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Cerebrovascular reactivity alterations due to subconcussive repetitive head trauma in asymptomatic high school football players

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CEREBROVASCULAR REACTIVITY ALTERATIONS DUE TO SUBCONCUSSIVE REPETITIVE HEAD TRAUMA IN ASYMPTOMATIC HIGH SCHOOL FOOTBALL PLAYERS

A Thesis

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To my Parents

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I thank my family, Mom, Dad, Grandma for their blessings. Whatever I have been able to achieve to date, is the result of constant support and motivation from my parents. Thank you mom and dad for making me what I am today.

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LIST OF ABBREVIATIONS

CVR	Cerebrovascular Reactivity
mTBI	mild Traumatic Brain Injury
BH	Breath Hold / Breath Holding
fMRI	functional Magnetic Resonance Imaging
CTE	Chronic Traumatic Encephalopathy
CBF	Cerebral Blood Flow
BOLD	Blood Oxygen Level Dependent
MR	Magnetic Resonance
HDR	Haemodynamic Response
PLA	Peak Linear Acceleration
HIT	Head Impact Telemetry
GM	Grey Matter
WM	White Matter
LOC	Loss of Consciousness
RMSE	Root Mean Squared Error

ABSTRACT

Joshi, Chetas. M.S.E.C.E., Purdue University, May 2015. Cerebrovascular Reactivity Alterations due to Subconcussive Repetitive Head Trauma in Asymptomatic High School Football Players. Major Professor: Thomas Talavage

Chronic neurological damage as a result of chronic repetitive head trauma is a major concern for football athletes today. Repetitive concussions have been linked to many neurological disorders. Recently, it has been reported that repetitive subconcussive events can contribute to long-term neurodegeneration. For these reasons, it is important to understand the effect repetitive subconcussive head trauma has on brain health in young athletes. Past research has demonstrated that cerebrovascular reactivity (CVR), an important mediator of cerebrovascular regulation, is impaired following mild traumatic brain injury (mTBI). This impairment increases susceptibility to secondary injury following mTBI. In this study, Breath-Hold (BH) task based functional Magnetic Resonance Imaging (fMRI) was used to track CVR changes in asymptomatic high school football athletes across three competition seasons. Athletes in the first competition season had higher exposure to head impacts than the athletes during the second and the third seasons. Baseline scans were acquired before the start of the season, and follow-up scans were obtained during and after the season to track the potential changes in CVR as a result of experienced trauma. Noncollision-sport athletes were scanned over two sessions as controls during the first and third competition season. CVR decreased significantly in football athletes during the first half of their season in the first completion season but not in any other competition seasons. Controls did not show any significant changes in CVR. The results suggest that athletes getting higher exposure to head impacts in short duration of time drives cerebrovascular changes that may place athletes at higher risk of getting injured. These results also indicate that the brain may not be able to adapt quickly to

abrupt increases in contact activity (as associated with the beginning of practice and competition), transiently increasing risk for symptomatic injury.

1. INTRODUCTION

1.1. Motivation

Concussion in athletes involved in collision sports is very common and Football ranks first in both the number of participants as well as the incidence of head injuries in the United States [1]. Chronic brain damage resulting from repetitive head trauma is a major concern for the American Football athletes. A study conducted in 1999 showed that football accounted for 65% of all high school sports-related concussions [2]. The observation of chronic traumatic encephalopathy (CTE) in NFL retirees without history of diagnosed concussion [3-5] has shifted the attention toward the repetitive subconcussive blows. Discussion in the neuropathology community has implicated repetitive sub-concussive events as a significant source of accrued brain damage [3, 7]. In addition to that, some recent studies [6] have reported neurophysiological changes in high school football players without observable symptoms of concussion. Together, these studies show that it is important to understand the effects sub-concussive repetitive head trauma has on the brain starting at an early age.

Cerebral Blood Flow (CBF) is essential for the maintenance of the neuronal activity. An important mediator of cerebrovascular regulation is cerebrovascular reactivity (CVR) to CO2, a compensatory mechanism where blood vessels dilate in response to hypercapnia to regulate CBF. Animal studies [8] and human studies [9, 10], have demonstrated CVR reduction following mTBI. Also, there is mounting evidence that CVR reduction plays a significant role in the evolution of mTBI sequelae [8]. To date, many studies using neurocognitive and neurophysiological testing have been conducted but no CVR studies have been conducted on asymptomatic athletes subjected to repetitive sub-concussive head trauma nor have studies been conducted on young athletes. The neural activity leads to changes in CVR which can be tracked by Blood Oxygen Level

Dependent (BOLD) signal using fMRI technique. In this work, CVR in asymptomatic high school football players was tracked for 3 consecutive seasons throughout the competition period using functional magnetic resonance imaging (fMRI) with a hypercapnic breath hold task [11]. Athletes involved in non-collision sports (controls) were also studied so that CVR changes in football athletes could be compared to CVR changes in controls who did not receive repetitive head trauma but were still physically active.

Recent research indicated significant relationships between the number of blows sustained by a subject and the ensuing neurophysiological change [12]. In this work, an attempt had been made to find out the correlation between the exposure to the blows (Helmet-sensor impact data) and CVR changes observed using the brain-imaging scans performed before, during and after each season.

2. BACKGROUND

2.1. mTBI (mild Traumatic Brain Injury)

The hallmark of mTBI is the presence of functional symptoms after a head collision event despite no identifiable structural injury [13]. Because diagnosis is based on symptoms, the definition of mTBI has evolved over time. Before 1999, mTBI was typically defined as an incident involving loss-of-consciousness (LOC) [1]. After the emphasis on LOC was removed, it was hypothesized that symptom severity was correlated with symptom resolution leading to the adaptation of mTBI grading scales. The grading scales still focus primarily on the presence of LOC and amnesia to define the various grades of mTBI; athletes with no LOC are often allowed to return to play in the same game [14-15]. Research has now shown symptom severity to be a poor predictor of symptom resolution showing that "mild concussions" show neurocognitive deficits on par with those of "moderate and severe concussions" [14-15]. As such, there has been a shift away from grading of mTBI [16]. At the forth International Conference on Concussion in Sport mTBI was defined as "a complex pathophysiological process affecting the brain, induced by traumatic biomechanical forces" where "clinical symptoms largely reflect a functional disturbance rather than structural injury" resulting in "a set of clinical syndromes that may or may not involve loss of consciousness" [28]. According to this definition, the clinical symptoms have to be present in mTBI. In addition to that, most clinicians still believe that in the absence of the clinical symptoms there is no problem. Recent research (PNG: Purdue Neurotrauma Group) have shown neurophysiologic changes and changes in functional connectivity due to sub-concussive repetitive trauma [6, 34]. Along the same lines, the work presented in this document has identified the presence of neurophysiologic impairments in the absence of overt neurocognitive symptoms, suggesting the existence of subconcussive mTBI by observing CVR changes.

