognitive, structural, and functional baseline data.

At day1-3 and 30 after injury, the boxers will undergo the MRI structural and MRI BOLD (with hypercapnia) procedures at UT Southwestern Medical Center Advanced Imaging Resource Center. The subjects will also undergo neurpsychological testing, and blood/saliva collection for tests to determine the efficacy of sildenafil. A randomization schedule will be prepared by the study statistician. Randomization will be 1:1 sildenafil:placebo, and will be in blocks of 2. Investigators associated with this study will not have access to the randomization schedule. The principal investigator, study team, and study subjects will be blinded in this study. To monitor the drug administration, a daily diary will be filled out by the participant. The tolerability of sildenafil in boxers will also be assessed.

A total of 130 boxers will be screened and only 100 boxers will be enrolled in this study to complete the study aims. The study participants will not be responsible for any research related costs, which include travel and parking when visiting UTSWMC for the collection of blood/saliva, neuropsychological testing, and MR imaging. The participants will be compensated for their time.

The participants in **group 1** (single therapy) will consist of the active boxers/MMA fighters that experience significant sub-concussive blows or a concussion during competition. In this group, 65, professional male/female boxers/MMA fighters within the North Texas region will be screened and about 50 concussed athletes will be enrolled. The athletes that have at least 25 blows to the head and a Symptom Score > 1 on at least 3 items from the Rivermead Post-Concussion Questionnaire following their fight will be enrolled in this study. The research subjects will be treated with sildenafil (60mg), once at 2 hours after experiencing a concussion.

Group 2 will consist of the active boxers/MMA fighters that experience significant sub-concussive blows or a concussion during competition. In this group, 65, professional male/female boxers/MMA fighters within the North Texas region will be screened and about 50 concussed athletes will be enrolled. The athletes that have at least 25 blows to the head and a Symptom Score > 1 on at least 3 items from the Rivermead Post-Concussion Questionnaire following their fight will be enrolled in this study. Once enrolled, the subjects will be treated with sildenafil (60mg) once-a-day for 14 days.

Healthy Control group: In this study, 80, age (between 18 and 35)-matched healthy controls will be enrolled. The control subjects will participate in neuropsychological testing, blood/saliva collection, and the MR imaging procedures. Healthy controls will be tested in group 1 (n=40) and 2 (n=40) and randomized to receive either sildenafil or placebo in this group.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT:

To determine if sildenafil administration improves CBF/CVR, reduces symptoms, and improves cognitive performance in concussed boxers/MMA fighters. *Hypothesis: Both single and multiple sildenafil therapy improves CBF/CVR, symptoms, and cognition in concussed boxers. Also, long-term administration of daily doses of sildenafil to concussed boxers/MMA fighters is well tolerated.* Following injury, we will enroll 100, active boxers/MMA fighters and 80, controls into a double-blind, placebo-controlled study. At a 1:1 ratio, the subjects will receive a single treatment of either placebo or sildenafil. The purpose of this study is to determine if sildenafil treatment improves CBF/CVR, reduces symptoms, and improves cognitive outcomes. In this study we will treat the injured athletes once (within 2 hours of injury) or daily (for 14 days) to determine if sildenafil improves outcomes. Previous clinical studies conducted by our research group, we found that sildenafil treatment (60mg) is tolerable within the TBI population. The athletes will maintain a daily diary of symptoms. At day 1-3 and 30 after injury, the CBF/CVR MR imaging procedures, the Rivermead Post Concussion Symptom Questionnaire, and neuropsychological assessments (NIH Common Data Elements) will be conducted to study the effect of sildenafil therapy. Blood (8cc) and saliva will also be collected to measure brain biomarkers of injury. Tolerability (blood testing and symptom reporting) assessments will be conducted throughout the study to ensure that the symptomatic boxers tolerate multiple, daily doses of sildenafil.

4.2.1.a. MRI

Preparation for the MRI procedure:

At baseline and post-concussion time-points (day 1-3, 30), the research coordinator will schedule the imaging procedure with the subject. There will be a 3-day window to facilitate scheduling of the MRI studies. Once the subject arrives to UT Southwestern Medical Center (Dallas, Texas), the research coordinator will escort the participant to the Advanced Imaging Resource Center for the MR imaging session. Prior to conducting the imaging procedures, the subjects will be screened for MRI safety using the Screening Questionnaire. The subjects may be asked to lie still for up to 7-10 minutes at a time. While in the scanner they will hear loud knocking noises, and they will be fitted with earplugs or earmuffs 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 anytime. Participants will first have a structural MRI scan.

The structural MRI sequences we will use do not require contrast. The subjects will not be required to do any tasks during the structural MRI. Sequences used for the structural MRI 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.

The BOLD sequence with hypercapnia challenge:

The participants will have a BOLD MRI scan (baseline, day 1-3, and 30 after injury) with CVR hypercapnia measurements. Global and 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 after the initial 45-minute structural scan and fitted with a breathing mask that covers the mouth and nose (similar to a C-PAP mask). After

the mask 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. A research assistant will be inside the magnet room throughout the BOLD scan with hypercapnia challenge to switch the valve and monitor the subject. Physiologic parameters, including end-tidal ($EtCO_2$), breathing rate, heart rate, and capillary oxygenation will be monitored continuously during the experiment using a capnograph and pulse oximeter. The type of gas breathed is switched every minute similar to a block design fMRI experiment, while Blood Oxygen Level Dependent (BOLD) MRI Images are acquired for 7 minutes.

Times are close approximations which may be altered depending on the cooperation of the patient. The subject will not engage in the hypercapnia challenge for more than the specified 7 min. Total scan time with structural and BOLD sequences will be approximately 1 hour.

