Title

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Neuroimaging, Wearable Sensors, and Blood-based Biomarkers Reveal Hyperacute Changes in the Brain after Sub-concussive Impacts

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Abstract
Impacts in mixed martial arts (MMA) have been studied mainly in regard to the long-term effects of concussions. However, repetitive sub-concussive head impacts at the hyperacute phase (minutes after impact), are not well understood. The head experiences rapid acceleration similar to a concussion, but without clinical symptoms. We utilize portable neuroimaging technology – transcranial Doppler (TCD) ultrasound and functional near infrared spectroscopy (fNIRS) – to estimate the extent of pre- and post-differences following contact and non-contact sparring sessions in nine MMA athletes. In addition, the extent of changes in neurofilament light (NfL) protein biomarker concentrations, and neurocognitive/balance parameters were determined following impacts. Athletes were instrumented with sensor-based mouth guards to record head kinematics. TCD and fNIRS results demonstrated significantly increased blood flow velocity (BFV) ($p=0.01$) as well as prefrontal ($p=0.01$) and motor cortex ($p=0.04$) oxygenation, only following the contact sparring sessions. BFV increase after contact was correlated with the cumulative angular acceleration experienced during impacts ($p=0.01$). In addition, the NfL protein blood biomarker demonstrated positive correlations with angular acceleration ($p=0.03$), and maximum principal strain and fiber strain ($p=0.01$). On average athletes experienced 23.9±2.9 g peak linear acceleration, 10.29±1.1 rad/s peak angular velocity, and 1,502.3±532.3 rad/s^2 angular acceleration. Balance parameters were significantly increased following contact sparring for medial-lateral (ML) center of mass (COM) sway, and ML ankle angle ($p=0.01$), illustrating worsened balance. These combined results reveal significant changes in brain hemodynamics and neurophysiological parameters that occur immediately after sub-concussive impacts and suggest that the physical impact to the head plays an important role in these changes.

Keywords: Functional Near-Infrared spectroscopy; sub-concussive impacts; hyperacute; transcranial doppler ultrasound

1. Introduction

Mixed martial arts (MMA) is an extreme combat sport that allows for fighting techniques such as wrestling, boxing, kickboxing, as well as others, in an attempt to win matches by knockout, submission, or the decision of the referee (1). In many cases, the participants do not wear any type of protective headgear and sustain repetitive head impacts (RHI) which can result in disorientation or loss of consciousness, often meeting criteria for a concussion (2). The incidence of match-ending head trauma is 31.9% for professional MMA participants which is higher than reported rates in any other combative and contact sport (3).

An increasing concern with MMA fighters is the occurrence of numerous mild traumatic brain injury (mTBI) or concussion injuries, which have demonstrated evidence of long-term neurodegenerative pathology known as chronic traumatic encephalopathy (CTE) (4–6). However, there has been less focus on repetitive sub-concussive impacts and defining the effect of these lower-level head accelerations on brain's function. Sub-concussive impacts are described as generally having a lower impact force and acceleration to the head, compared to concussive impacts, and not resulting in clinical symptoms of concussion (7). The repetitive exposure to sub-concussive head impacts also appears to alter brain activity and cause cognitive impairments and long term neurological deficits for some individuals (8). Previous studies found association between diminished neurocognitive performance and increased sparring exposure in amateur MMA/boxers, suggesting that repeated sub-concussive blows may be just as harmful (9–11). Currently there is no consensus using conventional MRI/CT to diagnose concussion (12). Consequently, it remains challenging to diagnose sports-related concussions (SRC) quickly and accurately to understand the immediate underlying functional responses at both the concussive and sub-concussive levels quickly and accurately.

1.1 Portable Neuroimaging and Biomarkers of Injury

Little is known about how repetitive sub-concussive head impacts affect cerebral perfusion at the hyperacute level, within minutes after the impact. MRI and functional MRI (fMRI) techniques have been used to demonstrate structural and physiological changes days, weeks, or months following concussion (13), demonstrating reduction in cerebral blood flow (CBF) (14,15), as well as regional brain oxygenation changes (16,17). Measuring immediate changes using these imaging modalities have been lacking given the complexities of scheduling and time for travel for scanning (18,19).

There are currently several advanced non-invasive neuroimaging devices with unique portable capability, such as transcranial Doppler (TCD) ultrasound and functional near infrared spectroscopy (fNIRS). These methods allow for real-time measurements of real-world head impact effects on cerebral hemodynamics (20,21). Thus, results can be obtained on the field to observe the most immediate neurophysiological effects from head impacts. We have recently reported observable changes in brain hemodynamics via fNIRS and TCD measures following sub-concussive impacts from soccer heading comprising more mild impacts (22). By comparison, MMA athletes sustain a greater number, and greater level of head impacts (23). The use of fNIRS in the context of concussion has been limited and the method of data processing is still being researched (24). fNIRS has shown

potential applications for rapid diagnosis, especially in oxygenation and autoregulation monitoring (25), making it suitable to measure immediate changes in brain function. TCD measurements from athletes have been common with concussion, however, most studies take measurements days or weeks following concussion (26–28).