For purposes of this document, symptomatic mTBI will be referred to as “concussion” while the term “subconcussive” will be used to address impairments found in the absence of symptoms.

2.2. fMRI (functional Magnetic Resonance Imaging)

fMRI is the medical imaging technique used to measure the rapid delivery of blood (Haemodynamic Response) to the active neuronal cells in relation to the neural activities [21]. The functional imaging signal used in fMRI is the Blood Oxygenation Level Dependent (BOLD) signal, magnitude of which depends on the concentration of deoxyhemoglobin [22]. Neuronal activities and CBF are coupled. In the region of the brain where there is an increase in the neural activity, there is also an increased cerebral blood flow. This increase in CBF produces an increase in the ratio of oxygenated hemoglobin relative to deoxygenated hemoglobin in that specific region. The difference in magnetic properties of oxygenated and deoxygenated hemoglobin is what generates BOLD contrast and provides the information about active neurons and the inactive neurons. In the area of increased neuronal activity, there is an improved Magnetic Resonance (MR) signal due to the presence of diamagnetic blood (oxyhemoglobin) which interferes with the MR signal less. However, Paramagnetic blood (deoxyhemoglobin) causes destructive interference in the MR signal by dephasing the local magnetic field which leads to less signal in the areas of less or no neuronal activities. Thus, fMRI can be used to measure the functionality of the brain. mTBI/Subconcussive mTBI is about the detection of the functional disturbance. Hence, fMRI can be used to detect the presence of mTBI/subconcussive mTBI.

2.3. CVR (Cerebrovascular Reactivity) and the BH Task

Metabolic activities in neurons and CBF are tightly coupled and an increased metabolic demand is usually linked with increased CBF. CVR is a compensatory mechanism where blood vessels dilate in response to changes in the partial pressure of CO2 to regulate CBF. Dilation of blood vessels in response to CO2, leads to increased blood flow which leads to an increase in the BOLD response that can be measured using fMRI. Studies [9-10] have shown that CVR is impaired following mTBI. There are

several methods and procedures out there to assess CVR in healthy and diseased subjects. Breath-hold (BH) induced Hypercapnia (increased levels of CO_2) is a reliable method and it provides repeatable results in measuring CVR [17-18]. Although even short breath-holds (3 seconds) result in measurable BOLD responses, with an increase in the duration of the BH, the number of voxels exhibiting significant BOLD signal changes is increased [29]. Also, longer breath-holds offer more robust and repeatable BOLD responses across scanning sessions spanning weeks [30]. Research have shown that BH for 20 ~ 30 seconds of durations are acceptable and they provide an optimal response [19-20]. In this work, a hypercapnic BH (duration: 20 sec) task separated by paced breathing was used to assess the effect subconcussive repetitive head trauma has on CVR. BH challenges were separated by paced breathing as it provides a more consistent baseline condition for the extraction of the BH signal, reducing the inter-trial variability [31]. In this work, end-expiration BH technique was used since studies have suggested that end-expiration BHs result in greater reproducibility of CVR measures than end-inspiration BHs because end-expiration offers a more natural resting equilibrium state before the initiation of BH challenge [31, 35].

3. METHODS

3.1. Participants

Forty eight male high school football athletes were recruited (ages 14-18; average = 16.7) from 3 local high schools (HS1, HS2 and HS3) across three competition seasons (Season 4: 2012-13, Season 5: 2013-14 and Season 6: 2014-15). In addition 24 non-collision sport controls (ages 14-18, average = 16.65) were also studied from 2 different high schools across 2 competition seasons. Participants were not excluded on the basis of prior concussion and no included participant was diagnosed with a concussion during the study period of the competition seasons. During S4, along with 16 male high school football athletes from 2 high schools (HS1 and HS2), 10 controls involved in non-collision sports were studied. During S5, 12 male football athletes from HS1 were studied. During S6, along with 20 male football athletes from 2 high schools (HS2 and HS3), 14 controls were studied.

All athletes underwent fMRI scanning sessions prior to the onset of the contact practices (*Pre-season/baseline/reference)*, within the first 6 weeks (*In-season1*) of the contact/competition season and within the second 6 weeks (*In-season2*) of the contact season. During S4 and S5, the athletes underwent additional scanning 5-6 months after the end of the contact season (*Post-season2*). All the controls (S4 and S6) underwent two sessions of fMRI scanning (*baseline*/follow-up) 4-6 weeks apart during periods of comparable physical activity. During S5 and S6, the athletes underwent scanning 1-2 months after the end of the contact season (*Post-seasont1)*. Head collision events, during both games and practices, were monitored using Head Impact Telemetry (HIT) System during S4 and S5 and using xPatch (X2 Biosystems) during S6. The HIT System has been validated for counting hits and estimating the Peak Linear Acceleration (PLA) magnitude [23-24] (in multiples of the gravitational constant [Gs]) but has been observed

to provide inaccurate rotational acceleration data (rad/s^2) [12]. The RMSE (Root Mean Squared Error) of LA (Linear Acceleration) estimated using HIT system was also observed to be very high (above 900%) for some of the locations of the sensors on the helmet. Therefore xPatches were used during S6.

3.2. Imaging and Data Acquisition

fMRI with a hypercapnic breath-hold task was used to measure CVR in asymptomatic high school football athletes and non-contact sport controls. Imaging was performed on a 3T Signa HDx (General Electric; Waukesha, WI) with a 16-channel brain array (Nova Medical; Wilmington, MA). One breath-hold fMRI run (4 breath holds, 20 seconds duration each separated by paced breathing: inhalation:4 seconds, exhalation:4 seconds) was acquired in each scanning session using a gradient-echo echo planar sequence (TR/TE = 1500/26 msec; 20cm FOV; 64 × 64 matrix; 34 slices; 3.8mm thickness; 117 volumes). The task was cued visually using PsychoPy software with instructions presented via a fiber optic-goggle system (NordicNeuroLab, Bergen, Norway). A respiratory belt was used to monitor task compliance. A T1-weighted anatomical was acquired using a 3D spoiled gradient echo sequence (TR/TE 5.758ms/2.032ms, flip angle=73°, 1mm isotropic resolution).

3.3. fMRI Data Processing

fMRI data were pre-processed using AFNI, including slice timing correction, motion correction, spatial smoothing, alignment to the structural scan, normalization to Talairach space, and conversion to percent signal change. Additionally, the FAST automated segmentation tool in FSL was used to create a grey matter (GM) mask from the talairach template. CVR in white matter (WM) is significantly lower than that in the GM [25] (Most of the blood vessels carrying blood to the neuronal tissues pass through GM voxels) and hence CVR was observed only in the GM voxels.

