STUDY TITLE: Novel protection against potential brain, hearing and vision injury during Blast Wave Exposure

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CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER

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(1) ABSTRACT:

Significant morbidity, mortality, and related costs are caused by traumatic brain injury (TBI). A simple, effective, and lightweight device worn by athletes or war fighters in the field, designed to mitigate TBI resulting from blast trauma or concussive events, would save lives, and the huge costs incurred for life-treatment of surviving victims. An externally-worn medical device that applies mild jugular compression according to the principle of the Queckenstedt Maneuver (the Device) is being developed by Q30 Sports Science, LLC (Q30). Preliminary research suggests that the Device has the potential to reduce the likelihood of TBI. The currently developed collar (Smith 2009; Smith 2011; Smith 2011; Smith 2012) has been approved for studies in humans and the results indicate safety for use during high demand and maximal exertion activities, *Study ID: 2013-2240, Institutional Review Board - Federalwide Assurance #00002988).* Regarding safety, the externally worn collar is meticulously designed to mimic the body's own omohyoid muscle actions upon the jugular veins that will provide similar pressure and volume increases not to surpass that of a yawn or the mere act of just lying down.

This study will investigate the effectiveness of this device in tactical team members exposed to blast waves during tactical training. Subjects participating in this study will be randomly assigned to one of two groups: 1) Device wearing during the tactical training or 2) Non-device wearing during the tactical training. The helmets of all participants will be outfitted with an accelerometer which will measure the magnitude of every concussive blast wave sustained by the subject. Effectiveness of the device will be determined via differences in brain MRI and EEG, vision and hearing testing prior to and following standardized breaching and diversionary device scenario training.

(2) PURPOSE OF STUDY:

The purpose of the study is to monitor changes in brain structure and function between the pre-training and post-training, in a population of tactical team members wearing the Device and compared to a similar population not wearing the device. Secondly, the purpose is to determine the protection of the device relative to amount and magnitude of sustained head impacts.

(3) BACKGROUND:

The Device has the promise of providing a novel mechanism for reducing or preventing the likelihood of TBI, and may be used in conjunction with other protective equipment. TBI is the leading cause of death in individuals under age 45. The cost of TBI in the U.S. is estimated at

anywhere from $50 to $150 billion, annually. The January, 2008 New England Journal of Medicine reports, “Head and neck injuries, including severe brain trauma, have been reported in one quarter of service members who have been evacuated from Iraq and Afghanistan”(Okie 2005; Xydakis 2005; Hoge 2008). The vast majority of these injuries have resulted from exposure to improvised explosive device (IED) blast waves**.** Head injuries, concussions and the resulting trauma have been in public discussion recently as the National Football League (NFL) deals with a lawsuit regarding head injuries by about one-third of living former NFL players.

According to NASA, “The oscillation of a fluid caused by an external force, called sloshing, occurs in moving vehicles containing liquid masses, such as trucks, etc.” This oscillation occurs when a vessel is only partially filled. It is hypothesized that the brain faces similar slosh energy absorption during external force impartation.(Turner 2012) Slosh permits external energies to be absorbed by the contents of a partially filled vessel or container by means of inelastic collisions. Tissues of differing densities can decelerate at different rates creating shear and cavitation. If the collisions between objects or molecules are elastic, the transfer of energies to those objects diminishes, minimizing the energies imparted by slosh.(Smith 2012)

Woodpeckers, head ramming sheep and all mammals (including humans) have small, little known and misunderstood muscles in their necks called the omohyoid muscles. Highly G-tolerant creatures of the forest have utilized these muscles to gently restrict outflow of the internal jugular veins thereby “taking up” the excess compliance of the cranial space and ultimately protecting themselves from TBI like tiny “airbags” in a motor vehicle. Rat studies by have demonstrated that we can easily and safely facilitate this muscle’s actions by a well-engineered gentle compression over those muscles.(Smith 2012; Turner 2012)

The medical Queckenstedt Maneuver devised to detect spinal cord compression, gently places pressure over the external jugular veins to increase cerebral spinal volume and pressure. In this maneuver, the veins are compressed while a lumbar puncture monitors the intracranial pressure. “Normally, the pressure rise to the higher ‘plateau’ level occurs instantly upon jugular compression to fall again equally fast upon release of the compression”(Gilland 1969). This incredibly simple principle can be employed to protect soldiers and athletes from TBI by safely, and reversibly, increasing intracranial volume and pressure. The neck collar device is made of Outer collar - hytrel (thermoplastic elastomer), Inner collar - TPSiV (thermoplastic elastomer), metal insert (stainless steel), and is fitted to the neck to provide a comfortable and precise jugular compression that potentially mitigates cerebral slosh (Figure 1). The device will be worn inside the collar of an athletic compression shirt.

Although the skull, blood, and brain are “almost incompressible,” the vasculature tree of the cerebrum is quite reactive and compressible. As volume is added to the cranium, eventually the compensatory reserve volume is surpassed and the intracranial pressure increases slightly. Increasing cerebral blood volume by just 1-3% safely and reversibly reduces compliance of the cerebral vascular tree and diminishes absorption of slosh energies. Jugular compression

increases cerebral blood volume almost instantaneously. As mentioned, this degree of increase has significantly mitigated slosh and TBI in laboratory animals and mimics the highly concussion resistant wild animals that are able to reflexively increase cerebral blood volume through natural jugular compression.