MRI patient procedures:

1. Safety screening
2. 45 min structural scan
3. Out of the magnet and fitted with the mask for hypercapnia challenge
4. 7 min. BOLD scan with mask and hypercapnia

4.2.1.b. TBI Neuropsychological Assessments

In this clinical study, we test whether sildenafil improves cognitive, mood, and functional outcomes in injured boxers. The testing procedures will be conducted in a quiet room at UTSWMC by a neuropsychologist or trained coordinator. We anticipate that the testing procedures will take approximately 1 hour to complete. The subjects will be given frequent rests and they have the right to refuse to answer any questions or to stop a test at any time and for any reason. TBI phenotyping instruments recommended for evaluation in all TBI cohort or natural history studies have been labeled the TBI Common Data Elements (CDEs)(58).The subject will be interviewed, complete questionnaires, take pen-and-paper tests, and perform simple actions.

In this study we will include the following selected subset of assessments taken from the CDE's:

- Hopkins Verbal Learning Task (HVLT). The **Hopkins Verbal Learning Test** (HVLT) is a brief verbal learning and memory test with six alternate forms of 12 words.
- The Sport Concussion Assessment Tool (SCAT5), from the Concussion in Sport Group, is a standardized tool for evaluating injured athletes for concussion and can be used in athletes aged 13 years and older. This test will take approximately 30 minutes.
- Satisfaction with Life Scale (SWLS): is a short 5-item instrument designed to measure global cognitive judgments of satisfaction with one's life. The scale usually requires only about one minute of a respondent's time.
- Rivermead Post-Concussion Symptom Questionnaire: rate the severity of 16 different symptoms commonly found after a TBI (5 min).
- Galveston Orientation and Amnesia Test (GOAT)-modified for sports athletes

4.2.2 SECONDARY ENDPOINT:

To determine if sildenafil reduces the blood/saliva levels of brain biomarkers of injury after concussion. The goal of this study is to produce pilot efficacy data to design a definitive study aimed at testing the efficacy of the administration of sildenafil at reducing the blood/saliva levels of Von willebrand factor (vWF), glial fibrillary acidic protein (GFAP), neurofilament-H (NFL-H), creatine kinase-B (CKBB) and tau. In brief, about 8cc of blood and saliva will be collected at each time-point. The blood and saliva will be collected once at baseline and on day 1-3 and 30 after injury. Upon collecting the samples, the blood will be centrifuged at 4 degrees Celsius for 10 minutes at 1500 rpm. The plasma/saliva will be collected and aliquoted into 1 ml tubes and immediately frozen at -80 degrees Celsius. To measure the levels of brain biomarkers, the plasma levels of vWF, GFAP, NFL-H, CKBB, and tau will be measured using the enzyme-linked immunoassay according to the manufacturer's instructions. Each sample will be loaded onto the 96-well plate in triplicate. Each sample will be tested in triplicate. The plate will be incubated for 2 h at room temperature, washed, and 200 µl of the substrate will be added for 90 min at room temperature. After 90-min, the absorbance will be detected at 405 nm. The values will be compared to the standard curve and the concentration of the biomarker will be determined.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

FOR ATHLETES

1. Age 18-35
2. Male or female professional boxers/MMA fighters
3. Ability to undergo MR imaging procedures
4. At least one of the following:
   a. KO/TKO scored by fight referee.
   b. Greater than 25 blows to the head.
5. Significant post-concussive symptoms (Symptom Score > 1 on at least 3 items from the Rivermead Post-Concussion Questionnaire)

FOR CONTROLS

1. Age 18-35
2. Male of female who do not participate in contact sports
3. Screen negative for mTBI using Ohio State TBI ID

5.2 PARTICIPANT EXCLUSION CRITERIA

1. Contraindication to sildenafil which includes the following:
   a. Current use of organic nitrate vasodilators
   b. Use of ritonavir (HIV-protease inhibitor)
   c. Current use of erythromycin, ketoconazole, or itraconazole

d. Current use of cimetidine
e. Current resting hypotension (BP < 90/50 mm Hg)
f. Current severe renal insufficiency (Creatinine Clearance ≤ 30mL/min)
g. Current hepatic cirrhosis
h. Current cardiac failure or coronary artery disease causing unstable angina
i. Retinitis pigmentosa
j. Known hypersensitivity or allergy to sildenafil of any of its components

2. Daily therapy with a PDE5 inhibitor within the past 2 months
3. Immediate hospitalization for severe concussion
4. History of neurological or psychiatric disorder not related to TBI
5. Known inclusion in another interventional clinical trial
6. Subjects with metal implants that would interfere with the MR imaging procedures
7. Sickle cell disease
8. History of priapism

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Within the North Texas area there are approximately 130 boxing training centers. Over the last 6 years we have built relationships with trainers at these facilities and about 125 boxers have previously been enrolled in both observational and interventional studies conducted by the Gatson laboratory. We have been successful with enrolling athletes (boxers, MMA fighters, etc.) and completing the clinical studies with a very low dropout rate (10%). Thus, we believe that will meet study recruitment and experience very little dropouts.Our target sample size is 100 athletes. We will also recruit 80 healthy controls.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

In this study, the participants will be allowed to withdraw at any point. Also, the PI and/or study investigators may choose to withdraw a participant at any time. The reason for withdrawing participants may be for administrative purposes, non-compliance, and/or medical reasons.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

If a subject withdraws or is withdrawn from the study, any remaining unused samples and/or data identified as theirs will be destroyed at their request. If the PI or study investigators do not receive a request, then the samples will contiunue to be used in the analysis as applicable.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

Drs. Joseph Minei and Christopher Madden, who are the medical monitors, have the authority to halt the study, remove individual subjects from the study, and take whatever steps are necessary to protect the safety and well-being of research subjects. An independent monitor from UTSWMC will also have the capability to stop a study for safety reasons.