CVR values measured using the GM mean fMRI time series have been observed to achieve significant repeatability across different BH duration scans [35]. So, mean time series were calculated in the GM as a whole and in the frontal, occipital, parietal and temporal lobes (GM only during S4). The onset times of each task (inhalation, exhalation

and BH) were found using the belt data processing algorithm. The onset times were shifted by 9s to account for hemodynamic delay [26-27] (maximum correlation between BOLD signal change and task was found at this delay) and were used to calculate the durations of inhalation, exhalation, and breath-holds. These timing files (onset times and durations) were used for regression against the mean time series from the fMRI data. The weight of the BH regressor was used as the metric for CVR (B-weight, units %BOLD).

3.3.1. Respiratory Belt Data Processing

To make the mapping between the fMRI data and respiratory belt data precise, an automated algorithm was developed to calculate scanning session- and subject-specific task timing from the respiratory belt data signal, for regression against each subject's GM fMRI time series. The proprietary algorithm implemented low pass filtering, peak picking, and thresholding of waveform length to calculate onset times and durations for each regressor (inhalation, exhalation, breath hold) and generated timing files. The signal was passed through a Hamming-window based, linear-phase low pass FIR (Finite Impulse Response) filter so that the highest frequency that is passed was 0.14 Hz corresponding to T (time period) = 7.14 seconds (which corresponds to a sinusiodal wave produced by inhalation and exhalation) and so that all the high frequency noise is removed. The Figure 3.1 shows the filtered respiratory belt data signal. Next, a peak detection algorithm was implemented to get all the local maximas and local minimas from the filtered data. The local maximas represent the beginning of the inhalation and local minimas represent the beginning of the exhalation (Figure 3.1). To detect the boundary between inhalation/exhalation and breath-hold, a sliding window of N = 160 samples (equivalent to 4 seconds; corresponding to inhalation/exhalation duration) based algorithm was developed that used waveform length as a feature to segregate inhalation/exhalation from breath-hold. Waveform length (WL) as defined in equation (1) was determined by measuring the cumulative changes in amplitude from time sample to time sample over the entire time period of a window.

$$WL = \sum_{n=1}^{N-1} |x_{n+1} - x_n| \quad (1)$$

The waveform length is quite high for the inhalation/exhalation (sinusoidal) region as compared to the breath-hold region (almost flat) of the signal. A threshold of 200 on the waveform length was found to give a clear identification of the boundary and hence was used. Research has shown that BOLD CVR can be mapped using breath-holds, with high inter- and intra-subject repeatability, despite variations in duration and consistency of BH performance [35]. In that research [35], BHs of durations 10 ~ 20 seconds were assessed. In addition, long BHs offer repeatable BOLD response [30]. So, Breath-holds having duration above 11 seconds were considered proper; others were removed. Now, the local maximas and the local minimas identified by the peak detection algorithm that appeared in the breath-hold region were removed. The onset times of inhalation, exhalation and breath-holds were then used to find the durations of each inhalation, exhalation and breath-hold. Certain thresholds were also applied on the durations as well as the change in the amplitude of the signal over a time period (slope) to remove the spurious onset timings. At the end, the onset times were shifted (i) backward by 1.25 seconds (50 sample points) to compensate for the shift (group delay) introduced by the linear-phase FIR filter and (ii) forward by 9 seconds to incorporate the delay in the hemodynamic response. Figure 3.2 shows a complete process implemented in the algorithm. This algorithm provided a robust mechanism to track the task compliance and find out the precise locations and durations of the tasks (inhalation, exhalation and breath-hold). A Matlab code was written implementing the algorithm which took the respiratory data files as the input and output the timing files having onset times with durations.