Some studies have also turned to examining blood-based biomarkers which can provide information on neuronal and glial cell damage, metabolic abnormalities, inflammation, axonal injury, and other pathophysiological changes that can aid in diagnosis, monitoring injury progression, or determining return to play timeframes (29). For example, previous mTBI studies have reported increased levels of neurofilament light (NfL), tau protein, S100B, glial fibrillary acidic protein (GFAP), galectin 3, occludin, plasma soluble cellular prion protein, as well as decreased levels of copeptin in plasma (29–31). Few studies have examined if there are changes in these protein concentrations closely following sub-concussive impacts.

1.2 Neurocognitive Functional Response Following Head Impacts

Standardized assessments have demonstrated effective use in measuring parameters from individuals diagnosed with concussions, since they commonly suffer from deficits in sensorimotor function or balance (32). There is a validated test for predicting neurocognitive and motor performance called the upper extremity function (UEF) dual-task test (33). This test uses wearable sensor technology to measure cognition and physical function status by monitoring simultaneous performance of repetitive arm movement and counting, known as dual-tasking (34). The effectiveness of UEF dual-task actions to identify cognitive status has been previously demonstrated in older adults (35) and also used to assess various neurological related diseases including frailty (34), patients with mild cognitive impairment (36), and Alzheimer's disease (33). We expect this measure to be sensitive to changes in motor and cognitive performance after repetitive head impacts.

1.3 Head Impact Kinematics and Strain-Based Injury Metrics

Aside from the physiological responses to brain injury, previous sports studies have focused on head kinematics by recording real time linear acceleration and angular velocity on the field using 6 degrees-of-freedom (DOF) wearable sensor technology embedded into mouth guards (37–39). Although on-field experiments have provided useful information on the external results of injury, they do not provide information about the extent of internal damage to the brain. Thus, computational head injury models have shown to be a good alternative to provide more information than kinematics alone, for detecting concussion risk by informing impact-induced brain strains or deformation patterns causing brain injury (40–44). This aids in predicting mechanical pathways of brain damage to improve diagnosis and neurological assessment (45). Outputs of computational models such as maximum principal strain (MPS) of the whole brain, has been associated with diagnosed concussion (46), but fiber strains that characterize stretching along white matter tracts is important in brain injury as well (40) and improve injury prediction performance (47). This is especially evident in highly vulnerable areas such as the corpus callosum (48). These methods have been effective in tracking head motion

and simulation during impacts; however, its association with hyperacute physiological response of the brain has not been studied rigorously, which is why neuroimaging would be beneficial at the hyperacute phase.

The purpose of this study was to examine the immediate measurable effects, *i.e.*, within minutes, of head impacts sustained during MMA training sessions in terms of brain blood characteristics and neurological function, with a secondary aim to determine changes in blood-based biomarkers within 12 hours. We postulated that there would be significant changes in brain oxygenation, blood flow velocity, cognition and balance parameters that will be similar to hemodynamic trends observed in concussion studies but to a lesser extent. We also hypothesized that some of these hemodynamic characteristics would be correlated with head kinematics and the brain strain experienced during sub-concussive head impacts.

2. Methods

2.1 Participants

The study protocol was reviewed and approved by the Human Subjects Institutional Review Board at the University of Arizona (IRB 1911128385). Nine male MMA athletes (age>18) were recruited to participate in this study (Table 1). These were amateur athletes recruited from local gyms with high fitness level. Exclusion criteria comprised diagnosed diseases associated with motor performance deficits or known history of brain injury or concussion within the last six months, which was determined by a pre-screening based on self-reporting. Written and informed consent was obtained from all subjects before participation.

2.2 Participant Instrumentation

Participants were equipped with custom fitted mouth guards with an embedded six degrees-of-freedom accelerometer and gyroscope (sample frequency = 1000 Hz, Sports & Wellbeing Analytics Limited, United Kingdom) to record head kinematics (Figure 1A). Approximately 104 ms of kinematic data was transmitted to a wireless receiver if an impact exceeded the 10 g threshold of head acceleration (37,49–51). Assessments took place at the participants' home gym.