The medical monitors, PI, Independent monitor, and IRB 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 medical monitor, UTSWMC monitor and IRB of record. Only when these entities agree in writing that the problems have been satisfactorily addressed,the protocol may be re-activated.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

Sildenafil (60mg) and placebo tablets will be ordered from Pfizer and delivered to the UTSWMC pharmacy. The UTSWMC pharmacy will be responsible for packaging the study drug and devising the randomization schedule.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Sildenafil is supplied as blue, film-coated, rounded-diamond-shaped tablets containing sildenafil citrate equivalent to 60 mg of sildenafil. The placebo pills will be compounded and have similar appearance to the investigational drug.

6.1.3 PRODUCT STORAGE AND STABILITY

The drug will be stored at room temperature (25°C [77°F]) with excursions permitted (59-86°F).

6.1.4 PREPARATION

The study drug is in pill format. The participant will self administer the sildenafil pill. The study participant will maintain a daily diary to document the administration of the study drug.

6.1.5 DOSING AND ADMINISTRATION

In the first and second group, the participants will be administered either a single-dose or 14 pills of placebo or sildenafil (60mg) after suffering a concussion. The first dose will be taken at 2hours after injury.

6.1.6 ROUTE OF ADMINISTRATION

Oral

6.1.7 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

N/A

6.1.8 DURATION OF THERAPY

The duration of the study for the primary and secondary endpoints will be 30 days.

6.1.9 TRACKING OF DOSE

During the course of this study, the participant will maintain a drug diary and document when the treatment was administered. Once the participant completes the study, the pill bottle will be collected and the remaining pills in each bottle will be counted.

6.1.10 DEVICE SPECIFIC CONSIDERATIONS

N/A

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

The UTSWMC pharmacy will create the randomization schedule. The study coordinator will follow the randomization schedule and deliver the pill bottles to the participant. Instructions will be given the research subject pertaining to daily documentation of drug administration and returning the pill bottles at the completion of the study.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

Prior to performing any study procedures, consent will be obtained. All of the evaluations and procedures will performed for research purposes only. None of the tests will be for clinical purposes. In table 2, the study procedures are listed for the athletes that will be treated with sildenafil after competition and procedures for the controls.

Boxers (n=100); **Controls** (n=80)	**Baseline**	**Day 1-3**	**Day 7 (phone)**	**Day 15 (phone)**	**Day 22 (phone)**	**Day 30**
1. Inclusion/Exclusion Criteria	X					
2. Informed Consent	X					
3. Blood/saliva collection (side effects, tolerability, brain biomarkers)	X	X				X
4. Structural MRI (T1 MPRAGE, FLAIR, DTI, rsBOLD)	X	X				X
5. Randomization		X				
6. Dispensing study drug (sildenafil or placebo)		X				
7. Pill counting for compliance						X
8. Monitoring for adverse effects		X	X	X	X	X
9. Symptom inventory		X	X	X	X	X
10. Neuropsychometric CDEs	X	X				X

7.1.2 STANDARD OF CARE STUDY PROCEDURES

N/A

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Biochemistry:
Blood (~8cc) will be collected and the blood albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, and coagulase levels will be measured from from the participants at day 1-3 and 30 after injury. By performing these tests we will be able to detect side effects that may be associated with the study drug. For example, if the AST or ALT ratio is out of the normal range, the participant(s) will be withdrawn from the study. Also, if the participant reports any side effects that may be linked to the study drug, the study participant will be withdrawn from the study. The IRB and FDA will be notified within 7 business days. The

testing will be performed by Quest diagnostics, Dallas, Texas. Blood will also be collected to measure the levels of brain biomarkers of injury within the athletes.

7.2.2 OTHER ASSAYS OR PROCEDURES

N/A

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

At baseline and on day 1-3 and 30 after TBI, the blood/saliva samples will be collected. About 8cc of blood will collected and after clotting, the sample will be centrifuged at 4 degrees Celsius for 10 minutes at 1300g. The blood will be centrifuged within 1 hour of collection. The plasma and saliva will be collected and aliquoted into 1 ml cryovial tubes and immediately frozen at -80 degrees Celsius. Information pertaining to each sample will be added to the specimen log. All personnel will be trained in accordance to the CDE recommendations.

7.2.4 SPECIMEN SHIPMENT

For the biochemistry, we will schedule the Quest Diagnostics courier to collect the samples at UTSWMC to deliver the blood samples to Quest Diagnostics for analysis.

Quest Diagnostics
11613 North Central Expressway #120
Dallas, Texas 75243

7.3 STUDY SCHEDULE

7.3.1 SCREENING

Boxers that meet the baseline study criteria will be approached and if interested they will be consented to participate in the study procedures. Medical and concussion history will be reviewed at the baseline time-point to determine which boxers will be considered to participate in the study. The research coordinator/boxing consultant will identify the athletes that have experienced greater than 25 blows to the head or a Symptom Score > 1 on at least 3 items from the Rivermead Post-Concussion Questionnaire following the boxing match. Once the subject has been identified, the research personnel will review the subject's chart to determine if they meet criteria for enrollment into the study. If so, the participant will be approached to obtain consent where the patient has the right to ask questions about the study.