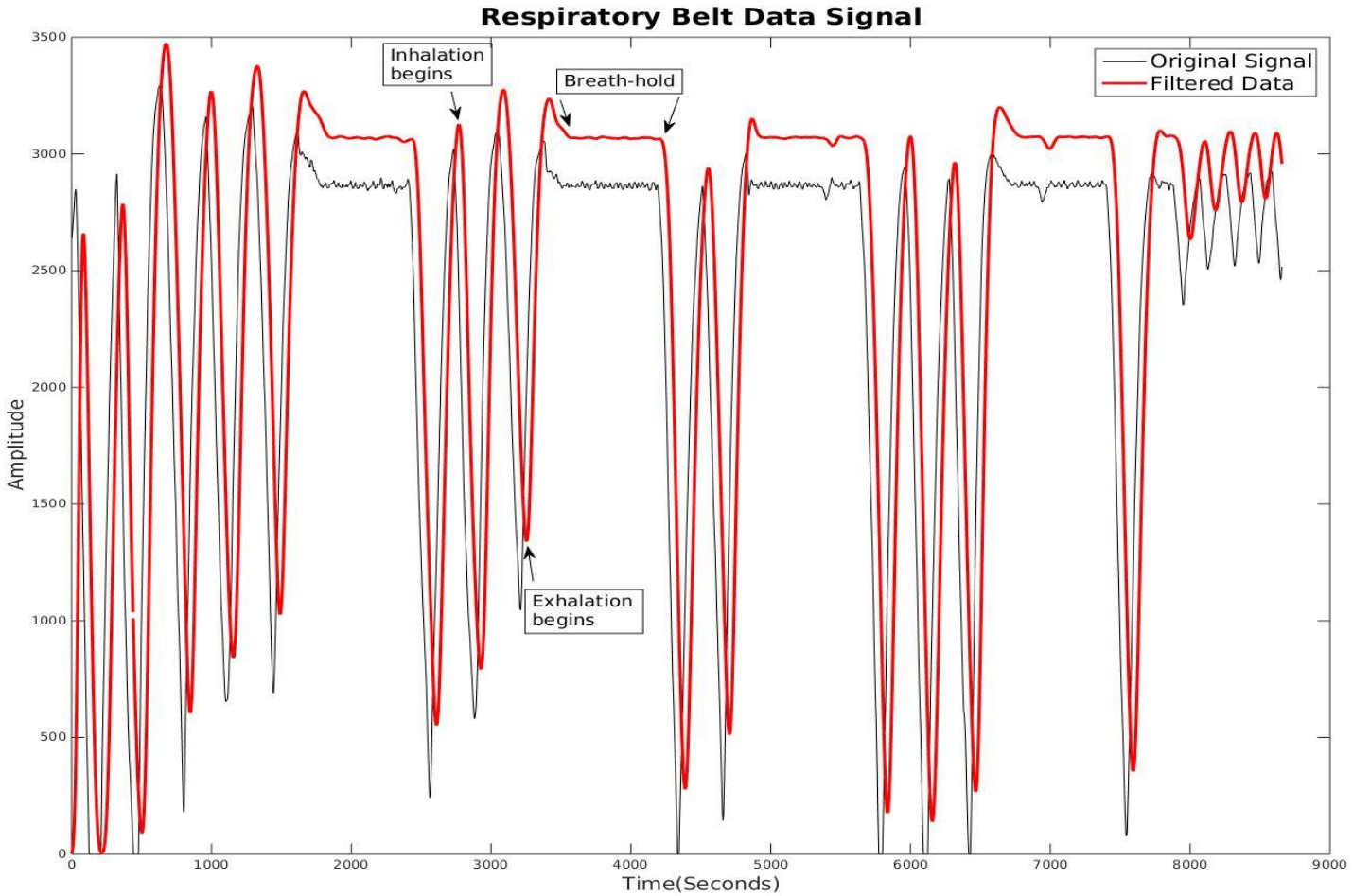

Figure 3.1 Filtered respiratory belt data signal showing regions of inhalation, exhalation and breath-hold

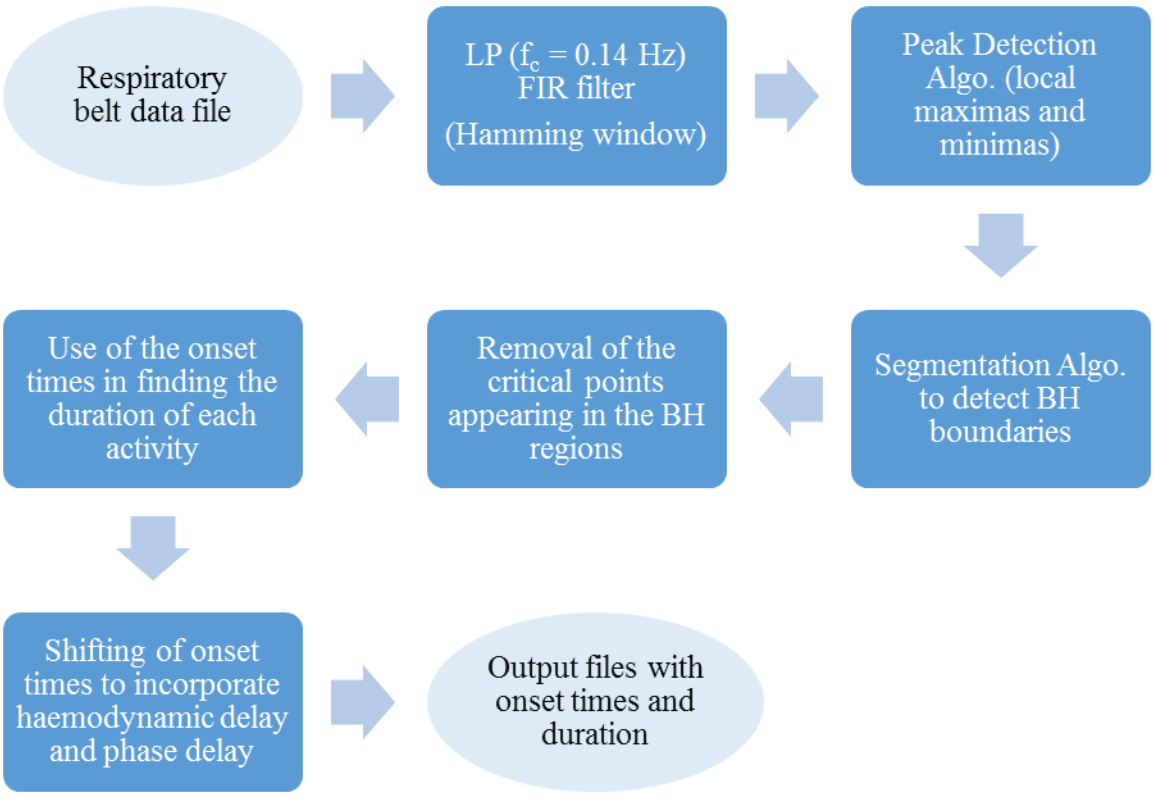

Figure 3.2 Flowchart of respiratory belt data processing algorithm

The algorithm was tested on all the football athletes' and controls' data and exhalations and inhalations were modelled as single BLOCK regressor of duration equal to 4 seconds. However, due to large variance in intra-subject breath hold durations, holds were modeled as different stimulus of the same class, using the dmBLOCK (duration modulated BLOCK) response model in AFNI.

In S4, the B-weight was found in GM as a whole as well as in 4 different lobes. However, in S5 and S6, the task time series were used for regression against the mean time series of fMRI data only in the GM as a whole since it was observed in S4 that athletes exhibit global changes in all the GM voxels irrespective of the lobe. The weight of the breath-hold regressor was used as the metric for CVR (B-weight, units %BOLD).

3.4. Analysis of fMRI Data

For all tests, a significance level of 0.05 was used.

3.4.1. CVR Deviation from Reference/Baseline

For S4, to assess whether CVR in control or football athletes deviated from reference during or after the season, the distributions of breath-hold B-weights from the GM regression of each follow-up scan (in-/*post-season*) was compared to the distribution of the breath-hold B-weights from the GM regression of the reference scan (baseline or pre-season) using a Wilcoxon Signed Rank Test (non-parametric alternative to paired t-test). For sessions showing significant deviation from reference, additional Wilcoxon Signed Rank Tests were conducted using the distributions of the B-weights from each lobe (Frontal, Occipital, Parietal and Temporal) to test whether the deviance observed in GM was localized in a particular lobe.

Since the athletes exhibited global changes in all the GM voxels during S4, for S5 and S6, the distributions of the breath-hold B-weights from GM as a whole for each follow-up scan were compared to the distribution of the breath-hold B-weights from GM as a whole of reference scan using Wilcoxon signed rank test.

During S4, only those athletes were brought back for follow-up scanning sessions who were observed to experience high cumulative loading. In S5 and S6, all the football athletes were scanned irrespective of their exposure to head impacts. S5 and S6 football athletes went through less contact practices than the S4 athletes. So, S4 athletes were analyzed separately from S5 and S6 athletes. S5 athletes were found having significantly lower cumulative hits per week values than the S6 athletes belonging to the top 50% of the pool during *in-season1* (Figure 4.6). Hence, S5 athletes were studied separately from S6 athletes. Overall, athletes in different competition seasons were analyzed separately.

3.4.2. Subject-wise CVR Deviation

Since the controls did not show significant difference between sessions neither during S4 nor during S6, a giant pool of controls was made by combining the controls from S4 and S6. To compare the CVR deviation at different follow-up sessions during different competition seasons, GM B-weight at *baseline* was subtracted from the GM B-

weight at each follow-up session for every football athlete and control. The difference was termed "ΔB-weight ($B_{session} - B_{reference}$)". Box and whisker plots were drawn for the distributions of ΔB-weights for all the follow-up sessions.