During assessments, before and immediately after sessions, participants were equipped with the TCD headset (Rimed, Digi-Lite, New York, USA) to measure both the left and right MCA velocity and heart rate, using 2 MHz transducers fixed in place (Figure 1B). The TCD M1 MCA depths were assigned as 65 to 45 mm distance from the transducer, accessed via the trans-temporal window and confirmed with insonation angle and direction of blood flow according to previous studies (52). The same trained personnel recorded all TCD signals. Participants were also equipped with the fNIRS (Brite 24, Artinis Medical Systems, Netherlands) device to measure brain oxygenation at a sample frequency of 50 Hz via multi-wavelength LED's situated on a soft neoprene head-cap containing 8 receivers 10 transmitters 30 mm apart, allowing for approximately 15mm penetration depth. As indicated in Figure 1C, the probe positions of the fNIRS detection device covered the area linking Fp1, F3, F7 and Fp2, F4, F8 corresponding to the left and right prefrontal cortex respectively, as well as C3, Cz, C4, corresponding to the precentral/postcentral gyrus containing the primary motor cortex, somatosensory cortex, according to the international EEG 10–20 system (53,54). The differential path length factor (DPF) was calculated for each individual in relation to their age (55). Data included concentration signals of oxyhemoglobin (O2Hb), deoxyhemoglobin (HHb), total hemoglobin (tHb), and hemoglobin difference (HbDiff) collected via OxySoft (Artinis

Medical Systems, Netherlands) software. To ensure good signal quality from the fNIRS optodes, the cap was tightly fitted, properly secured, and any hair was moved aside and signal quality was verified in real time.

During fNIRS measurements participants also wore two wireless accelerometer and gyroscope sensors (sample frequency = 100 Hz, BioSensics LLC, Brookline, MA, USA) on the bicep and wrist of the dominant arm, secured with a Velcro band, to perform the motor function UEF test. Parameters taken from these sensors were used to determine a UEF cognitive score. The same sensors were then used to measure balance parameters when placed on the waist and ankle as the participant stood still for 30 seconds.

2.3 Experimental Protocol

Participants were first instructed to complete ten days of non-contact training to serve as a control measurement in which no data was collected. Athletes were allowed to perform cardio workouts, weight-lifting or training that did not involve contact with the head during these ten days. The athletes chose the specifics of the cardio workout to perform during the non-contact session; we only asked that it raise their heart rate similar to when sparring. Participants were then evaluated using a design that involved a pre-test, exposure or non-exposure, and a post-test, with a total of five separate data collection sessions (Figure 2). The same assessments were completed before and within 15 minutes following a session to determine hyperacute responses. The first data collection meeting took place at the end of the 10-day period which included pre- and post- measurements from a non-contact training session of approximately 45 minutes. Following this, were four contact training sessions, which took place on separate days, each with the same pre- and post-measurements obtained. During the exposure period, participants performed multiple rounds of sparring within a period of 30-45 minutes, while wearing the custom-fit mouth guard. Kinematic data was transmitted for each impact that exceeded 10 g of linear acceleration and was confirmed via video.

Pre-and post-measurements consisted of TCD recordings, fNIRS with UEF test, and balance measurements in this order. Participants were seated during TCD and fNIRS measurements. First, resting velocities of the MCA were recorded for approximately 2 minutes using the TCD headset. Following this, participants wore the fNIRS cap and the wireless arm sensors, and performed the UEF dual-task motor and cognitive test (35). This consisted of rest,single task, rest, and dual task, in that order, with each period lasting approximately 2 minutes and only run-through once for the pre- and post-measurement (Figure 1C). The single-task movement consisted of bending and straightening the dominant arm as consistently as possible. The dual-task movement consisted of the same motion while also counting backwards by three, from a given number. Participants followed instructions of when to conduct each task from a visual PowerPoint presentation on a laptop screen placed in front of them. A practice trial was provided before fNIRS data acquisition to minimize learning effects. Following this, participants performed separate balance test where they stood still in place, legs shoulder-width apart and arms crossed in front, for 30 seconds with the wireless sensors placed on the waist and ankle.

Biospecimens were collected at two timepoints, baseline (12 hours after the non-contact training) and 12 hours after the last sparring session (post-sparring), by trained study personnel in a certified blood collection room following all safety protocols. At each timepoint, 12-hour fasted blood samples were collected from study participants by venipuncture into vacutainer tubes (2 x 7.5 mL powdered glass clot activator, 2 x 10 mL ethylene diamine tetra-acetic acid or EDTA) for 35 mL of whole blood. The clot activator vacutainer tubes were allowed to clot for 30 min prior to being centrifuged at 3000 rpm for 15 min. EDTA vacutainer tubes were centrifuged at 3000 rpm for 15 min within 2 min of collection. The aliquots of serum (clot activator tube), plasma (EDTA tube), and red blood cells (EDTA tube) within the tubes were immediately transferred to polypropylene vials and stored at −80 ℃ until analysis. Blood-based biomarkers of brain injury included serum neurofilament light (NfL) concentrations (pg/mL) a biomarker of axonal injury and plasma glial-fibrillary acidic protein (GFAP), an astrocyte injury biomarker, and were analyzed by Quanterix Corp using the NF-Light Simoa Assay Advantage Kit (Quanterix Corp, MA, USA) and R&D Systems human GFAP duoset ELISA (Bio-Techne, R&D Systems kit, Minneapolis, MN, USA) respectively. Plasma IL-6 (pg/mL) concentrations were determined using the Human IL6 Quantikine ELISA (Bio-Techne, R&D Systems high-sensitivity kit, Minneapolis, MN, USA). In most cases, the data session occurred in the evening, and the blood draw occurred first thing in the morning for the fasting.

