STUDY TITLE: Novel protection against potential brain injury during competitive non-helmeted sport in females.

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CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER

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INVESTIGATOR INFORMATION:

Gregory D. Myer, PhD (513) 636-0249
Principal Investigator Name Telephone Number

(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 (the Device) that applies mild jugular vein compression according to the principle of the Queckenstedt Maneuver, 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 and Fisher 2011, Smith and Fisher 2011, Smith 2012) has been approved for studies in humans (IRB 2013-2240) and the results indicate safety for use during high demand and maximal exertion activities. 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 high school athletes playing a non-helmeted sport such as soccer. Athletes participating in this study will be randomly assigned to one of two groups: 1) Device wearing during the season or 2) Non-device wearing during the season. This study will focus on the use and effectiveness of the device solely in females, as male football and hockey players have previously been investigated. All participants will be outfitted with an adhesive patch-like accelerometer (which will be placed behind the ear (*Xpatch–X2 Biosystems http://www.x2biosystems.com/x2_x_patch/)* which will measure the magnitude of every impact to the head sustained by the athlete. Effectiveness of the device will be determined via differences in longitudinal brain imaging and functional testing following competitive soccer participation. A subset of athletes who report a diagnosed concussion will also receive additional brain neuroanatomical and neurophysiological testing within a week following the diagnosed concussive event. At each of these time points participants will also undergo various neurocognitive assessments outlined below.

(2) PURPOSE OF STUDY:

The purpose of the study is to monitor longitudinal changes in brain structure and function between the preseason and postseason, in a population of soccer playing athletes 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. Concussion in female high school soccer players have been noted to occur at a rate of 4.5 concussions per 10,000 athletic exposures (Comstock, Currie et al. 2015).

Anatomically and physiologically, there remain many differences in the male/female genders. Interestingly, there has also been disparate prognosis and incidence of TBI in female athletes exposed to concussive impacts. We propose that *Slosh Theory* can explain these differences offering a mechanistic approach that could help shed light on further ways to alleviate the TBI burden on society. Note that *Slosh Theory* teaches us that hydrodynamics (fluids moving within moving containers) contribute to, or are even the main etiology for, energy absorption of the cranial contents and that mitigation of *SLOSH* (increased compensatory reserve volume) may mitigate TBI.

Young female athletes appear to suffer many of the highest incidences of TBI in the sports arena. Specifically in the sport of soccer, girls have a concussion incidence rate of 0.36 per 1000 athlete exposures (AE), the highest for all girls' sports.(Gessel, Fields et al. 2007) Others have contemplated a lack of neck muscle strength or endurance that seemingly just doesn't justify the differentiation noted. On the other hand, cranial vault differences are quite real and starkly different. Specifically, females and especially younger females are known to have a greater compensatory reserve volume that trends towards males as they age. This metric does seem to follow that of known TBI predisposition. Further, daily or weekly changes in hormonal metrics could alter the day to day risk of suffering a TBI and could explain the erratic data accumulated to date if not controlled for in a scientific manner.

In this light, we explore the effects of estrogen and progesterone on the risk for suffering TBI and the need to study these effects when performing prospective studies of young female athletes. Fluctuations in endocrine status may have a global impact on cerebral circulation and can also cause regional alterations in the blood flow of specific brain regions(Szelke, Mersich et al. 2008).

During the early follicular phase (day 1 to 5 of cycle), women exhibit greater basal CBF than men, but similar vasodilatory responses to hypoxia and hypercapnia (Peltonen, Harrell et al. 2015). There remain differences in cerebral vascular reactivity between the sexes and these should be highlighted due to the obvious alterations in CO2 in sport (Kastrup, Thomas et al. 1997, Kastrup, Dichgans et al. 1998)Aging is also known to contribute to CO2 reactivity (in women only) and thus one would expect greater changes in the younger athlete verse the aged (Kastrup, Dichgans et al. 1998). Further, "results demonstrate that the CO2 sensitivity of the hemispheric vessels is sex hormone dependent. Estrogen and progestin treatment have opposite effects on this cerebral circulatory parameter(Szelke, Mersich et al. 2008)."