7.3.2 ENROLLMENT/BASELINE/FOLLOW-UP

Sildenafil Study:

Baseline Visit (Visit 1, Day 0)

- Obtain informed consent of potential participant verified by signature on study informed consent form
- Verify inclusion/exclusion criteria
- Obtain demographic information, medical history, head injury history, medication history, alcohol and tobacco use history
- Record vital signs, results of examinations, other assessments.
- Baseline symptom questionnaire
- Collect blood/saliva for biochemistry, biomarker testing
- MRI procedures
- Neuropsychological testing
-

Follow-up Visit (Visit 2, 2 hours after injury)

- Administer sildenafil (60mg) or placebo once of daily for 14 days

Follow-up Visit (Visit 3, Day 1-3)

- Record adverse events
- Collect blood/saliva for biochemistry, biomarker testing
- Administer sildenafil or placebo (once)
- MRI procedures
- Neuropsychological testing

Follow-up Visit (Visit 4, Day 30)

- Symptom questionnaire
- Collect blood/saliva for biochemistry, biomarker testing
- MRI procedures
- Neuropsychological testing

Follow-up Visit (Visit 5 [Phone Visit], Day 14 after completion of the study)

- Symptom questionnaire
- Safety Assessment

7.3.3 EARLY TERMINATION VISIT

If the study is terminated early, the final visit would consist of collecting a blood/saliva sample and administering the neuropsychological testing and symptom questionnaire (at time-

points after day 10 after injury). The MRI scan would also be necessary in cases where the study ends after the 10-day post-enrollment.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

In this study, placebo will be used as a comparator to assess whether sildenafil improves CBF, potentiates CVR, reduces symptoms, and improves cognitive outcomes in the brain injured athletes.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

1. Sildenafil is contraindicated for use in people that are hypersensitive to any component of the tablet.
2. Sildenafil has a systemic effect vasodilatory effects, which may be augment the effect of other anti-hypertensive agents.
3. Caution should be used when using Viagra and ritonavir, since concomitant administration of the protease inhibitor, ritonavir, substantially increases serum concentrations of sildenafil.
4. Caution is advised when Phosphodiesterase Type 5 (PDE5) inhibitors are co-administered with alpha-blockers.
5. Combinations of Viagra with other erectile dysfunction medications (Revatio) is not recommended.
6. Sildenafil use is contraindicated with regular and/or intermittent use of organic nitrates.
7. Sildenafil should not be taken with other PDE5 inhibitors.
8. Co-administration of Cimetidine (800mg), erythromycin, or saquinavir with sildenafil is cautioned since a combination of any of these drugs with sildenafil results in a great increase in sildenafil systemic exposure.

7.6 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

In cases of overdose, standard supportive measures should be adopted as required. Renal dialysis is not expected to accelerate clearance as sildenafil is highly bound to plasma proteins and it is not eliminated in the urine.

7.7 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

Adverse events will be reported in accordance with Federal NIH requirements. All adverse events, serious and non-serious, expected and unexpected, related and unrelated will be tracked by the study coordinator.

Serious or unexpected adverse events and other unanticipated problems will be reported orally as soon as possible and in writing within 7 days if life-threatening and within 15 days otherwise. Expected or non-serious adverse events will be reported at the time of continuing review.

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

Unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2 ADVERSE EVENTS

8.2.1 SEVERITY OF EVENT

Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.

Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 EXPECTEDNESS

Table 4. Adverse events attributed to sildenafil in placebo-controlled randomized trials

Adverse Event	% of patients on sildenafil reporting AE (n = 734)	% of patients on placebo reporting AE (n = 725)
Headache	16%	4%
Flushing	10%	1%
Dyspepsia	7%	2%
Nasal Congestion	4%	2%
Urinary Tract Infection	3%	2%

Abnormal vision*	3%	0%
Diarrhea	3%	1%
Dizziness	2%	1%
Rash	2%	1%
*Abnormal Vision: Mild and transient, predominantly color tinge to vision, but increased sensitivity to light or blurred vision. In these studies, only one patient discontinued due to abnormal vision		

Table 5. Adverse Events Reported by Participants in Placebo Controlled RCTs, but Equally Common in Placebo Group (occurred in <2% of participants; relationship to Viagra unknown but plausible)

System	Adverse Event
Body	Face swelling, light sensitivity, shock, pain, chills, accidental fall , abdominal pain, allergic reaction, chest pain, accidental injury, body or muscle weakness
Cardiovascular	Chest pain, AV block, migraine, syncope, tachycardia, palpitation, hypotension, postural hypotension, myocardial ischemia, cerebral thrombosis, cardiac arrest, heart failure, abnormal electrocardiogram, cardiomyopathy.
Digestive	Vomiting, glossitis, colitis, dysphagia, gastritis, gastroenteritis, esophagitis, esophagitis, stomatitis, dry mouth, liver function tests abnormal, rectal hemorrhage, gingivitis.
Hemic and Lymphatic	Anemia and leukopenia.
Metabolic and Nutritional	Thirst, edema, gout, unstable diabetes, hyperglycemia, peripheral edema, hyperuricemia, hypoglycemic reaction, hypernatremia.
Musculoskeletal	Arthritis, arthrosis, myalgia, tendon rupture, tenosynovitis, bone pain, myasthenia, synovitis.
Nervous	Ataxia, hypertonia, neuralgia, neuropathy, paresthesia, tremor, vertigo, depression, insomnia, somnolence, abnormal dreams, reflexes decreased, hypesthesia.
Respiratory	Asthma, dyspnea, laryngitis, pharyngitis, sinusitis, bronchitis, sputum increased, cough increased.
Skin and Appendages	Urticaria, herpes simplex, pruritus, sweating, skin ulcer, contact dermatitis, exfoliative dermatitis.
Special Senses	Sudden decrease or loss of hearing, mydriasis, conjunctivitis, photophobia, tinnitus, eye pain, ear pain, eye hemorrhage, cataract, dry eyes.

Urogenital	Cystitis, nocturia, urinary frequency, breast enlargement, urinary incontinence, abnormal ejaculation, genital edema and anorgasmia.

Table 6. Post-Marketing Experiences Reported in Temporal Association with the use of Viagra.