2.4 Data Analysis

All kinematic sensor data from the mouth guard were filtered with a fourth-order low pass Butterworth filter with 300Hz and 184Hz cutoff frequencies for the accelerometer and gyroscope, respectively (56). The sensor's kinematic data were converted to the head's coordinate system at the head center-of-gravity according to previous rigid body transformation equation (50,57). Since previous research demonstrated that mechanical strain and stretching in brain tissue correlates with injury (40,46), we inputted the kinematic data to pre-trained convolutional neural network (CNN) models for strain estimation, based on brain response samples available in a published study (58). The rotational velocity profile was first preprocessed to a fixed input size for the CNN, and resulting strains calculated include maximum principal strain (MPS) of the whole brain, MPS of the corpus callosum (CC), and fiber-oriented strain (FS) of the CC, all assessed at the 95^{th} percentile levels (59).

Images from the TCD ultrasound were exported and analyzed in 8 second time increments in a graph digitizing software. The step interpolation algorithm was used to obtain the contour trace of the velocity profile as data points with known axis limits. The right and left MCA blood flow velocity data were averaged together for each measurement. Additionally, flow resistance was assessed by the pulsatility index (PI), which is a frequently used output TCD parameter. This is calculated by subtracting end-diastolic velocity from peak systolic velocity and dividing the value by mean flow velocity (60). PI is independent of the angle of insonation and is dimensionless. It is also independent of the diameter of the insonated vessel which allows for comparison between participants and is therefore a commonly used metric in TCD studies (60).

The fNIRS signal was down sampled to 5 Hz when exported from Oxysoft software (Artinis Medical Systems, Netherlands). All data was read into MATLAB Fieldtrip Toolbox for analysis. Channels were visually inspected. Bad channels due

to poor scalp coupling were removed, and motion artifacts were corrected for by means of thresholding using functions in Fieldtrip Toolbox. The data was then converted into concentration values using the modified Beer Lambert Law. Cardiac (1- 2 Hz) and respiration (0.2- 0.4 Hz) interference in the signal were removed using a band-pass filter with cut-off frequencies of 0.001-0.1 Hz (61), while still extracting important physiological signal due to the task (stimulation frequency = 1/180sec = 0.005 Hz). Next, Principal Component Analysis (PCA) was done to try and remove global interference. Each component had an associated eigenvalue, and the component with the largest value was likely due to the most interference from surface tissue (62). This was the first principal component and the time course was then used in later analysis. A preferred method of removing additional physiological factors in fNIRS experiments is using the General Linear Model (GLM) (63,64), which consists of regressing fNIRS data with a linear combination of regressors and an error term. GLM measures the temporal variational pattern of signals rather than their absolute magnitude and incorporate regressors such as scalp blood flow, into the statistical framework (64,65). The GLM regression analysis was applied to all channels using the MATLAB FieldTrip Toolbox (66). Briefly, we read in the events, which are represented as triggers, indicating the samples in the data in which the task started or stopped. We use these to make some additional continuously represented channels that represent the onset, offset, and the motion. We add two channels for a constant offset, and for a slope. These are used to remove the baseline and a constant drift in the signal over time. We perform GLM analysis where each channel is represented as a pixel in the statistical parametric map (SPM) which is done to statistically analyze and compare groups of images to highlight neurological differences (64). To create a regressor of interest, the hemodynamic response is predicted by convolving the canonical hemodynamic response function (HRF) and its temporal and dispersion derivatives included, with a single boxcar function regressor that represents the task segments (67–69). A boxcar function was used due to the required sustained stimulation of performing motor and cognitive tasks in our study (67,70). The first and second derivatives control the timing of the peak response as well as the width. Thus, design matrix consisted of one regressor of interest (single or dual task) convolved with the HRF, and added nuisance regressors including the signal drift and the time course of the first principal component to be removed. After GLM analysis of the channels, results were grouped together based on functional regions, *i.e.*, prefrontal, motor/sensorimotor for concentration changes in O2Hb.

Kinematic measures are computed from the wireless sensors for the UEF test and were analyzed using an in-home detection MATLAB algorithm to determine an overall UEF cognitive score for pre- and post-measurement comparison. These measures include speed, rise time, speed reduction, and the total number of flexions. The score is based on slowness, exhaustion, weakness, flexibility, and body mass index, which is determined based on logistic model from previous studies (71,72). Similar analysis was used for the same wireless sensors used during the balance test.