3.4.3. Comparison: Baseline for Football Athletes vs Baseline for Controls

Significant increase in CVR at *post-season2* in S5 engendered interest in knowing at what level the *baseline* would have stabilized for the football players as compared to the controls before the beginning of a new season. Hence, the distribution of GM B-weights at *pre-season* for football athletes was compared to the distribution of GM B-weights at *baseline* for controls for all the competition seasons using Wilcoxon Rank Sum test.

3.5. Analysis of fMRI Data based upon HIT system/xPatch Data

Significant CVR decrease at *in-season1* compared to *pre-season* in S4 but not in S5 and S6 led me to compare the hits that the athletes took until *in-season1* scanning date during 3 competition seasons. It was hypothesized that the accumulation of hits affect the CVR. The HIT system data (S4 and S5) and xPatch data (S6) were used to find out the cumulative hit count for each athlete until the *in-season1* scanning date. The distribution of cumulative hits of each competition season was compared to the distribution of the cumulative hits of every other competition season using Wilcoxon rank sum test. No competition season was found significantly different from any other competition season. Hence, in S4 and S6, for each competition season, athletes were sorted in descending order of their cumulative hits and the pool was divided into 2 groups (i) athletes in top 50% of the pool (ii) athletes in bottom 50% of the pool. Due to a small sample size in S5, no grouping was done in S5. The distribution of cumulative hits for each group was compared to the distribution of cumulative hits of every other group. The distribution of B-weights at *in-season1* was compared to the distribution of B-weights at *baseline* for each group using Wilcoxon signed rank test.

As the cumulative hit count could not explain the CVR decrease in S4 completely, frequency of hits (cumulative hits per week) was used as a feature. The distribution of cumulative hits per week of each competition season was compared to the distribution of the cumulative hits per week of every other competition season using Wilcoxon rank sum

test. No competition season was found significantly different from any other competition season. Hence, again the S4 and S6 athletes were divided into two groups based upon their cumulative hits per week values and the analysis was done the same way as it was done when cumulative hits was used as the feature.

4. RESULTS

4.1. Analysis of fMRI Data

Controls (S4) exhibited no significant difference between sessions. In S4, football players exhibited significant CVR deviation from *pre-season* at *in-season1* ($p < 0.03$) but not at *in-season2* (Figure 4.1A) and all the lobes were found significantly deviant at *in-season1*. In addition to that, a significant CVR deviation was also found at *post-season2* ($p < 0.05$), relative to the *pre-season* measure (Figure 4.1A). The Line plots revealed a decrease in CVR (relative to *pre-season*) in 11 of 16 subjects at *in-season1* (Figure 4.1B) and in 12 of 16 subjects at *post-season2* (Figure 4.1C).

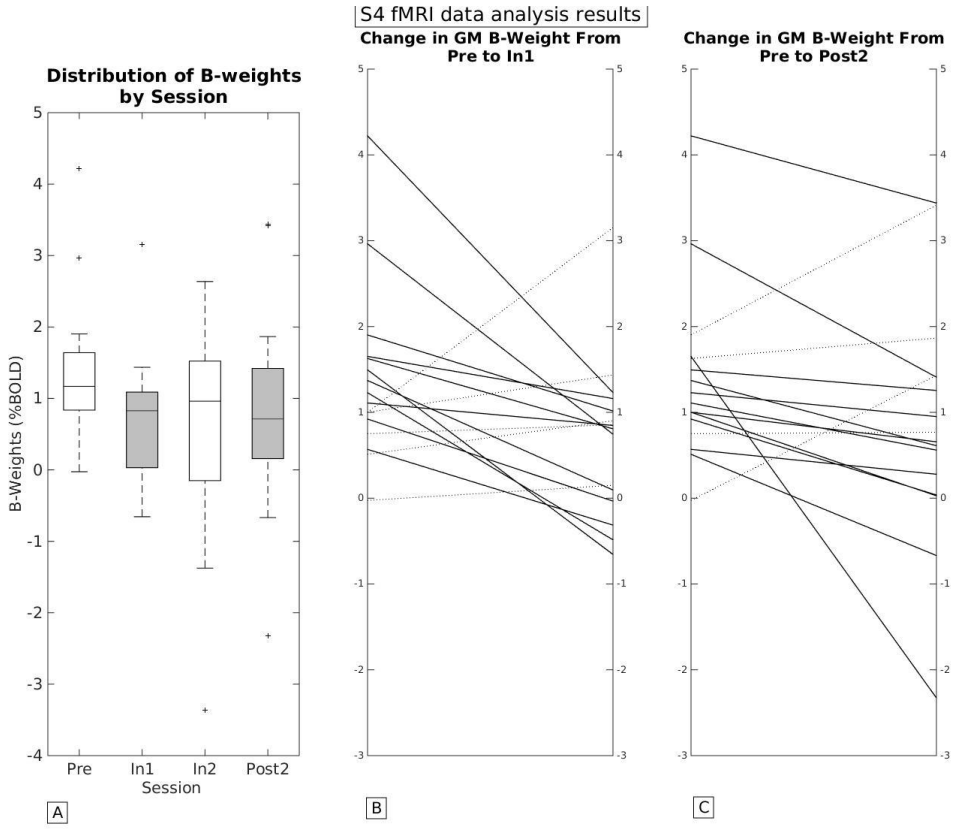

Figure 4.1 (A) Box and whisker plots of the GM B-weight distributions for S4 at *pre-season*, *in-season1*, *in-season2*, and *post-season2*. Outliers in each distribution are indicated by (+) and sessions deviating significantly from *pre-season* are indicated by shading. (B) Change in GM B-weight from *pre-season* to *in-season1*. (C) Change in GM B-weight from *pre-season* to *post-season2*. Solid lines in (B) and (C) show players who exhibited a decrease in B-weight from *pre-season* while dotted lines indicate players who exhibited no change or an increase from *pre-season*.

In S5, the football players exhibited significant CVR deviation from *pre-season* only at *post-season2* ($p < 0.03$) (Figure 4.2A). The Line plots revealed a significant increase in CVR (relative to *pre-season*; Figure 4.2B) at *post-season2*.