A landmark article, published in the *Journal of Neurosurgery*, used a standard acceleration-deceleration impact laboratory model of mild TBI. The study showed a successful and marked reduction of axonal injury following Internal Jugular Vein (IJV) compression as indicated by immunohistochemical staining of Amyloid Precursor Proteins (APP) (Smith 2012; Turner 2012). It is argued that IJV compression reduces slosh-mediated brain injury by increasing intracranial blood volume and reducing the compliance and potential for brain movement within the confines of the skull. The potential for such technique to mitigate both linear and rotational brain injury in humans by "internal protection" represents the most novel approach to mitigating TBI.

Summary of Prior Work

A. Safety testing in athletes has been approved by the local IRB and was completed in the Cincinnati Children's Hospital Human Performance Laboratory *(Study ID: 2013-2240; PI: Gregory Myer)*. Evaluation of monitored vital signs, biomechanics, cardiorespiratory capacity, postural control, dynamic stabilization, reactive index, concentration and cognition, memory, strength and power in a population of athletes showed no statistically significant adverse effect of wearing a mild jugular vein compressive neck collar compared to a sham arm band.(Myer 2013) Cumulatively, the pre and post safety measures indicate that neurologic parameters of executive function, eye hand coordination, balance, memory and reaction times were unchanged following two hours of physical testing wearing the collar prototype. Acceptance of the compression collar was not different in physiological biomarker response to the non-collared condition during maximal oxygen uptake and maximum effort power testing.(Myer 2013)

B. Magnetic Resonance Elastography was established at CCHMC in collaboration with The Mayo Clinic to support these studies. Under jugular vein compression with the collar, all participants tolerated the procedure without any untoward effects. The preliminary studies of dynamic shear strain showed no consistent pattern of wave propagation and elasticity placed upon the vascular and cranial tissues. Analysis of these data continues.

C. We studied 410 participants (ages 12 to 68 years of age) via a middle ear power analysis (MEPA) with and without the compression collar, and no complaints or untoward effects were noted and no decline in the auditory perception was recorded. The expected changes of reduced Acoustic Reflectance of the inner ear and middle ear (indicative of reduced compliance) were noted only in subgroup analysis of those with jugular vein compression. The results of this study indicate that the neck compression collar prototype may have the potential to safely reduce energy impartation into cranial structures (i.e., the inner ear); however, further work is needed with advanced collar designs to establish this effect.

D. fMRI and CO_2 reactivity was performed on 12 adults before and after application of jugular vein compression. Results comparing before and after jugular vein compressions (with the collar) yielded no alterations in O_2 uptake or glucose metabolism to any portion of the brain.(Fisher 2013).

E. An *in vivo* clinical trial was approved by CCHMC IRB and was completed in the Cincinnati Children's Hospital Human Performance Laboratory and Radiology Department (Study ID: 2014-5009; PI: Gregory Myer) An *in vivo* clinical trial was performed in hockey players of the proposed intervention device used during sporting competitions to test its effect in ameliorating neuroanatomical and neurophysiological changes to the brain using two widely accepted techniques [diffusion tensor imaging (DTI), and event related potentials (ERPs) utilizing electroencephalography.](Reches 2014) For athletes in the non-intervention group, radial diffusivity (RD, DTI parameter associated with white matter structural integrity(Song 2003; Song 2005)) increased significantly from pre-season to mid-season. By comparison, the athletes in the intervention group did not show a significant change in RD with similar accumulated g-force head impacts. In kind, ERP analysis showed concomitant changes in brain network dynamics in the non-intervention group—the level of change was strongly correlated with the accumulated g-force of the collisions, whereas the intervention group showed no significant change. These group differences indicate that mild jugular vein compression may provide protection from the detrimental effects of collisions and resultant brain injury. These prospective longitudinal data utilized an internal (*in vivo*) approach and demonstrate, for the first time, that it is possible to protect the brain from sports related head impacts.

F. An *in vivo* clinical trial was approved by CCHMC IRB and was completed in the Cincinnati Children's Hospital Human Performance Laboratory and Radiology Department (Study ID: 2015-2205; PI: Gregory Myer) The *in vivo* clinical trial was performed in football players implementing the proposed intervention device used during sporting competitions to test its effect in ameliorating neuroanatomical changes to the brain using evidenced by diffusion tensor imaging (DTI), Based on pre-clinical data we hypothesized that collar imparted jugular compression that minimally restricts venous outflow to encourage cerebral venous sinus engorgement would reduce brain injury biomarkers in athletes exposed to head impacts during a competitive football season. This project utilized a prospective controlled trial to evaluate effects of mild jugular vein (i.e., neck) compression (collar; n=31) relative to controls (no-collar; n=30) during a competitive football season (males; 17.04 ± 0.67 years). Helmet sensors were used to collect daily impact data in excess of 20 g (games and practices) and the primary outcome measures, which included changes in white matter microstructure, were assessed by diffusion tensor imaging (DTI). Specifically, four DTI measures including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were analyzed using a Tract-Based Spatial Statistics (TBSS) approach—a voxel based analysis. The final analyses included both an intent to treat (ITT) and per protocol evaluation of the collar intervention. The ITT analysis indicated a consistent vascular response by the athletes to collar compression, as indicated by internal jugular vein dilation (IJV) superior to its application ($p < .01$). Both groups experienced similar overall g-forces and total head impacts during the competitive football season (impacts > 20 g; collar 16983 vs no-collar 17750 ($p > .05$). Significant pre- to post-season reduction in MD, AD, and RD (corrected $p < .05$) was evidenced by extensive WM areas in the no-collar group, while no statistically significant longitudinal change was indicated for any of the DTI measures in any WM region in the collar group. Comparing the two groups, the no-collar group demonstrated significantly larger pre- to post-season DTI change in many WM regions (corrected $p < .05$). Correlation analysis also showed initial evidence of significant correlation between the change in AD in some WM regions and the number of impacts and/or the cumulative G-force experienced in the no-collar group (all $p < .05$). Per protocol, results were consistent with presented ITT findings with an