Occurred predominantly in patients who had preexisting cardiovascular risk factors. Undetermined if these events are related directly to Viagra, to sexual activity, to the patient's underlying conditions, or to a combination of these factors.

Cardiovascular and Cerebrovascular	Myocardial infarction, sudden cardiac death, ventricular arrhythmia, cerebrovascular hemorrhage, transient ischemic attack, hypertension, subarachnoid and intracerebral hemorrhages, and pulmonary hemorrhage	
Special Senses	Sudden decrease or loss of hearing	
Other Events	**Nervous**	Seizure, seizure recurrence, anxiety, and transient global amnesia
	Urogenital	Prolonged erection, priapism, and hematuria.
	Special Senses	Diplopia, temporary vision loss/decreased vision, ocular redness, ocular burning, ocular swelling, increased intraocular pressure, retinal vascular disease, vitreous detachment/traction, paramacular edema, and epistaxis.
	Non-arteritic anterior ischemic optic neuropathy (NAION)	Decreased vision including permanent loss of vision. NAION reported rarely post-marketing in temporal association with use of Viagra mostly in patients with risk factors including: Low cup to disc ratio, age over 50, diabetes, hypertension, coronary heart disease, hyperlipidemia, and smoking.

Table 7. Incidence of Clinical Worsening and Most Frequent Adverse Events in Daily Dosing of Sildenafil and Placebo (dose listed was administered 3 times each day for 12 weeks)

Dose	Placebo	Sildenafil		
Number of Subjects (percent Female)	**-- n=70 (81%)**	**20mg TID n=69 (71%)**	**40mg TID n=67 (70%**	**80mg TID n=71 (79%)**
Clinical worsening	7 (10%)	3 (4%)	2 (3%)	5 (7%)
Death	1 (1%)	0	1 (1%)	2 (3%)
Hospitalization for pulmonary arterial hypertension	7 (10%)	2 (3%)	2 (3%)	2 (3%)
Initiation of prostacyclin	1 (1)	0	0	0
Initiation of bosentan	0	0	1 (1)	0
Adverse Events	**Placebo**	**Sildenafil**		
		20mg TID	**40mg TID**	**80mg TID**
Headache	27 (39%)	32 (46%)	28 (42%)	35 (49%)
Flushing	3 (4%)	7 (10%)	6 (9%)	11 (15%)
Dyspepsia	5 (7%)	9 (13%)	6 (9%)	9 (13%)
Back pain	8 (11%)	9 (13%)	9 (13%)	9 (8%)
Diarrhea	4 (6%)	6 (9%)	8 (12%)	7 (10%)
Limb pain	4 (6%)	5 (7%)	10 (15%)	6 (8%)
Myalgia	3 (4%)	5 (7%)	4 (6%)	10 (14%)
Cough	4 (6%)	5 (7%)	3 (4%)	6 (8%)
Epistasis	1 (1%)	6 (9%)	5 (7%)	3 (4%)
Pyrexia	2 (3%)	4 (6%)	2 (3%)	7 (10%)
Insomnia	1 (1%)	5 (7%)	4 (6%)	3 (4%)
Influenza	2 (3%)	4 (6%)	4 (6%)	3 (4%)
Visual disturbance	0	0	3 (4%)	5 (7%)
Gastritis	0	2 (3%)	2 (3%)	3 (4%)

FDA WARNING: There is a potential for cardiac risk of sexual activity in patients with preexisting cardiovascular disease. Therefore, treatments for erectile dysfunction, including VIAGRA, should not be generally used in men for whom sexual activity is inadvisable because of their underlying cardiovascular status. We expect this will be rare in our study, which will not enroll subjects older than 50 years of age. Participants with hypotension, cardiac failure, or coronary artery disease with unstable angina are excluded from this study.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

After enrollment into the study, a telephone follow-up will occur every 7 days. Telephone follow-up and clinic visits will give physician investigators an opportunity to interface with participants. Participants will be instructed to call 911 or go to the nearest emergency room in the event of a serious symptom. Investigators will carry a pager and will be available to the study participants for questions or conserns about side effects. Study participants will be cautioned against taking medications that may have possible adverse interactions with sildenafil. Licensed physician-investigators may prescribe medication for headache or other minor side effects. Otherwise, patients will be instructed to seek medical attention from their personnal physician.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Adverse events will be reported in accordance with Federal requirements. All adverse events, serious and non-serious, expected and unexpected, related and unrelated will be tracked by the study coordinator and medical monitors. Serious or unexpected adverse events and other unanticipated problems will be reported orally as soon as possible and in writing within 7 days if life-threatening and within 15 days otherwise to the IRB and FDA. Expected or non-serious adverse events will be reported at the time of continuing review.

8.4.2 REPORTING OF PREGNANCY

Although extensive testing in animals has demonstrated no negative effects on the fetus, sildenafil has not been studied in pregnant women. Sildenafil is listed as Pregnancy Category B. Pregnancy is an exclusion criteria in this study. There is no effect of sildenafil on sperm count or motility of sperm in men. If a participant has been enrolled and tests positive preganancy at a later visit, she will be withdrawn from the study.

8.5 STUDY HALTING RULES

Administration of study agent will be halted when three grade 3 AEs determined to be “probably related” are reported to the study coordinators and /or study PIs. The PIs will notify the IRB and FDA immediately when the third grade 3 event is reported and enrollment screens will stop accepting new study participants. The PI will inform the DSMP members within 24 hours of this occurrence and will provide the DSMP group with AE listing reports. The DSMP members will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMP members will provide recommendations for proceeding with the study to the study sponsor. The PI will inform the FDA of the temporary halt and the disposition of the study.

8.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMP composed of individuals with the appropriate expertise, including Neurology/Radiology. The DSMP members will only meet to discuss reported AEs. A written reported will be submitted to the DSMP group every 6 months. At this time, each data element that the DSMP group needs to assess will be clearly defined. The DSMP group will provide its input to the PI and IRB.