All statistical data were assessed in GraphPad Prism 9.0 (73) software and statistical significance was set at $p=0.05$. The four contact sessions were combined and then compared to the non-contact measurements. Tests for normal distribution were done using the Shapiro-Wilk test (74). The fNIRS data was analyzed by fitting a mixed-effects model that is fit using Restricted Maximum Likelihood (REML) with

Geisser-Greenhouse correction applied. This method gives the same analysis values and multiple comparisons tests as repeated measures ANOVA and thus, interpreted the same. The concentration values obtained from GLM analysis acted as the measurement variable, with contact, task, and time as the nominal repeated measures. If statistically significant differences were identified between pre- and post-measurements, the Holm-Sidak method was used to correct for multiple comparisons. The TCD data was analyzed using a two-way ANOVA with blood flow velocity as the measurement variable, and contact and time as the nominal repeated measures. Pearson correlation coefficients were calculated for normally distributed groups and Spearman correlation was calculated for non-normally distributed groups to determine significant correlations.

3. Results

3.1 Kinematics

There were 707 impacts recorded for the entire study resulting in 23.9±2.9 g peak linear acceleration, 10.29±1.1 rad/s peak angular velocity, and 1,502.3±532.3 rad/s^2 angular acceleration on average (Figure 3A). This ranged between approximately 10-70 impacts per participant within a session and between 100-200 impacts per contact session. No brain injuries were diagnosed during the sparring sessions. Figure 3B shows an example of the mouth guard data recorded during an impact. Most impacts occurred near the front of the head resulting in a negative acceleration posteriorly or backwards, according to the sensor orientation which showed greater negative values in the z axis compared to x and y (Figure 1A). Averages did not vary significantly among the four different contact sessions (Figure 3A). As shown in figure 3C, average MPS for the whole brain was 0.11±0.04, MPS of the CC was 0.10±0.03, and FS of the CC was 0.07±0.03. Head kinematics during non-contact sessions were only recorded for one participant, whose head acceleration exceeded the 10g threshold (leading to MPS: 0.11±0.006; MPS_CC: 0.102±0.01; FS_CC: 0.07±0.004).

TCD Neuroimaging

Results from the TCD ultrasound blood flow velocity measurements include 7 participants – the MCA was not able to be located for two participants and the temporal windows were determined to be absent which may be related to thickness and porosity of the bone (75). The results of the two-way ANOVA reveal an interaction effect between time and contact for the MCA blood flow velocity changes ($p=0.03$). Multiple comparison analysis reveals a significant increase in the post-measurements compared to pre-measurements, for all contact sessions ($p=0.01$) (Figure 4A). Average blood flow velocity values for all contact sessions were 74.54±19.8 cm/s and 80.06±22.1 cm/s for pre- and post-measurements, respectively (Table 3). However, this significant increase was not evident in the non-contact sessions with average blood flow velocity values at 75.31± 21.7 cm/s and 75.54± 23.01 cm/s for pre- and post-measurements respectively ($p=0.99$). Average heart rate was measured before and after each training session and showed significantly increased heart rate from 69.6±15.2 bpm to 108.04±26.3 bpm for pre- and post-measurements, respectively, for non-contact training ($p<0.0001$), and from 68.2±13.8 bpm to 101.7±22.1 bpm for pre- and post-measurements respectively for all contact trainings ($p<0.0001$) (Figure 4A). Although change in heart rate seems to affect blood flow velocity as well, we did not observe any significant correlation between HR changes and the resultant TCD measurements

within the specific range in the current study ($p>0.06$). Two-way ANOVA revealed also a significant interaction effect of time and contact for the pulsatility index changes ($p=0.04$). Multiple comparisons analysis revealed PI was significantly decreased following the contact sessions with values of 1.1 ± 0.1 a.u. and 0.84 ± 0.13 a.u. for pre- and post-measurements respectively, ($p=0.0002$). This again was not the case for the non-contact session with values of 1.03 ± 0.2 a.u. and 0.97 ± 0.3 a.u. for pre- and post-measurements, respectively ($p=0.69$), as reported in Figure 4A and Table 3. In addition, we observed a correlation of post values for blood flow velocity with cumulative angular acceleration after contact ($p=0.01$) (Figure 4B).

3.2 Pre- and Post-Impact fNIRS Changes

Results from the fNIRS measurements following GLM analysis, reveal significant increases in O2Hb concentration values following the contact sessions across all 9 subjects. The mixed-effects model analysis revealed an influence of time in terms of pre- and post-measurements in the left and right prefrontal region ($p=0.01$, $p=0.007$), as well as in the left motor region ($p=0.01$). Multiple comparisons tests revealed pre-and post-measurement significant differences only for the head contact sessions in the prefrontal cortex (left: single task ($p=0.04$) and dual task ($p=0.01$); right: dual task ($p=0.02$)), as well as the left motor cortex during the dual task ($p=0.04$) periods (Figure 5, Table 4). The right motor cortex did not show significant changes. There were no significant differences in O2Hb concentration changes following the non-contact sessions for any of the brain regions ($p>0.11$) (Table 4). Additionally, there were no changes in pre- and post-measurements of oxyhemoglobin concentrations during rest for contact ($p>0.14$) or non-contact ($p>0.28$). Although change in heart rate seems to affect fNIRS concentration changes, we did not observe any significant correlation between oxygenation values and the changes in heart rate in this study for non-contact (single task $p>0.17$; dual task $p>0.11$) nor contact (single task $p>0.33$; dual task $p>0.41$).