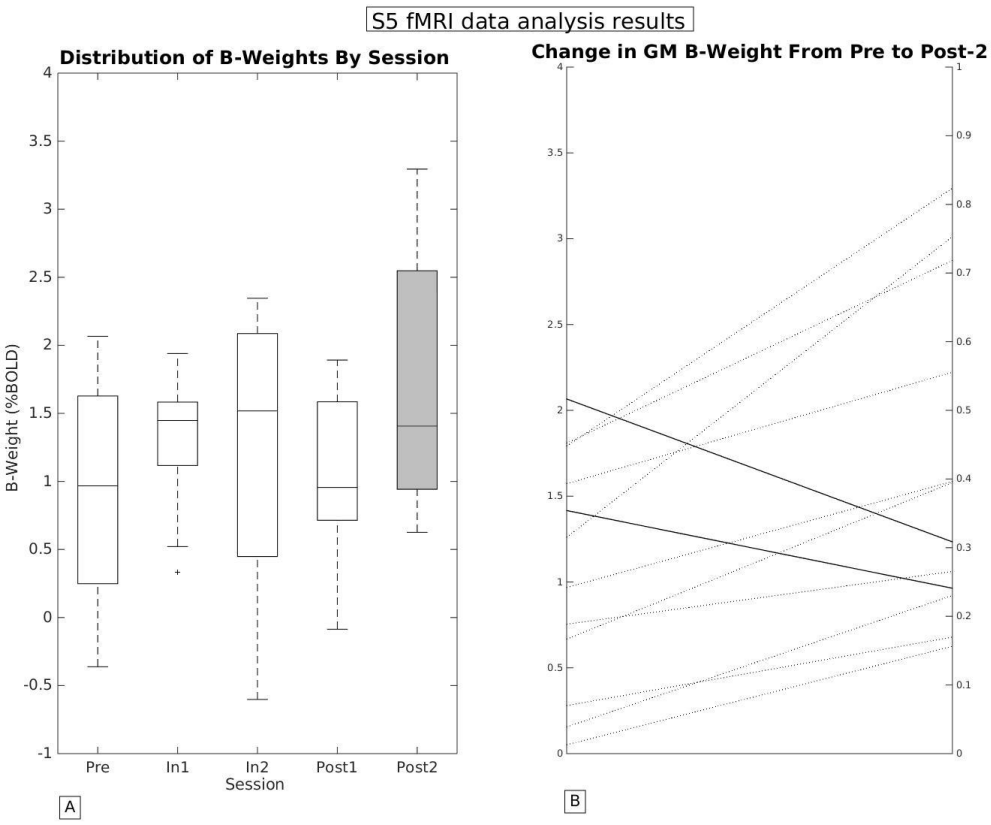

Figure 4.2 (A) Box and whisker plots of the GM B-weight distributions for S5 at *pre-season*, *in-season1*, *in-season2*, *post-season1* and *post-season2*. Outliers in each distribution are indicated by (+) and sessions deviating significantly from *pre-season* are indicated by shading. (B) Change in GM B-weight from *pre-season* to *post-season2*. Solid lines show players who exhibited a decrease in B-weight from *pre-season* while dotted lines indicate players who exhibited no change or an increase from *pre-season*.

In S6, controls did not show any significant difference between sessions. The football players exhibited no significant CVR deviation from *pre-season* at any of the follow-up sessions as well.

The subject-wise deviation in CVR from reference for the controls (S4 and S6 combined) was not found significant with a median equal to zero (Figure 4.3). The distributions of ΔB-weights were significantly different (lower) from zero for S4 *in-season1* and *post-season2* sessions. The distribution of ΔB-weights was found significantly different (higher) from zero for S5 *post-season2* session (Figure 4.3).

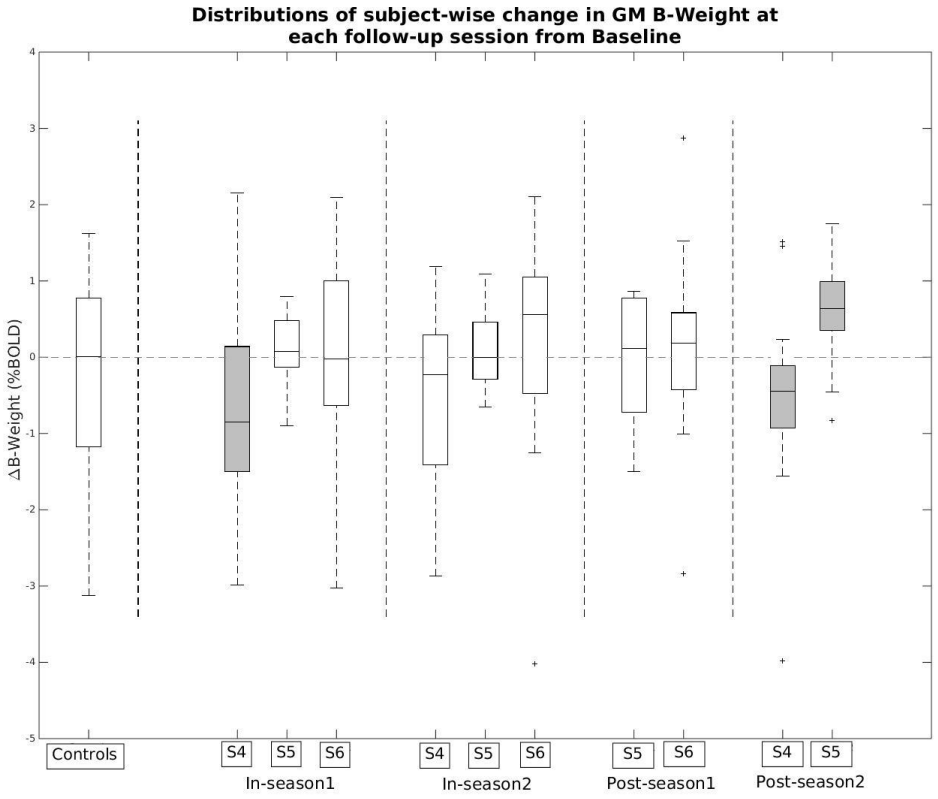

Figure 4.3 Box and whisker plots of the GM ΔB-weights distributions for controls and football players at all the follow-up sessions for all competition seasons. Outliers in each distribution are indicated by (+).Outliers in each distribution are indicated by (+) and distributions deviating significantly from zero are indicated by shading.

The comparison of *pre-season* distribution of B-weights for football athletes with that of the controls revealed that the *baseline* for S4 was significantly higher ($p < 0.05$) than the *baseline* of controls (Figure 4.4). The baselines of S5 and S6 were not significantly different from the baseline of controls but the medians were higher than the median for controls (Figure 4.4).