expected increase in effect sizes noted in most voxel analyses. Our findings, based on four DTI measures known to relate to brain injury, indicate a consistent reduction of change in diffusivity parameters noted in the no-collar group at post-season. This is a literature driven sign of sub-threshold white matter injury due to repetitive head impacts during the competitive season. The smaller and statistically non-significant change in diffusivity in the collar group evidences a protective effect from the induced jugular outflow impedance. Restated, the approach to impede IVJ blood flow appears to have ameliorated the detrimental effects that resulted from a season of head impacts. The current study presents the first football related prospective longitudinal data and demonstrates a novel, in vivo, approach to protect the brain from football related head impacts. These results build on prior research and evidence the need for future work to determine if this novel method for brain injury prevention is both safe and effective.

(4) STUDY DESIGN:

The current project will be designed following a prospective longitudinal study design. All MRI scanning will be performed on 3 Tesla Philips MRI scanners (3T Achieva in S-Building and 3T Ingenia in T-Building) located in Imaging Research Center (IRC) in the Cincinnati Children's Hospital Research Foundation (CCHRF). Sedation will not be used for any of the test visits. The entire MRI protocol will include high resolution T1-weighted 3D images, a 61 direction diffusion tensor imaging sequence, resting state fMRI, susceptibility weighted imaging, and task-based fMRI. The fMRI tasks will be focused on assessment of short-term memory and motor function and will include presentation of visual or auditory stimuli such as words, numbers, or simple sentences. Participants will be asked to respond via button-press or lower extremity movement such as knee extension. The MR scan will be completed in 90 minutes or less. Peripheral pulse oximetry and respiration waveforms will be collected for data analysis in order to minimize the potential confounding effect from the physiological changes. A practice session of the fMRI paradigms will be completed just prior to scanning to allow the participant to ask any questions and be familiar with the protocol. All functional and neurocognitive testing will be performed at the Cincinnati Children's Hospital Human Performance Laboratory.

(5) DURATION:

Each participant will participate in 2 planned study visits (pre and post tactical training) that may take up to 2.5 hours. Data analysis will continue for a 1 year period following the final enrollment.

(6) SELECTION & RECRUITMENT OF PARTICIPANTS:

We will recruit up to 100 study participants. The participants (all over age 18) will be recruited from the Hamilton County Police Association. Questions regarding participation will be answered during the presentation or through e-mail or phone. Participants will be contacted via telephone to further explain the study, answer any additional questions and to enroll them in the study. The participants who voluntarily agree to participate will be scheduled to complete the pre-participation testing. The participant will read and sign the "Consent to Participate in a Research Study" form, approved by the Institutional Review Board of Cincinnati Children's Hospital. If the participant does not read or sign the form, they will not participate in the study. Once the potential study participants has been identified, he/she will be allocated to one of two

groups: 1) Device wearing during the tactical training or 2) Non-device wearing during the tactical training.

Inclusionary criteria include:

- Normal healthy volunteer
- Able to provide written consent

Exclusionary criteria include:

- Unable to provide written consent
- History of neurological deficits, previous cerebral infarction, or severe head trauma as indicated through pre-tactical training screening:
- Medical contraindications to restriction of venous outflow via the internal jugular veins (known increased intracerebral pressure, metabolic acidosis or alkalosis)
- Glaucoma (Narrow Angle or Normal Tension)
- Hydrocephalus
- Recent penetrating brain trauma (within 6 months)
- Known carotid hypersensitivity
- Known increased intracranial pressure
- Central vein thrombosis
- Any known airway obstruction
- Any known seizure disorder

(7) PROCESS OF OBTAINING CONSENT

Once a participant is identified as a potential participant, is contacted by a CCHMC/Sports Medicine representative and verbally agrees to participate, the process to obtain consent will begin. A copy of the informed consent will be provided to the participant at this time. The study coordinator will review the informed consent and the participant will have an opportunity to ask any questions regarding the study and/or the study protocol. At that time, the participant will be given time to decide whether or not they wish to participate and if so, asked to sign the informed consent. Once the signature is obtained, the participant will be given a copy of the consent and testing will commence. At no time will the participant be coerced into participation. Receiving the informed consent prior to enrollment will allow the participants to review the study information prior to participation in the study. This will aid the participant to make an informed, unforced decision regarding election to participate in the study. The coordinator will ensure that all necessary forms have been signed prior to any data collection.

(8) STUDY PROCEDURES:

Location I – Brain Imaging-Performed at CCHMC Imagining Research Center

MR imaging data Acquisition

Magnetic Resonance Imaging (MRI), are all based on the concept of using magnetic fields and radio waves to make chemical, anatomical and physiological assessments with in the living tissue. This technology has been utilized for diagnostic and research purposes since the early 1980s.

This testing will consist of 2 MRI sessions (pre-training, and post-training) all inside a 3T scanner at the CCHMC Imaging Research Center. During the acquisition of MR images, the study participants will lie on the scanner table. For most portions of MR acquisition, the study participants will only be instructed to lie still. For other parts of the acquisition, study participants will be asked to answer questions that will assess their cognitive ability and working memory. Participants will be allowed to communicate with the MR operator via an always-on, two-way intercom at any time. In addition, the participants have a hand-held air ball to squeeze in the event that they elect to be removed from the magnet immediately. The study participants have control over their presence in the magnet, which in turn tends to minimize feelings of claustrophobia. As magnetic resonance imaging employs the use of strong magnets, patients will receive a standard preoperative screening questionnaire regarding the potential for ferromagnetic objects within their bodies to ensure their safety during the study. Participants will be screened for MRI specific contraindications such as:

- Braces or permanent metal dental work
- Insulin pump
- Cardiac pacemaker
- Cochlear implants
- Hearing aids
- Aneurysm clips
- Orthopedic pins, wires, screws, or plates
- Any other exclusionary criteria as documented on the MRI safety screening poster included with recruitment materials

Those participants with any aforementioned contraindication will be excluded from the imaging portion of the study but will still be eligible to participate in the rest of the study procedures.