3.3 Blood-based Biomarkers

There was no significant difference between non-contact baseline (0.94-4.58 pg/mL) and post-sparring (1.42-4.06 pg/mL) serum NfL concentrations ($p=0.46$). Post-sparring serum NfL values were consistent with levels found in studies of other high-contact sports (2.5-19.2 pg/mL), where repetitive sub-concussive impacts are common such as football and soccer (76–78). In addition, plasma GFAP and IL-6 concentrations did not differ between non-contact baseline (0.15-13.21 pg/mL, 0.82-3.16 pg/mL) and post-sparring (0.13-13.11 pg/mL, 085-6.12 pg/mL), respectively. One subject had GFAP levels (>20.98 pg/mL), above assay sensitivity at both timepoints, and results were not included in the analysis.

The blood-based biomarkers were additionally correlated with kinematic values including linear acceleration, angular velocity, angular acceleration, the cumulative values for each of these parameters, and lastly the total number of impacts. There was a positive correlation with the NfL biomarker post-measurement levels and angular acceleration ($p=0.01$) as well as cumulative angular acceleration, as a measure for impact exposure ($p=0.02$) (Figure 6A). The final blood draw was performed 12-hours following contact session four; therefore, we also looked at possible correlations for values in this session. We found positive correlations with the NfL biomarker post-measurement levels and the angular velocity ($p=0.03$) as well as the cumulative angular velocity ($p=0.02$) (Figure 6B). These biomarker values were also correlated with the brain strain parameters including MPS, and

MPS and FS of the CC. There was a positive correlation for each parameter with the NfL biomarker when looking at the pre- and post-measurement difference of the biomarker in strain estimations for the fourth contact session (Figure 6C).

3.4 Balance and Function

There were significant differences in balance parameters following contact sessions which included a significant increase in medial-lateral (ML) ankle angle from baseline (0.08-0.38 deg) to post-contact (0.12-0.62 deg) ($p=0.01$) and ML center of mass (COM) sway from baseline (0.03-0.14 cm) to post-contact (0.04-0.24 cm) ($p=0.01$) relating to greater range of motion in balance. On the contrary, balance parameters between pre- and post-measurements showed no significant differences after the non-contact session ($p>0.11$). Similarly, the UEF neurocognitive score was not significantly different following the non-contact session ($p>0.07$), nor the combined contact sessions ($p>0.1$).

4. Discussion

The present study associates immediate changes in brain hemodynamics and blood biomarkers following multiple sub-concussive head impacts, with head kinematics in MMA. Significant changes were observed within minutes after contact training sessions via TCD ultrasound of the MCA blood flow velocities, as well as oxygenation changes in the cortex of the brain via fNIRS. These changes in terms of blood flow velocity were also correlated with the angular acceleration of the head, experienced during the training sessions. In addition, concentration levels of the NfL blood-based biomarker of injury demonstrated correlations with kinematics of the head including angular acceleration, angular velocity, and brain strain. This information from portable, real-time neuroimaging, biomedical sensors, and injury biomarkers can give more insight into the brain response from head impact. Further, in combination with current on-field metrics of concussion diagnosis, these methods could improve monitoring head impact injuries in contact sports.

4.1 Neuroimaging

Advanced neuroimaging techniques such as TCD and fNIRS allow for insight into the microstructural and functional impairments of the brain. The MCA blood flow velocity values as well as the oxygenation status within different regions of the brain, were only significantly increased following the contact sessions. However, all values still were comparable to average healthy values reported in the literature (79). These combined findings may suggest that the physical impact on the head plays an important role in why these physiological changes are observed within minutes after the contact session, regardless of the heart rate increase of the athlete. There is limited data showing immediate changes in cerebral blood flow velocity and fNIRS measurements immediately after sub-concussive impacts. In one study, no changes were reported in MCA velocity following 40 soccer headers (26). Another study reported increased cerebral blood flow within 48 hours after injury using arterial spin labeling (80). On the contrary, other more serious injury cases report decreased cerebral blood flow and are measured days to weeks after injury (15,81,82). The PI measure has been described as cerebrovascular resistance or compliance of the artery. Some studies report increased PI levels after injury suggesting the injury may reduce the capacity of the cerebrovascular bed to respond to dynamic changes in blood flow (83). However, there are a few studies that report decreased levels of PI in mTBI populations compared to controls during

the first 48 hours after injury (81,84), which is a similar pattern to our results even though there are no injuries.