Evidence indicates that sex steroid hormones influence the functionally unique capillary bed of the brain and blood-brain barrier permeability(Krause, Duckles et al. 2006). Estrogen treatment of young, ovariectomized rats significantly reduces dye extravasation into both the olfactory bulb and hippocampus, indicating a general tightening of the barrier (11). We have found in rat cerebral blood vessels that endotoxin-mediated induction of COX-2 and iNOS is increased when animals are treated chronically with progesterone or medroxyprogesterone. Induction of inflammatory mediators by endotoxin was dramatically enhanced in female cerebral vessels on the day of estrus when the plasma level of 17-estradiol is low and progesterone is high. During pregnancy and the postpartum period in rats, aquaporin 4, a water channel associated with brain edema, is increased around intracerebral blood vessels(Krause, Duckles et al. 2006).

There is a vast amount of evidence for hormone-dependent modification of function and behavior during the menstrual cycle, but little is known about associated structural alterations of the brain(Kastrup, Dichgans et al. 1998, Krause, Duckles et al. 2006, Szelke, Mersich et al. 2008, Hagemann, Ugur et al. 2011, Peltonen, Harrell et al. 2015) During the menstrual cycle estradiol levels show a marked peak at ovulation and progesterone levels rise afterwards with a broader peak towards the next menses. On the other hand the brain volume change, and even more so the associated loss in CSF-volume between t1 and t3 which by itself was not significant, correlates well with changes in progesterone levels. We demonstrate for the first time that brain morphology varies during the menstrual cycle, with a (grey matter) volume peak at time of ovulation which can be estimated to be ,13,5 ml for a ‘‘standard’’ brain (Hagemann, Ugur et al. 2011).

We would expect alterations in TBI susceptibility based on the altering levels of estrogen and progesterone in menstruating females (due to brain volume changes presumably affected by alterations in cerebral blood volume) whereas this is obviously not the case in males. As a theory of TBI pathophysiology gaining ground is the alteration in Blood Brain Barrier and estrogen/progesterone does contribute to this physiology as well, we also anticipate variations in injury and prognosis of the female athletes based on the timing of their menstrual cycles.

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, Naser et al. 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, Bailes et al. 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, Bailes et al. 2012, Turner, Naser et al. 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, Chin et al. 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)..

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, Bailes et al. 2012, Turner, Naser et al. 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, Edwards et al. 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, Edwards et al. 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, Duffin et al. 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, Laufer et al. 2014) For athletes in the non-intervention group, radial diffusivity (RD, DTI parameter associated with white matter structural integrity(Song, Sun et al. 2003, Song, Yoshino et al. 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 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 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, DTI, resting state fMRI, SWI, HARDI, ASL and BOLD will be completed in 60 minutes or less (see Table 1 for detailed specifications). Peripheral pulse oximetry and respiration waveforms will be collected for signal correction in functional MRI in order to improve data analysis for resting state fmri to minimize the potential confounding

effect from the physiological changes. All functional and neurocognitive testing will be performed at the Cincinnati Children's Hospital Human Performance Laboratory.

Table 1. Image sequence, mode and analysis. Imaging will be completed in 60 minutes or less.

Sequence	Resolution	Mode	Analysis	Time (min)
3D T1	1x1x1 mm	Anatomy	Visual /Volumetrics/registration	5
DTI	2x2x2 mm	WM microstructure	Visual/ROI/TBSS/structural connectivity	12
rs-fMRI	3x3x4 mm	Network Connectivity	Functional connectivity	10
N-back fMRI	3x3x4 mm	BOLD activation	Quantitative	6
HARDI	2x2x2 mm	WM microstructure	Tractography, structural connectivity	12
SWI	SWI	Hemorrhagic Injury	Visual examination for incidental finding	6
			Total Time	~57 min

(5) DURATION:

The study recruitment and intervention will occur during one season. Each participant will participate in at least 2 planned study visits that may take up to 3 hours. Data analysis will continue for a 2 year period following the final enrollment.