Location 2 -Physical and Cognitive Testing Performed at CCHMC Human Performance Laboratory

Station I – Occulomotor Assessment

All participants will sit in front of a desktop monitor. During this process, the participant will look at several points in order to calibrate the desk-top eye tracker (Tobii X2 60). All participants will participate in three oculomotor performance tests: (1) a reflexive saccade test, (2) a self-paced saccade test, and (3) a smooth-pursuit tracking test. All tests will be presented on a PC monitor approximately 1.5 m away from participant, and stimuli will be automated using customized *MATLAB* (Mathworks, Natick, MA) routines.

<u>*Reflexive Saccades*</u>. Reflexive saccades will be tested as participants track discrete target motion that will jump randomly by 14, 16, 18, 20, 22, or 24˚ on the screen in a horizontal and vertical direction, at intervals varying pseudo randomly between 1.0 and 2.0 s. The current fixation target will be extinguished at the same time as the next peripheral target appears. The test sequence will take 30 s/trial (2 trials) and all participants will be instructed to follow the targets as quickly and accurately as possible. Dependent measures for reflexive saccades will include: (1) saccade latency (ms), (2) saccade velocity (˚/s), (3) mean absolute position error of the final eye position, (4) gain of the primary saccade, and (4) gain of the final eye position.

Self-paced saccades. Self-paced saccades will be assessed as the participant glances back and forth as quickly and accurately as possible between two constantly visual targets at ± 15˚ horizontally from one another. This test will take 30 s per trial and the participant will perform 4 trials. The dependent measures will be: (1) the number of refixations within 30 s, and (2) the mean intersaccadic interval (ms).

Self-paced saccades combined with smooth pursuit tracking. A series of self-paced saccade tasks will be presented in which either, (1) a stationary and moving target (horizontal or vertical) is presented, (2) two horizontally moving target are presented, or (3) two vertically moving targets are presented. The participant will be instructed to glance back and forth between the targets as quickly and accurately as possible. Each test will take 30 s per trial and the participant will perform a total of 22 trials over ~ 11 min. The dependent measures will be: (1) the number of refixations within 30 s, and (2) the mean intersaccadic interval (ms), (3) average eye peak velocity (˚/s) after removal of all saccades from the tracking performance, and (4) the tracking lag (ms). Total oculomotor testing time will take approximately 10 minutes, and all eye data will be recorded using Tobii Studio software and will be sampled at 60 Hz.

Station II: Hearing Testing

This station will help determine the effectiveness of the device by measuring the auditory response of a clicking noise that will be non-invasively imparted into the subject's outer ear. For both tests, a soft silicone tip is placed into the outer ear, and sounds are delivered via a miniature speaker. For the wideband absorbance test, absorbed acoustic responses will be monitored to provide information on the operation of the collar device. These are measured as changes in the acoustic absorbance of the ear. For the oto-acoustic emission test, tones or clicks will be presented to the ear with the same soft ear probe and recording microphones pick up the oto-acoustic emission coming back from the outer hair cells of the inner ear. The computer averages and processes the responses in reference to a noise floor across a specified frequency range, displaying the results on the computer screen for the tester. The oto-acoustic emissions test also provides a brief hearing screening to determine if hearing is normal.
Evidence indicates that wideband absorbance and oto-acoustic emissions will be altered with increased intracranial pressure, thus this simple, non-invasive exam will provide feedback on the effectiveness of the device.

During the wideband absorbance and oto-acoustic emissions testing the study participants will also be fitted with a compressive circumferential neck collar which can provide variable and specific levels of pressure. The pressure is achieved by inflatable pods that sit adjacent to the trachea affixed to a non-stretch adjustable collar. Manual inflation is regulated and monitored by an experience operator/ technician through a bulb style pump and an electronic gauge. Testing will occur without pressure and then with pressures applied up to 40mmHg.

Otoscopy will be performed by an audiologist to note any ear blockages, such as wax or drainage for exclusion purposes. The oto-acoustic emissions test will be interpreted to determine if normal responses were obtained in each ear. If abnormal, a hearing screening using pure tone audiometry screening will be provided free of charge. In the event that the hearing screening is determined to be abnormal, the subject will receive the results which may be shared with their primary care provider to determine any need for referral for follow-up.

Station III – Electroencephalogram (EEG)

The following cognitive tests will be performed while EEG/ERP data is collected:

Auditory oddball task. The oddball task is a classic EEG paradigm that has been extensively used for many years, in numerous studies and in many neurological patient populations. It is considered to involve executive functions, attention and memory processes. The main ERP components are N100 (low level stimulus processing) and P300 (attention allocation and stimulus evaluation), which is maximal at fronto-central areas for novel stimuli and centro-parietal for Target stimuli. In this task sounds are presented, at an average rate of 1 every 1.5sec. A total of 80% of the sounds ("standard') are tones of repeating frequency and intensity. A total of 10% of the sounds ("target") are tones of another frequency to which participants respond by pressing a button. The remaining 10% of sounds ("novel") are multi-frequency sounds, different for each trial. The test takes approximately 11 min to complete.