9 CLINICAL MONITORING

Roles and Responsibilities
The PI, Dr. Ding, will be responsible for working with the IRB to ensure the participants’ safety on a daily basis. In addition, we assembled independent safety monitors (ISM) as a part of our

DSMP to oversee trial safety, data management, analysis and quality assurance. Marco Pinho, M.D. (Assistant Professor, UT Southwestern Medical Center), Donald Glass, M.D., Ph.D. (Assistant Professor, UT Southwestern Medical Center) and Kimbra Kenney, M.D. (Neurologist, Walter Reed National Military Medical Center) will oversee the study protocols, monitor the safety of the participants, and ensure data security. Drs. Pinho, Glass, and Kenney (outside member) are not associated with the trial and have no conflicts of interest with the study team or proposed institutions. Dr. Pinho will be responsible for reviewing protocol adherence, quality control, regular data verification and protocol compliance. Over the last 4 years, Dr. Pinho has assisted Dr. Gatson on various pilot clinical studies at UT Southwestern Medical Center. His role of those studies was to examine computed tomography (CT) and magnetic resonance imaging (MRI) to screen for intracranial injuries within research subjects enrolled in Dr. Gatson's clinical studies. Drs. Glass and Kenney will be responsible for monitoring data management, analysis and quality assurance by reviewing the data sources, the security measures in place to protect data sources (how data will be labelled and stored), and adherence to Good Clinical Practice and regulatory requirements. At UT Southwestern Medical Center, Dr. Glass has previously served on a number data safety monitoring boards.

The ISMs will meet with the investigative team at the start of the trial. The study coordinator will complete monthly reports detailing the study progress enrollment status, other medications taken by the participants during the trial (to screen for concomitant drug use), any adverse events (AEs), and any protocol deviations.

Trial Safety

Throughout the study, the study coordinator and investigators will monitor the participants for AEs. The study coordinator will contact the study participants every 7 days to monitor for adverse events. To monitor for drug toxicity, at the 2nd visit to UT Southwestern Medical Center (day 1-3), 8cc of blood will be collected to determine toxicology, liver function, and coagulation after taking the study drug (sildenafil) for 1-3 days. The study participants will be asked to notify the study team if planning to take any other drug besides the study drug. The participants will also be asked to maintain a daily log of medications taken other than the study drug to monitor drug to drug interactions.

Safety oversight will be under the direction of study PI, medical monitors (Drs. Joseph Minei and Christopher Madden) and the research coordinator. A written reported will be submitted to the DSMP members every 6 months. The ISMs will only meet to discuss reported AEs. Events determined by the PI and co-investigators to be unexpected adverse events involving risks to subjects or others will be reported by the contact PI to the IRB and FDA within 7 business days. With respect to unexpected adverse events, the contact PI will inform the ISMs within 24 hours of this occurrence and will provide the ISMs with AE listing reports. The ISMs will convene an ad hoc meeting by teleconference or in person as soon as possible and provide recommendations for proceeding with the study to the study sponsor and IRB. The ISM will provide its input in the report to the PI and IRB. The contact PI will inform the FDA if there is a temporary or permanent halt of the study. All study members will be informed by the contact PI about any unexpected adverse events.

Upon the reporting of unexpected adverse events a participant, the study coordinator will collect a blood sample (8cc) to determine blood chemistry and will conduct follow up medical monitoring. The Quest Diagnostics laboratory (Dallas, Texas) will perform the biochemical tests. The subject information, adverse event report (if any), and research chart will be kept in a locked file cabinet in the research office (South Campus H7.126). Only study investigators and coordinators will have access to the subject's file.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

STATISTICAL ANALYSIS PLAN

Study design and randomization scheme

Once eligibility is confirmed, based upon defined inclusion and exclusion criteria, randomization will occur within 2 hours of a bout. The placebo-controlled double-blind design will diminish concern about predictability of treatment assignments. The unblinded study biostatistician will prepare a randomization schedule. Randomization will be 1:1 sildenafil:placebo, and will be in blocks of 2. Treatment allocation will be determined via a computer program that automatically generates random digits. Investigators associated with this study will not have access to the randomization schedule. The randomization program will also record the assignments to the corresponding boxers in the database and will display the assignments on the screen for action by the study coordinators.

Outcomes

Primary endpoints- The absolute changes in global and regional CBF/CVR to hypercapnia and improved symptoms and cognitive performance.

Secondary endpoints- Effect of sildenafil on brain biomarkers in blood after injury.

Statistical analyses

Preliminary analyses: Prior to the formal analyses, we will conduct exploratory data analysis by examining the distributions of the key variables using descriptive statistics and graphic tools. In particular, baseline variables (demographics, concussion history and injury severity characteristics) will be examined to determine whether outliers are present. The baseline characteristics of the 100 enrolled boxers/MMA fighters will be compared with 80 controls using linear regression and two sample testing. Continuous variables (primary and secondary outcomes) will be summarized through standard measures of central tendency and spread including means, medians, standard deviations and IQRs. Frequency distributions will be calculated for categorical variables (CBF/CVR, post-concussion symptoms, neuropsychological measurements). Data transformations will be used if needed to produce variables that conform to the distributional assumptions underlying the analytic techniques. Both primary and secondary outcomes will be evaluated under the intent-to-treat principle, meaning that all available data on all randomized participants, regardless of adherence to the study protocol, will be included and analyzed according to the treatment assignment, although compliance will be closely monitored via pill counting. Statistical analysis will be performed with SAS (SAS Institute, Cary, NC, USA) and R software.