(6) SELECTION & RECRUITMENT OF PARTICIPANTS:

We will recruit up to 150 study participants. The participants (age 14-19 years old) will be recruited from a local school district and local sports clubs and teams. Participants will be recruited using a top-down approach. We have gained the consent and the cooperation of the school district administrations. Presentations and letters from the research team will be given to each participating school to detail the participation requirements and study risks.. Questions regarding participation will be answered during the presentations 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 and parents/guardians who voluntarily agree to participate will be scheduled to complete the pre-participation testing. The participant and parent/guardian 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 and parent/guardian does not read or sign the form, they will not participate in the study. Once the potential study participants/school teams are identified, they will be allocated to one of two groups: 1) Device wearing during the season or 2) Non-device wearing during the season. This research does not mandate that the participant maintains participation in their sport. The desire to participate in the sporting event is an independent, personal decision separate from the decision to enter into this study. If any athlete choses to stop participation in the sporting activity then they will also be withdrawn from the study, due to no longer meeting inclusion criteria.

Inclusionary criteria include:

- Female

- Normal healthy volunteer
- Able to provide written consent
- Must be 14 years or older and a participant on a high school soccer team

Exclusionary criteria include:

- Unable to provide written consent
- History of neurological deficits, previous cerebral infarction, or severe head trauma as indicated through pre-season 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. 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.

Because we will be testing teens, we will be using the Parent Consent Form to obtain both the participant assent and the parent consent. The participants and their parents will be given adequate time to review the study materials and ask questions. If they choose to participate, the patient and parent will sign the IRB approved consent forms. It will be made clear to the patient and their parents that participation in the study is voluntary.

In the event that a parent or guardian will not be present at the scheduled testing appointment, consent/assent forms will be provided ahead of time for review. 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), including sequences outlined in Table 1 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 a minimum of 2 MRI sessions (preseason, and post season) and additional scans following any clinically diagnosed concussions, 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 may 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: Anthropometric Measurements.

Anthropometric measures will be recorded at Station IV. Height, weight, and body composition (bioelectrical impedance) will be recorded and body mass index (BMI) calculated.

Height: A measure of height will be recorded with a digital stadiometer (accurate to 0.25 cm).

Weight: A measure to the nearest 0.5 kg will be taken on a calibrated physician scale with the participants' shoes off.

Neck Measurement: We will measure the circumference of participant's neck with a measuring tape.

Station II – 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.

Reflexive Saccades. 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.

ADHD rating scale: The ADHD rating scale queries parents how often their child exhibits each of the DSM-V ADHD symptoms on a 4-point Likert scale.

Menstruation Questionnaire – In order to determine the status of menstrual cycle, each participant will complete a questionnaire monthly, while participating in the study. Answers from this questionnaire will allow study staff to determine the approximate status of their cycle. This questionnaire will be administered using the electronic data capture REDCap. REDCap is a secure, web-based application for building and managing online surveys and databases. It is a resource of the Center for Clinical and Translational Science and Training (CCTST) provided to researchers. Subjects will be emailed the link to complete the questionnaire each month during participation in the study. All data will be de-identified via a study ID, assigned to each participant when enrolled in the study. Participants will also complete a Pre-Season Menstruation Questionnaire at the first study visit.

Injury Surveillance

Device and Compliance Acceptance: The study coordinator will be responsible for providing the appropriate intervention (device or no-device) to each team based on their assignment prior to the season onset. 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. Following the initial fitting, each athlete will receive adequate instruction on how to properly use the device on a daily basis. Throughout the season, the coordinator will make routine visits to each team to monitor the proper usage and fitting of the device, tracking the use of the device by each athlete individually. At the end of practice/game the device will be collected by the athletic trainer and/or stored in the athlete's locked locker. The device is NOT to go home with the athlete.

New Concussion Surveillance and Follow-up: 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 below. .

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. If available, video of games and practices will be used to validate and confirm head impacts recorded by accelerometers.