Visual GO/NO-GO. This is a classical motor inhibition task. The stimuli consist of white English letters appearing over a black background in the center of a computer screen, between two vertical white lines which remain on the screen throughout the whole duration of the experimental block. The Go stimulus can be any of the letters B, C, D, E, F or G appearing in equal proportions, and the No-Go stimulus is always the letter X. Subjects are instructed to press a key as quickly as they can whenever presented with a Go stimulus, and to withhold pressing the key when presented with the NoGo stimulus. The inter stimulus interval is varied randomly between 1000ms to 3000ms with steps of 250ms. Two versions of the task are used. In one version (the "20 percent") the No-Go condition appears in 20% of the trials and the Go stimuli occupy 80% of the trials. In the second version (the "80 percent") the NoGo stimulus appears 80% of the time and the Go stimulus appears in the remaining 20%. The total number of trials is set to 360, resulting in an overall task duration of about 12 minutes

Visual Inter-Hemispheric Transfer Time (IHTT): This task is designated to evaluate the temporal delay in transfer time of visual information between homologous cortical areas across hemispheres, via callosal fibers. The task consists of measuring EEG from participants while they observe visual images presented randomly to either their right or left visual field. During the experimental block participants are instructed to ignore the laterally presented stimuli and fixate constantly at the center of the screen. Subsequently, ERPs for the lateral visual stimuli are computed, and IHTT is indexed as the peak latency difference between ERPs to the ipsilaterally presented stimuli and ERPs to the contra-laterally presented ones.

Resting EEG. 2 min resting EEG will be recorded with participant eyes closed.

***Device and Compliance Acceptance*:** The study coordinator will be responsible for providing the appropriate intervention (device or no-device) to each subject based on their random assignment. At first fitting of the collar a registered vascular technologist will utilize ultrasound to ensure that the collar fits correctly and is activated as prescribed.

***Accelerometers*:** Each helmet of the study participant will be instrumented with a GForce Tracker™ (GFT; gForceTracker, Markham, Ontario)) accelerometer device. The GFT will be affixed to the inside of each participant's helmet. The GFT is equipped with electronics that allow for the measurement of linear accelerations and rotational velocities of the head. The GFT measures 6 degrees of freedom by directly measuring three axes relative to linear acceleration and three axes relative to angular velocity. The GFT stores all impacts locally. These devices need to be connected physically to a computer, and data can only be accessed with a unique username and password only known to the research team.

Each participant will be provided with an X Patch accelerometer (X2 Biosystems; Seattle, Washington) The X patch adheres to the head, just behind the ear using an adhesive patch, as seen in the image. X2 is a small and durable device that is attached to the back of the neck behind the ear to track impacts, and it stores data for uploading to a PC. This X2 accelerometer accurately measures the severity of impacts by converting data such as high impact collision into usable data outputs. X2 data will be used in final analysis to normalize the exposures to potential concussive events. All impacts of greater than 10 g-force will be recorded and utilized in the post-season analyses. No one besides those approved by this IRB protocol will have access to any data.

Breacher Training

The study period will include a standardized breaching class for Law Enforcement and Military Operators. The course will be taught by current HPCA SWAT instructors and the comprehensive class will aim to teach officers and operators to identify the tools that are necessary when faced with a multitude of forced entry scenarios and how to use them both safely and effectively. In addition, a representative from a company that provides a variety of breaching equipment will be in attendance and will be a valuable resource to help ensure safety during the tactical training. Study participants assigned to either the collar or no-collar condition will engage in scenario based live-fire training all while incorporating the breaching techniques.

(9) DATA ANALYSIS/METHODS:

Data Storage.

The personal demographic data for each participant will be blinded from the researchers, and a coded identification number will be used to track all collected data. Data will be stored on password-protected computers and only pertinent research personnel will have access. Data forms will be stored by coded identification number in a locked cabinet to which only pertinent research personnel have access. All data will be collected for research purposes only.

Data Analysis.

Data processing and analysis will be performed using a series of existing software including FSL (FMRIB's Diffusion Toolbox in FSL Software, Oxford, UK), AFNI (Cox, 1996) , SPM (Statistical Parametric Mapping analysis package, Wellcome Department of Cognitive

Neurology, London, UK), DTIStudio (John Hopkins University, Baltimore, MD; Jiang et al., 2006), as well as additional customized software written in Matlab or IDL.

DTI data will first be subjected to preprocessing to correct for Eddy current and head motion artifact, followed by calculation of the three diffusion eigenvectors and eigenvalues. DTI measures, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) will be calculated. The regions of interest will be manually determined in major white matter areas such as corpus callosum, internal capsule, and external capsule. After being normalized to a common template, voxel based group analysis can be performed to explore brain regions that present significant group difference or longitudinal changes. Fiber tracking can be performed to generate white matter tracts in different areas in the brain, e.g., cortico-spinal tract, different segments in corpus callosum, optic radiation, cingulum superior longitudinal fasciculus, and others.

Functional fMRI (resting state fMRI) will also be subjected to routine image pre-processing pipeline. Functional connectivity analysis will be performed, using the CONN toolbox, http://www.nitrc.org/projects/conn/) between all brain regions that are involved in the proper functioning of default mode network, sensory motor network, visual network, and a series of other networks that are known to be strongly functionally connected during resting state. SWI, HARDI and ASL BOLD will undergo standard image post-processing.