In terms of brain oxygenation, one previous study utilized functional MRI to show increased functional activity within 24 hours after injury in college football players, followed by a 7-day impairment period post-injury (85). One reason suggested for this increase in oxygenation for participants when performing motor and cognitive tasks, is that the injured brain works extra for similar tasks, leading to overcompensation and increased oxygenation readouts (86). On the other hand, reduced brain oxygenation during cognitive tasks was reported using fNIRS 15 days post-injury (87). In this case of diminished oxygenation, studies hypothesize an altered cognitive resource allocation and compromised signaling messengers post-injury. However, the results of increased oxygenation and blood flow velocity in our study may be an immediate response of the brain after sub-concussive impacts, in contrast to regional hypoxia seen in more severe cases of TBI (88). During an evoked brain activity, regional changes in blood flow alter the concentration of O2Hb in the brain (89). Research has shown in healthy subjects, neural stimulation in the form of cognitive/motor tasks, can induce an increase in cerebral blood flow and O2Hb concentration, in which the increase in delivery of oxygen exceeds oxygen consumption as cerebral vasculature responds to physiological signaling messengers, *i.e.,* calcium ions, nitric oxide, other metabolites (90). The post values of blood characteristics were significantly greater in our study following head impacts even though our athletes were performing the same tasks before and after impacts.

4.2 Kinematics and blood-based biomarkers

Few studies have focused on the head impact biomechanics of MMA athletes (91–93). We provide immediate measurements from impacts for MMA athletes after contact and non-contact training sessions. Average values for peak linear acceleration and peak angular velocity were comparable to previous sub-concussive MMA sparring events (94,95). Some maximum values fall within reported concussion ranges however, no one was injured during this study. In addition, changes in MCA blood flow velocity values were correlated against angular acceleration, suggesting that the combination of intensity of angular head movement and the number of impacts, plays a role in MCA hemodynamic changes. The prediction of concussion based on head kinematics alone is not as accurate. The amount that the brain is deformed determined by strain calculations, also gives more insight to injury predictions. The brain strain reported in this study is comparative to other uninjured MMA brain strain reports (96) compared to concussion levels (46) suggesting this is at the sub-concussive level. Studies have found that the best indicator of concussion is strain within the corpus callosum region of the brain as 87.9% higher strains have been reported in injured MMA athletes compared to non-injured (91). For this reason, brain strain was calculated for this region.

Brain-specific biomarkers for use as diagnostic tools in brain injury have become more prevalent in recent years. Research on NfL chain peptides as potential biomarkers for axonal injury has been observed in certain contact sports studies since it is the most abundant neurofilament, with increased levels 24-48 hours following concussion (97,98). None of our athletes experienced a concussion which may explain why we do not see differences in any of the tested biomarker protein concentrations following all contact sessions. Post-sparring serum NfL values (1.42-

4.06 pg/mL) were consistent with levels found in studies of other high-contact sports (2.5-19.2 pg/mL), where repetitive sub-concussive impacts are common such as football and soccer (76–78). There were a number of head kinematic correlations with the NfL biomarker including angular velocity and angular acceleration. This suggests that a combination of the number of impacts and magnitude of rotation may affect the concentration changes of this biomarker without concussive injury.

4.3 Neurocognitive Function and Balance

Counting is considered a rhythmic task that involves working memory (99) and is more directly related to executive function (100,101). Combining this with other rhythmic tasks of different frequency such as repeated elbow flexion, may cause interference or be difficult to execute since dual-tasks recruit the same resources at the same time (102). According to previous studies, a more demanding cognitive task leads to a greater decline in the motor task performance (103). We hypothesized that the more challenging cognitive UEF dual-task would likely be affected after impacts compared to the single-task. However, there was no change in the cognitive score calculated from the arm sensor movements when comparing pre- and post-measurements, suggesting there was no change in cognition after one contact training session, or following four separate contact training sessions. Some studies have suggested neuropsychological function determined by neurocognitive tests is unaffected by repetitive sub-concussive level head impacts (104).

There were some differences in balance parameters following head impacts, however this occurred after individual contact sessions and not all four contact sessions overall. Even so, there were still no significant differences in balance parameters after the non-contact session, which suggests that the repetitive impacts the athletes sustained may have affected some balance characteristics as well.

4.5 Limitations

There are several limitations to this study. The smaller sample size of MMA athletes may have contributed to a reduced number of significant findings. Future work should include larger sample sizes. In addition, there was no control for the number of impacts each athlete sustained during each of their training sessions. The mouthguard trigger is based on a 10g threshold and therefore excludes lower impacts to the head. The frequency and severity of impacts were not controlled for either non-contact or contact sessions, however, given the multiple repetitions of contact sessions and the fact that the head kinematics follow previously reported values (94,95), we believe our sample covers a reasonable range that can be compared across the different sessions. Previous studies have reported mouthguard sensors with approximately 10% of kinematic error compared with ground truth measurements (105) which is even lower when translated to brain strain error estimates (106). Additionally, due to scheduling conflicts, some athletes completed all sessions in a shorter period compared to others. We included a 10-day period where the athletes were not subjected to head impacts and this was an estimation of returning to baseline levels before contact sessions. More research is needed to determine the accurate amount of time for baseline characteristics of experienced MMA athletes. Lastly, the lack of short separation channels in fNIRS is a limitation of this study, but PCA was performed a PCA approach to remove global interference.