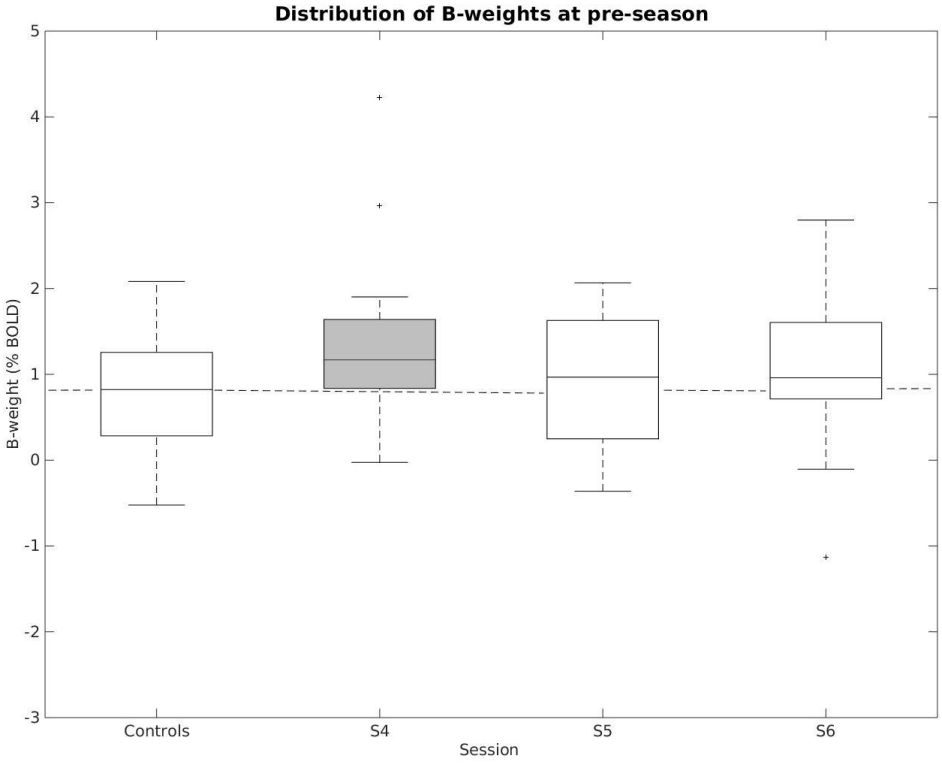

Figure 4.4 Box and whisker plots of GM B-weight distributions during *pre-season* for controls and S4, S5 and S6 football athletes. Outliers in each distribution are indicated by (+) and sessions deviating significantly from *pre-season* of controls are indicated by shading.

4.2. Analysis of fMRI Data based upon HIT system/xPatch Data

4.2.1. Feature: Cumulative Hit Count

No significant difference in the cumulative hits until *in-season1* was found between 3 competition seasons. However, after dividing S4 and S6 athletes in top 50% and bottom 50% based upon the cumulative hit count, the cumulative hits taken by S4 top 50% were found significantly different (higher) from those taken by S5 athletes ($p < 0.05$) (Figure 4.5). The cumulative hits taken by S4 top 50% athletes were still not significantly different from those taken by S6 top 50% athletes but they were higher in amount for S4

top 50% (Figure 4.5). Comparison of GM B-weights revealed that CVR was not significantly different at *in-season1* from *baseline* for any of the groups however, athletes in top 50% of the pool in S4 showed a decrease in CVR at *in-season1* with $p < 0.08$.

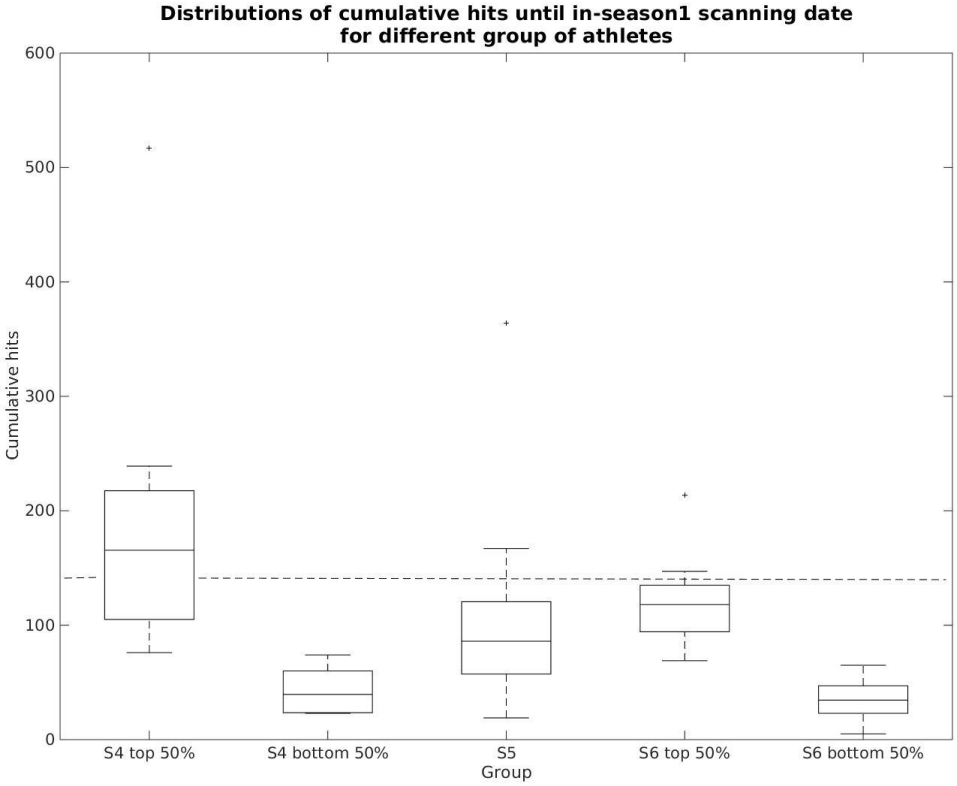

Figure 4.5 Box and whisker plots of cumulative hit distributions until *in-season1* for S4, S5 and S6 football athletes. Outliers in each distribution are indicated by (+).

4.2.2. Feature: Cumulative Hits per Week

No significant difference in the cumulative hits per week until *in-season1* was found between 3 competition seasons. Distribution of cumulative hits per week of S5 athletes was found to be significantly different (lower) from that of top 50% athletes in S4 ($p < 0.04$) and from top 50% athletes in S6 ($p = 0.04$). Cumulative hits per week of S4 athletes in top 50% of the pool were not significantly different from those of S6 athletes in top 50% of the pool; however, in S4, 5 out of 8 athletes belonging to top 50% of the pool were exposed to more than 38 hits per week whereas only 1 out of 9 athletes belonging to

top 50% of the pool in S6 were exposed to more than 38 hits per week (Figure 4.6). Comparison of B-weights revealed that there was a significant decrease ($p < 0.03$) in CVR at *in-season1* from *baseline* for the S4 athletes belonging to the top 50% of the pool (Figure 4.7A); however, S6 athletes in top 50% of the pool did not show a significant decrease in CVR at *in-season1*. Seven out of 8 athletes belonging to the top 50% of the pool in S4 exhibited a decrease in CVR at *in-season1* from *pre-season* (Figure 4.7B).