BNA Analysis EEG signals recorded will be cleaned by standard procedures, band pass filtered into overlapping physiological frequency bands, cut into epochs around sufficient pre and post stimulus onset times, and averaged to result with event related potentials (ERPs). For each band, the data is reduced into a set of discrete points that denote local extrema, the latencies and amplitudes of which are inputted to the algorithm. The algorithm then seeks a state-unique multi-sited spatiotemporal pattern. This is the BNA group network. Following that, a BNA score is computed for each participant individually. It is comprised of a set of similarity measures to a BNA group network or a group of BNA networks. Once normative data is established, patients who require assessment of their brain electrophysiological activity will undergo BNA analysis, and their scores will be compared to the BNA pattern of the normative data.

For the group analysis, the raw EEG of each participant undergoes three separate processing stages: (1) preprocessing (artifact removal, band-passing); (2) salient event extraction (discretization, normalization) and (3) network analysis (unitary events extraction, pair-pattern extraction) on all salient events gathered from all of the participants. The single participant level process involves three stages – the first two are identical to the first two stages of the group level process. In the third stage, the single participant activity is algorithmically compared to the set of patterns collected during the group analysis stage (see below).

The SIn (network) of a pair-pattern is 1 if it applies at all to a tested participant (i.e., the participant's electrode activity fulfilled the constraints set by the pair-pattern) and 0 if it does not. To assess the SIs (synchronicity) of all pair-patterns with an SIn of 1, the times of the discrete activity points of the individual participant are compared to the mean and SD of the activity times of the respective group pair-patterns. The SIa (amplitude) of the pair is evaluated

in a similar manner, by comparing the amplitudes of the activity points. The overall BNA score of the individual participant to each of the groups is computed by averaging the products WI (weight index) x SI (synchronicity index) of all pairs in all patterns of the group. For each participant, the classification score is computed separately for each similarity index such that three classification scores were computed: a network score, a synchronization score and an amplitude score:

$$Cnet = \frac{\sum_i (W_i * SIn_i)}{\sum_i W_i}, \quad Csync = \frac{\sum_i (W_i * SIs_i)}{\sum_i W_i},$$

$$Camp = \frac{\sum_i (W_i * SIa_i)}{\sum_i W_i}$$

Statistical considerations. The data analysis will begin with a review of descriptive statistics for all major variables and all major subgroupings of variables in the data set. For the inferential methods, we will use a number of different generalized linear modeling techniques, including linear regression models. All analyses will be conducted using SAS® version 9.3 (SAS Inst, Cary, NC), or Mplus (Muthén, 2007). Initial analyses will be undertaken to inspect data for errors, inconsistencies, and incomplete information. This will include examining the data with simple frequency tables and dot plots for univariate data and scatter plots and multi-way dot plots for bivariate and multivariate data. Data anomalies that cannot be resolved by the Biostatistical/data management team will be sent as queries via email to the project investigator for clarification and/or correction. During the verification process, outlying values will be corrected if necessary. Incomplete information will be corrected and the project investigator will receive updates. To summarize bivariate relationships among predictors and between predictors and outcomes, Chi-square of Fisher's exact test or Spearman's rank correlation coefficient, will be used, as appropriate. For reporting inferential statistics, such as differences in rates or means, 95 percent confidence intervals will be used extensively to quantify degree of clinical efficacy. Unless otherwise stated, statistical tests are considered two-sided and a .05 significance level is used. All models will be adjusted for potential independent predictor variables but will be limited to the number of predictors we can fit in a regression model while maintaining a valid and reliable model. Candidate predictors include amount of playing time, amount of practice time, previous mTBI, weight, age and height. Collinearity will be examined. Linearity assumptions will be checked and transformations examined. In those cases, a log transformation, polynomial terms, or restricted cubic splines will likely be used to relax the assumption of linearity in the regression models. We will remove the nonlinear terms or transformation only if the nonlinear test is non-significant with a $p \geq .10$. Additionally, overly-influential observations and distributional assumptions will be checked. Models will be interpreted graphically, predicted values will be examined, and appropriate significance tests will be utilized.

Statistical analyses. **-** Statistical analysis of outcomes measures will be done using SAS®, version 9.3 (SAS Institute, Cary, NC) and SPSS statistical software (SPSS Inc, Chicago IL). Comparisons between the testing conditions (collar vs. no collar) will be made using Analysis of Covariance, in order to control for time (pre vs post tactical training) and condition (collar versus no collar). We will also conduct correlation analysis to test the association between imaging biomarkers (as described above) with the results obtained from the impact

surveillance. The collision indices, including total number of collisions, number of collisions from front, back, left, right, top, bottom, G force, and timing of each collision will all be recorded and tested in the analysis. Secondary analysis to compare the intervention with no intervention and calculate the rates would involve a Poisson model, using an offset to account for the playing time and exposure to concussive impact for each of the study participants. We will calculate the rate and the associated 95% confidence interval. SAS®, PROC GENMOD will be used for analysis, which allows us to account for the fixed and random effects, use the appropriate link function, and the offset for amount of playing time exposure.

(10) FACILITIES AND PERFORMANCE SITES:

All MRI scanning will be performed on a 3 Tesla Philips Achieva MRI scanner located in Imaging Research Center (IRC) in the Cincinnati Children's Hospital Research Foundation (CCHRF). Sedation will not be used for any of the test visits. The entire MRI series, including anatomical imaging, 3d T1, DTI, rs-fMRI, SWI, and HARDI will be completed in 60 minutes or less (see Table 1. below for detailed specifications). All functional and neurocognitive testing will be performed at the Cincinnati Children's Hospital Human Performance Laboratory.

(11) POTENTIAL BENEFITS:

Participants of this study will not receive any direct or immediate benefits by completing this study. However, they will be contributing to research involving the potential for major contributions to future TBI/concussion prevention strategies.