Primary analyses: Our primary analysis (**Aim 1**) will be driven by the primary hypothesis that administration of single or multiple doses of sildenafil will improve CBF/CVR, lower symptoms and improve cognition. Boxplots of CBF and CVR of concussed boxers after sildenafil and placebo periods will first be plotted and compared with values at Day 1-3 and 30 post-TBI as well as with the 80 controls.

Also, we will assess the treatment effect of sildenafil on post-concussive symptoms and cognitive performance using two-sample t-test similarly as in the primary aim, but with the secondary endpoints of symptoms. On day 1 and 30 after injury we will then calculate the difference in CBF/CVR and each of the post-concussive symptom assessments and cognitive assessments after the single treatment of sildenafil. Also, we will assess the post-concussion correlations of CBF/CVR and cognitive performance by calculating the cross-sectional Pearson correlations of CBF/CVR with symptom inventory and neuropsychological CDEs collected at Day 1 and 30 after TBI. Correlations will be calculated separately and jointly at each time point, and for each of the subdomains of neuropsychological CDEs. Multiple comparisons will be adjusted by controlling the false discovery rate (FDR).

Our secondary analyses will consist of assessing the treatment effect of sildenafil therapy on brain biomarker levels using two-sample t testing, but with the secondary endpoints of symptoms. We will then calculate the difference in brain biomarker levels at the beginning of the sildenafil therapy and after the treatment period (Day 30 vs Day 1-3). Scatter plots including both arms will be generated based on the calculated differences and two linear regression models with changes in brain biomarkers as outcomes, and changes in symptoms as independent variables will be fit to assess the association between the recovery of the TBI-related markers. Potential confounders such as gender and treatment arm indicator will be adjusted in the model.

Alternative strategies:

For our primary analysis, if CBF or CVR is not normally distributed based as shown in Q-Q plot, a nonparametric Wilcoxon rank-sum test will be used instead. And for our secondary analysis, if we are not powered to identify correlations of biomarkers with subdomains of symptoms and neuropsychological CDEs after multiple comparison correction, we will use dimension reduction approaches such as principal component regression (PCR) on CDEs to group panels of symptom assessments. The resulting panels of symptoms are selected to explain most of the variations across subjects and will be most correlated with brain biomarkers.

Stopping rules

We do not anticipate a stopping rule for efficacy as the primary and secondary endpoints of the proposed study are clinically important, but intermediate. Even if we cannot reject the null hypothesis for the primary end point, we have multiple secondary endpoints that could still benefit our understanding towards the effect of sildenafil on concussed injury. Data collected in this study will be used to conduct a power analysis to design and implement the definitive study.

Sensitivity analyses and missing data

Although we do not expect large amount of missing data due to the close monitoring of the study implementation and great compliance rate from the past study experiences, we will perform sensitivity analyses to assess the potential impact of missing data. Since the analytic methods proposed in the primary and secondary aims will base on the missing at random (MAR) assumption, we will investigate the amount and patterns of missing data, and their influence on the final estimates. We will compare the rate of missing observations between the treatment and placebo groups using Fisher's exact tests. We will also evaluate model estimates using complete data only and data with imputed values to see whether the results suggest bias in analysis of the available data. For possible missing values that are "non-ignorable", we will utilize methods that jointly model the missing mechanism and the time-dependent outcomes to conduct valid statistical inference. In particular, shared parameter models, pattern-mixture models and potentially joint modeling of survival and longitudinal analysis methods could be used to assess potential impact of missing data. Shared parameter models link the outcome and missing probabilities using latent variables (random effects), and a logistic regression will

be used to estimate the probability of missing at each time point. While pattern-mixture models allow separate models for observations with different patterns of missingness. Results obtained from the shared parameter and pattern-mixture models will be compared to the estimates from the analysis under the MAR assumption. Statistical software including SAS and R will be used for the analysis.

Power analysis and sample size justification

We conducted a power analysis based on the primary endpoints of change in CVR and CBF and correlation of CBF/CVR and symptoms/cognition. Based on the preliminary results, we assume the average changes in CVR between treatment and placebo has an effect size of 0.86. With 50 concussed boxers in each arm, assuming that the correlation between the outcome measures at Day 1-3 and 30 is 0.2, we will achieve 80% power to detect difference in CVR changes comparing sildenafil and placebo effects. If the correlation is 0.5 among outcome measures at all time-points, then with 80% power we only need 34 concussed boxers in each arm. Table 1 demonstrates the needed sample size under a range of effect sizes and within-subject correlations. To understand the correlation between the CVR and post-concussive symptoms among the 50 enrolled boxers (for each group) assessed at three time points, we will have 82.9% power to detect a correlation of 0.4 based on the preliminary results in Table 1 with 95% confidence level.

	Correlation between 3, time-points (rho)						
	0	0.2	0.3	0.5	0.6	0.7	0.8
Corresponding SD	1.414	1.265	1.183	1	0.894	0.775	0.632
Effect size	Sample size needed						
0.7	46	38	33	25	21	16	12
0.86	32	26	23	18	15	12	9
1	24	20	18	14	12	10	8
1.2	18	15	14	11	9	8	6

Table 1: Sample size for each treatment and placebo group under a range of effect sizes and within-subject correlations to achieve 80% power.

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10.2 MEASURES TO MINIMIZE BIAS

10.2.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Recruitment Methods and Consenting Process:

At the baseline visit, the participants will be consented to participate in the study. After participating in the boxing match, the enrolled boxers will be re-screened and if the boxer has a Rivermead symptom score > 1 on at least 3 items from the Rivermead Post-Concussion Questionnaire or has greater than 25 blows to the head, the participant will be informed of their eligibility to participate in the study and the potential risks associated with this clinical trial.