5. Conclusion

Understanding the underlying mechanisms of head impacts that occur during MMA sparring sessions may give insight to the management of concussions in the future for many contact sports. The novel findings of this study include one, detectable changes of brain hemodynamics immediately after head impacts using portable, non-invasive, and real-time neuroimaging TCD and fNIRS of real-world impact data; and two, association of head impact kinematics and brain blood characteristics, such as blood flow and brain specific blood-based biomarkers. Reliable diagnostic measures for concussion and sub-concussive hits are of the utmost importance. Neuroimaging and brain-specific biomarkers are showing great promise and in combination with current on-field metrics, could be useful biomarkers or mitigation of risks for concussion in the future.

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Table 1 Demographic Parameters

Characteristic	mean±SD
n total	9
Age (years)	31.2 ± 2.8
Height (in)	70.3 ± 2.9
Weight (lbs)	182.8 ± 27.2
Race (n)	
American Indian of Alaska Native	0
Asian	0
Black of African American	2
Latino or Spanish Origin	2
Native Hawaiian or Other Pacific Islander	0
White	3
Two or more races reported	2
Ethnicity (n)	
Hispanic or Latino	5
Not Hispanic or Latino	4

Figure 1 Instrumentation. A) Mouth guard worn during sparring session. B) TCD headset and Axial slice view of MCA. C) fNIRS optodes arrangement based on the international EEG 10–20 system. Source and detector pairs are demonstrated in the 3D view: Yellow = source, Blue = detector. The Upper Extremity test protocol is performed during the fNIRS measurements system.

Table 2 Kinematics

Parameters	Mean ± SD
Linear acceleration (g)	23.9±2.9
Angular velocity (rad/s)	10.29±1.1
Angular acceleration (rad/s2)	1502.3±532.3
Max Principal Strain	0.11±0.04
Max Principal Strain Corpus Callosum	0.10±0.03
Fiber Strain Corpus Callosum	0.07±0.03

Results presented as mean ± SD. Bold-faced values show statistical significance (p<0.05)

Figure 2 Study Protocol. Summary of data collection time points

Parameters		Non-Contact	Contact
Middle Cerebral Artery Blood Flow Velocity (cm/s)			
	Pre-Impacts	75.31±21.7	74.54±19.8
	Post-Impacts	75.54±23.01	80.06±22.1
	p value	0.99	***0.01**
Pulsatility Index Middle Cerebral Artery (a.u.)			
	Pre-Impacts	1.03±0.2	1.1±0.1
	Post-Impacts	0.97±0.3	0.84±0.13
	p value	0.69	***0.0002**

Table 3 TCD Neuroimaging

Results presented as mean ± SD. Bold-faced values show statistical significance (p<0.05)

Brain Regions		Non-Contact		Contact	
		Single Task	Dual Task	Single Task	Dual Task
Left Prefrontal Cortex	Pre-Impacts: Post-Impacts: p value	0.35±0.2 0.49±0.2 p = 0.45	0.53±0.3 0.58±0.3 p = 0.98	0.42±0.1 0.6±0.2 ***p=0.04**	0.46±0.14 0.66±0.12 ***p=0.01**
Left Motor Cortex	Pre-Impacts: Post-Impacts: p value	0.37±0.16 0.44±0.15 p = 0.11	0.61±0.35 0.66±0.3 p = 0.99	0.42±0.2 0.62±0.25 p=0.15	0.46±0.13 0.73±0.26 ***p=0.04**
Right Prefrontal Cortex	Pre-Impacts: Post-Impacts: p value	0.49±0.54 0.53±0.3 p=0.99	0.43±0.25 0.52±0.25 p=0.73	0.44±0.14 0.6±0.18 p=0.15	0.49±0.21 0.75±0.2 ***p=0.02**
Right Motor Cortex	Pre-Impacts: Post-Impacts: p value	0.36±0.18 0.4±0.16 p=0.83	0.36±0.15 0.49±0.2 p=0.72	0.41±0.1 0.48±0.2 p=0.69	0.44±0.12 0.68±0.26 p=0.12

(p<0.0

Figure 4 Blood Flow Velocity. A) Difference in blood flow velocity, pulsatility index, and heart rate from pre- to post measurement for the non-contact and contact sessions, B) Post-measurements of blood flow velocity for 7 subjects following contact with correlation to angular acceleration

Figure 5 fNIRS Concentration Changes. A) Comparison of O2Hb concentration during pre- and post-measurements for non-contact and contact training in the left prefrontal cortex during the single task period (left) and the dual task period (right).

Table 4 fNIRS Neuroimaging