(12) POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS:

The Device partially circumnavigates and compresses the neck in the same way that a compression garment (non-medical apparel) behaves, and very similar to the compression exerted by a necktie (although this device is open over the trachea and can be pulled off if inadvertently gripped). These garments have been shown to gently facilitate natural response mechanisms in several small neck muscles and tendons (the Omohyoids), which are universally present in mammals and birds.

The physiologies imparted by these Omohyoids (and further facilitated by these garments) merely approximate natural physiologies, which occur when individuals lie in the prone, or supine position, and are also comparable to the simple act of yawning (which has been shown to collapse the jugulars). The Device will intentionally deliver an exacting, but gentle compression to the Omohyoid muscles in the neck allowing these muscles to optimize blood outflow of the neck vasculature. In the upright position (without the collar), the resultant vascular blood column siphons volume out of the neck, rapidly, creating a negative pressure on the cranium and resulting in a slight "under filling" and "sloshability" inside the skull.
The Omohyoid muscle raises the volume of the intracranial space by design. The Device does not contain any inherently rigid structures in its design. Similarly, neckties circumnavigate the neck, and safely raise intracranial pressure and volume comparable to the Device. The Device is manufactured of a soft rubber similar material and should be barely noticeable to the wearer. Careful MRI studies have confirmed an increase in blood volume in the brain but have also shown that there is no significant change in brain blood flow pattern with wearing a "tight necktie" (Rafferty 2010).

Although the venous jugular flow beneath the pressure cuff may be temporarily halted or slowed, the venous outflow from the cranium is never completely stopped, particularly from the anastomosis between the spinal vein and the basilar plexus and occipital sinuses *which are incompressible*."(Gregg 1944) Jugular compression has few known physiological effects besides the intended increase in cerebral blood volume and pressure. Only one innocuous physiology has ever been shown to alter with jugular compression. "Previous studies have shown that the decline in urinary sodium excretion which occurs normally in the sitting position, as compared with recumbency, can be partially but not completely prevented by compression of the neck (Lewis 1950; Torres 1970). This decline is urinary sodium excretion is minimal. There was no correlation between EEG changes and changes in systolic blood pressure occurring during jugular or carotid compression(Torres 1970). Further, studies on complete resection of the IJV note that, "the clinical observation that bilateral resection of the IJV is usually well tolerated suggests the presence of alternative, non-jugular pathways."(Gius 1950)

Effect of Body Position and Exercise on ICP: "At rest, compared with the reference 30-degree head-up position, the supine position increased intracranial pressure (ICP) by 6.21 mm Hg (35% with P<.01)."(Brimioulle 1997) Restated, just lying down increases ICP more than the Device (6.21mm Hg = 35% rise versus this device at only 25%). *Valsalva and raising ICP:* We define Valsalva, where a person tries to exhale forcibly with a closed glottis (windpipe), so that no air goes out through the mouth or nose. "When the Valsalva maneuver was performed during resistance exercise, the ICP rose to 31 mmHg (a rise of 138%). No complications were associated with participating in this investigation."(Haykowsky 2003) In other words, the Device facilitates the intended actions of the omohyoid with less pressure than the act of lying down or performing the Valsalva (holding one's breath and bearing down, which would be expected to occur regularly on a playing field).

Instead of letting three to five milliliters of blood rapidly flow out of one's brain upon standing, the Device will serve to retain that fluid inside the skull where it is believed to cushion the brain from external energy impacts and concussions. In rats, this simple action prevented 83% of TBI indicators during two 900 G impact studies at the West Virginia University.(Smith 2012; Turner 2012)Considering the above mentioned findings on jugular compression, this device can be considered not to meet the definition of a "significant risk device," as that term is defined in 21 C.F.R. § 812.3(m).

MR Imaging of the Brain: The risk the magnetic fields and the strengths, and radio waves is vanishingly small. Some patients can experience anxiety from the confined space of the magnet's bore. Therefore people with known claustrophobic tendencies will be excluded from the study. Another minor concern when using magnetic resonance technology is the noise the magnet makes when collecting data. Noise abatement measures are used; headphones and music with a selection of music options. Ferrous implants and or piercings can be affected in the magnetic field. Therefore participants will be advised to remove these and or scanned with a metal detector to screen for such objects.

Our colleague's previous experience with MRI experiments (who will be present and has a decade of experience with this technology) has provided confidence that there should be no psychological, physical, legal, or social risks involved with MRI experiments in general, though participants may be anxious about the scan, possibly causing them slight stress. The MRI scanning will be performed using the 3 T Siemens Trio MRI scanner. MRI does not involve ionizing radiation and scans up to 8 T are considered as non-significant risk. The risks common to all MRI scans can be described as: (1) ferromagnetic objects introduced into the magnetic field, (2) confinement in the scanner bore, (3) radio-frequency (RF) heat deposition in tissue which is monitored by the system to conform with FDA guidelines, and (4) acoustic noise. These risks are addressed below: Participants are allowed to communicate with the MR operator via an always-on, two-way intercom at any time. In addition, the participants have a hand-held air ball to squeeze in the event that they elect to be removed from the magnet immediately. Thus, the participants have control over their presence in the magnet, which in turn tends to minimize feelings of claustrophobia.

The MR imaging will be initially reviewed by a licensed radiologist just as it would be if it were being used as part of routine medical care. There is a possibility that while reviewing MR images we may see an abnormality that we did not expect to see in this study. In this event, we will notify the participant's legal representative (or participant is 18 years or older) if we see such an incidental finding. Depending on the type of incidental finding, we may contact the participant by mail or by phone. A member of the research team will discuss the incidental finding with the legal representative (or participant if over the age of 18 years). If the participant chooses, we will give information about this incidental finding to their primary doctor or we will refer them to an appropriate doctor for further evaluation. The costs for any care that will be needed to diagnose or treat an incidental finding would not be paid for by this research study.