A copy of the signed consent form will be provided to the participant/family, and one copy will remain in a locked storage facility. Both women and minorities will be enrolled in this study. Due to the study population, children will not be enrolled in this study. Previously, we have enrolled 125 boxers from the North Texas area to participate in both observational and interventional clinical trials. Recently we completed a Department of Defense (DOD) observational study aimed at measuring early brain biomarkers of injury in professional boxers. We identified numerous early predictors of outcome in these athletes. Our dropout rate is very low and previously the boxers were compliant with the study procedures (self-administering study medication, neuropsychological testing, MRI procedures). We expect a dropout rate of 10-20% and will enroll a total of 110-120 boxers to complete the proposed studies.

Drug Treatment and Randomization:

We will enroll 100 concussed boxers/MMA fighters using a double-blind, placebo-controlled design. The athletes will be randomized at a 1:1 ratio to either placebo or sildenafil for single or multiple doses (once-a-day for 14 days).

A randomization schedule will be prepared by the study statistician. Investigators associated with this study will not have access to the schedule. The PI, study team, and participants will be blinded.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

In compliance with ICH E6, regulatory and institutional requirements for the protection of confidentiality of participants, and the NIH, following enrollment into the clinical trial, the participant will be assigned a study numer. Also, all documents (information form, medical history, concussion history, neuropsychological and symptom forms) and imaging information collected during the course of the study will be placed into the participant study chart. The study chart will also contain a copy of the signed consent and HIPAA forms. The subject's research chart will be kept in a locked file cabinet in the research office (South Campus H7.126). Only study investigators and coordinators will have access to the subject's file.

12 QUALITY ASSURANCE AND QUALITY CONTROL

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the REDCap database will be generated. Any missing data or data anomalies will be communicated in writing to the study PIs and DSMP group. Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)). The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the irb, FDA, and NIH.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent

form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study agent, study procedures, and risks will be given to the participant and written documentation of informed consent will be required prior to starting intervention/administering study product.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator/study coordinator will explain the research study to the participant and answer any questions that may arise. 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 carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover MRI/neuropsychological testing, and collection and testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the PI and UTSWMC.

The representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The PIs will permit access to such records.

The study participant's contact information will be securely stored for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the IRB. Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in a locked cabinet at UTSWMC. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the UTSWMC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at UTSWMC.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES,SPECIMENS OR DATA

- Intended Use: Samples and data collected under this protocol may be used to study the efficacy of sildenafil at increasing CBF/CVR, reducing symptoms, and improving cognition. No genetic testing will be performed.
- Storage: Access to stored samples will be limited to the research team. Samples and data will be stored using deidentified subject codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data. All documents pertaining to the study will be stored under lock and key in secured rooms accessible only to the study personnel.
- Tracking: Data will be tracked using REDCap. Disposition at the completion of the study: All stored samples will be stored at UTSWMC. Study participants who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained for tracking.

13.5 FUTURE USE OF STORED SPECIMENS

Data collected for this study will be analyzed and stored at UTSWMC. After the study is completed, the de-identified, archived data will be transmitted to and stored at the UTSWMC TBI Data Repository, under the supervision of Kan Ding, for use by other researchers including those outside of the study. Permission to transmit data to the UTSWMC TBI Data Repository will be included in the informed consent.

With the participant's approval and as approved by local IRBs, de-identified biological samples will be stored at the UTSWMC TBI Data Repository with the same goal as the sharing of data with approved entities. The UTSWMC TBI Data Repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the masking of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed.
When the study is completed, access to study data and/or samples will be provided through the UTSWMC TBI Data Repository.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

We will follow data collection practices recommended by the NIH NINDS Common Data Elements. (1) Data entry and management: A database will be developed using REDCap. All data will be double entered. A comparison will be run between the primary and secondary entries to detect inconsistencies and data entry errors. Data will be routinely audited. If any systematic errors are found, data entry will stop and the data management system will be evaluated. (2) Data security: All data (e.g., survey, recordings, and electronic files) will be identified by alpha-numeric codes only, consent forms will be maintained separately from the data, data will be password protected, and all data will be stored in locked cabinets in lab offices. Only members of the research team will have access to these files.

14.2 STUDY RECORDS RETENTION

Study documents will be retained for a minimum of 3 years after the completion of the study.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. If deviations occur, corrective actions will be developed by study PIs and implemented promptly.

These practices are consistent with ICH E6:
- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of our research group to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents. Protocol deviations must be sent to our IRB. The site PI/study staff will be responsible for knowing and adhering to the IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

14.4 PUBLICATION AND DATA SHARING POLICY

To be considerd for publication in scientific journals, this clinical trial will be registered with ClinicalTrials.gov prior to initiaited any study related activities.

Resource Sharing: MRI data, neuropsychological data, and blood/saliva samples will be catalogued and stored for future study. Beyond publication of results in peer-reviewed journals and presentations at national meetings, Dr. Ding will make available data/samples to other investigators as a resource. All data requests will be considered by a Scientific Advisory Panel that will be established to determine access to data developed by the proposed study as well as subsequent ancillary studies. This Scientific Advisory Panel will be comprised of senior researchers and clinicians, including Drs. Christopher Madden MD andKan Ding MD,. No data that enable identification of individual patients will be collected or shared. All data will be stripped of patient identifiers upon collection; further de-identification to comply with HIPAA and Institutional Review Board regulations will not be necessary prior to sharing of resources with other investigators. Further, summary results will be posted on a laboratory website that will also provide access to data dictionaries, protocols, and ancillary study proposal forms. This website will describe the procedure for requesting data and proposing ancillary studies through the Scientific Advisory Panel.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

The Steering Committee will govern the conduct of the study. The Steering Committee will be composed of the study PI and Dr. Christopher Madden (Medical Monitor). The Steering Committee will meet in person at least annually.

16 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with UTSWMC has established policies and

procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

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