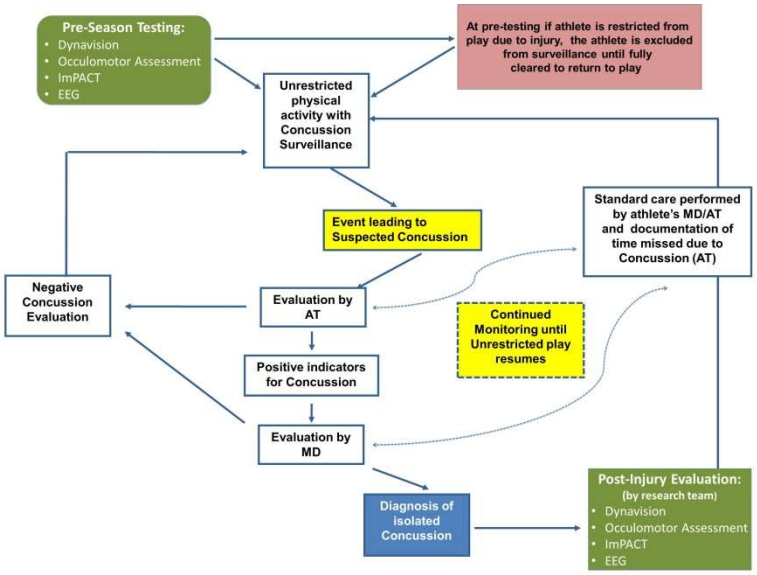

Figure 3. illustrates the steps that will be initiated when a concussive event is observed.

The study participants will also be monitored on a weekly basis for athletic exposures, and new injuries for a single season. Injury surveillance and concussion incidence will be monitored by the AT (K. Barber Foss) who will work directly with team athletes to visit each school and each team weekly following the initial screening. We will be monitoring injuries throughout the competitive sports season and collecting information about injuries from the school athletic trainer. Concussion will be reported according to criteria outlined in recent multi-investigator consensus documents (McCrory, 2013). The incidence of concussion will be calculated in this study using cases identified through two primary mechanisms: 1) Monitoring of concussion injuries and time missed from planned physical activity by the AT for each team, and 2) weekly inquiries to each coach by the Study Coordinator as to whether any participants are missing time from activity due to concussion. This dual query approach enables the Study Coordinator to cross check the injury and exposure data collected to improve the accuracy of the data. The data secured from the coach regarding each athlete's participation in individual practices and games combined with accelerometer exposure to impacts allows the research team to capture robust individual athlete exposures rather than just combined team exposures.

The athletic trainer will evaluate any athlete for whom a concussive event has been observed (within 24 hours). If criteria for concussive sequelae are not met, the athlete is cleared for return to play. In the event of a positive concussion evaluation by the AT, an MD will perform a second evaluation. Should the MD concur with the AT on a positive evaluation, a diagnosis of isolated concussion is made and the athlete is removed from play and participant is referred for to a post-concussion evaluation. The diagnosing physician will be blinded to the experimental treatment of the study participants.

Post-concussion and post season testing consists of the same battery of tests as performed for pre-season testing, which allows for direct comparison between typical and concussed data. In addition, concussed athletes will complete the Post-Concussion Symptom Inventory (PCSI) questionnaire at the beginning of the testing session. Diagnosis and treatment of a concussion is not effected in anyway by participation in the study. In addition, no post-

concussion testing completed as part of the study, will be used in making any clinical decisions for return to play.

(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.

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 season) 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.

Simple Reaction Time

A repeated measures mixed-model design will be employed. A total of up to three groups (Device vs. no device vs concussion) will be examined using a 3 x 2 (group by time point; pre-season vs. post-season) ANOVA.

Oculomotor Measures

Oculomotor control performance will also be compared using a 3 × 2 mixed-model ANOVA for each of the dependent variables. For smooth pursuit tracking performance, mean eye peak velocity and tracking lag will be submitted to separate 3 (group) × 2 (session) × 2 (random vs. sinusoidal task condition) mixed-model ANOVA.

(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).

(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, Quinn et al. 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 and Shipley 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, Buie et al. 1950, Torres and Ellington 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 and Ellington 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 and Grier 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, Moraine et al. 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, Eves et al. 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, Bailes et al. 2012, Turner, Naser et al. 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 may 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 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 $50 Clincard Mastercard® gift card for completing the first testing session and a $100 Clincard Mastercard® gift card for completing the final testing session. Participants who sustain a clinically diagnosed concussion will also receive $50 for each completed session following new concussion diagnosis. Registration in the Clincard payment system

requires a social security number, which will be acquired via a complete W-9 form for each participant.

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