Data Storage. There is also a minimal risk that the data collected for each participant may be viewed by individuals outside the research team. The risk that confidential data may be viewed is relevant for both the written forms and electronic databases. Precautions, such as password-protected computers, locked cabinets and coded identification numbers, are in place to minimize this risk.

Adverse Events. During the course of the investigation, injuries consistent with the sports being monitored are expected to occur (E.g. concussion, musculoskeletal injury, bone fractures). Care of all injuries will follow standard of care as directed by the team's athletic trainer and/or the participants treating physician. CCHMC will not be responsible for the medical treatment of any injuries that are not directly related to wear of the Q-collar device. In the case of an adverse event that is determined to be directly related to the wear of the Q-collar during competitive play, the principal investigator will report such event to Cincinnati Children's Hospital Medical Center IRB as any future funding organizations in a manner consistent with the requirements of each organization. As described in the consent, if a participant believes they have sustained an injury as a result of the study then they are instructed to contact the principal investigator or director of social services who in turn will then contact CCHMC IRB and necessary funding institutions, as aforementioned. If a participant sustains an injury during

testing they will be referred to the most appropriate medical facility or seek medical attention by the physician/medical specialist of their choice.

(13) RISK/BENEFIT ANALYSIS:

Participants will be approached for participation via the appropriate method. The purpose and the study protocol will be fully explained in conversation and with the informed consent process.

On the day of the study, the investigators will confirm that the volunteer participant has no health impairment as outlined in the exclusion criteria. Time will be taken to repeat the aims of the study, test protocol, and to answer any remaining questions posed by the participant. The methods described in this protocol have been used extensively in previous testing in the laboratory. During previous testing, there have been no reported injuries, adverse events or complications. Additionally, the investigators have considered potential risk for injury and have taken additional steps, described in the protocol, to minimize these risks.

Subject participation will also be halted should an adverse event while wearing the collar, such as syncope, occur. Any adverse events will be immediately reported. The safety officer will evaluate all adverse events and will determine if early stopping of the study due to safety concerns is warranted. Given the study design and sample, we do not deem futility or efficacy stopping rules are warranted.

(14) DATA SAFETY & MONITORING:

The Safety Officer (Dr. Paul Gubanich, MD) who has extensive experience in the management of concussion at the professional, collegiate, high school and middle school level will act in an advisory capacity to the Principal Investigator (PI) to monitor patient safety and progress for the clinical trial, "Concussion Prevention Device". Dr. Gubanich will be the contact person for severe adverse event reporting.

The Safety Officer's responsibilities are to:

- review the research protocol, informed consent documents and plans for data safety and monitoring;
- evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial site, and other factors that can affect study outcome;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- review study performance, make recommendations and assist in the resolution of problems reported by the PI;
- protect the safety of the study participants;
- report to the PI on the safety and progress of the trial;
- make recommendations to the PI concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;

- ensure the confidentiality of the trial data and the results of monitoring; and, assist the PI by commenting on any problems with study conduct, enrollment, sample size and/or data collection.

The Safety Officer and PI will hold meetings to review the data safety, the first of which will be held prior to initiation of the trial to discuss the protocol, approve the commencement of the trial, and approve the plans for monitoring the study. Meetings with the safety officer will be determined by the PI and will be closed to the public because of confidentiality considerations. An emergency meeting may be called at any time by the Safety Officer, or by the PI, should questions of participant safety arise.

Dr. Paul Gubanich, Division of Sports Medicine, will serve as a study monitor for this project, while the PI and study coordinators will be responsible for monitoring data quality and adverse events. The monitor will review adverse events and unanticipated events at the time they occur and will report his assessment of the event(s) to the PI.

This research study involves only minimal risk for participants (see Risk/Benefit Analysis section (15)). Further assurances regarding participant safety and protection of private and confidential participant information have been outlined in the Potential Risks, Discomforts, Inconveniences and Precautions section (14), the Privacy section (18) and the Confidentiality section (19). If during the, preliminary analyses the research team identifies strong evidence of harm from the Q-collar device the study will be stopped immediately.

(15) PRIVACY AND CONFIDENTIALITY:

The participant has the right to privacy. The investigators will protect participant privacy to the extent allowed by law. All facts about this study that can describe a participant's name will be kept private. Results of the study will be summarized regarding age, etc. but the investigators will take every precaution necessary to keep names private.

To maintain the privacy information of study participants, only pertinent research personnel will have access to participant information. Research personnel are employees of CCHMC and have been trained in human participants research and HIPAA compliance. To further insure privacy, all data will be analyzed and tracked using a coded identification number that does not use identifiable personal information. Personal information and identifiers will be securely recorded and filed by the administrative assistant. The data will be encrypted with a password and stored on a personal computer and backed up on a network drive. The participant identification code will be used on all data questionnaires.

The results of this study will be kept confidential. No participant identification will be made public record in any form unless the participant gives his or her expressed written permission of release of participant's name, photograph or likeness captured on video. The investigators will be available for any questions that may arise.

To further insure confidentiality, only pertinent research personnel will have access to participant information. Research personnel are employees of CCHMC and have been trained in human subjects research and HIPAA compliance.

(16) COST OF PARTICIPATION:

Participants will endure no costs other than time and effort in participating in this study. Insurance will not be billed for any of the tests associated with this study.

(17) PAYMENT FOR PARTICIPATION:

Participants will be compensated for their time and effort in participating in this study. They will receive a $500 Clincard Mastercard® gift card for completing the testing sessions.

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