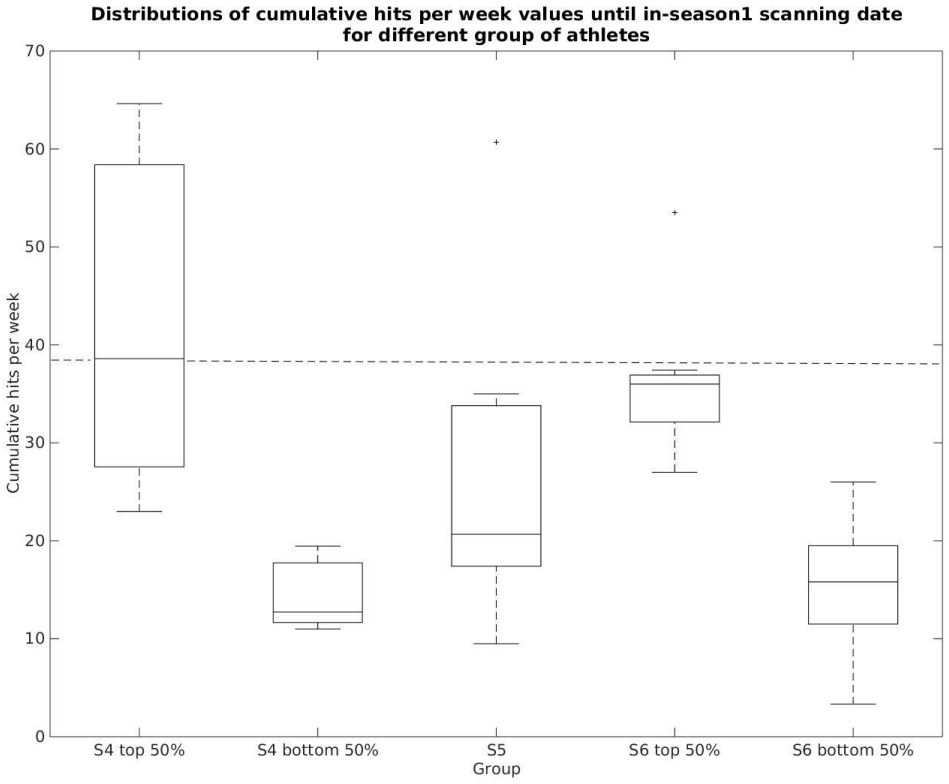

Figure 4.6 Box and whisker plots of distributions of cumulative hits per week until *in-season1* for S4, S5 and S6 football athletes. Outliers in each distribution are indicated by (+).

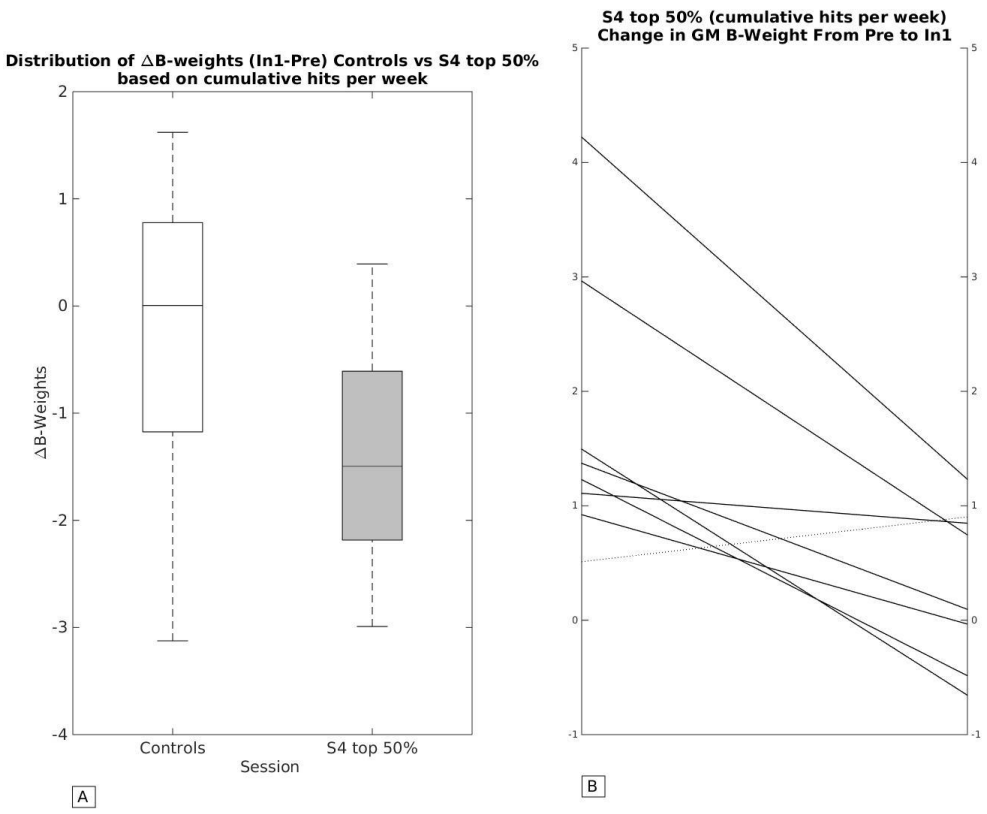

Figure 4.7 (A) Box and whisker plots of the GM ΔB-weight distributions (*in-season1 - pre-season)* for controls and the S4 athletes in top 50% of the pool based on cumulative hits per week value. Outliers in each distribution are indicated by (+) and sessions deviating significantly from *pre-season* are indicated by shading. (B) Change in GM B-weight from *pre-season* to *in-season1*. Solid lines in (B) show players who exhibited a decrease in B-weight from *pre-season* while dotted lines indicate players who exhibited no change or an increase from *pre-season*.

5. DISCUSSION

5.1. fMRI Data Analysis Results

Development of an automated algorithm for extraction of inhalation, exhalation and breath-hold task segments from respiratory belt data enabled enhanced detection of changes in cerebrovascular reactivity (CVR) in a large-scale study involving longitudinal assessment of football players, in comparison with non-collision sport controls. Controls did not exhibit global changes (GM as a whole) in CVR from baseline to follow-up while in S4, football players exhibited global changes in CVR following the onset of the season (first six weeks) that appeared to recover a bit in the latter part of the contact season (second six weeks). However, the global changes re-appeared sometime in the 5-6 months following the end of the football season. These findings indicate that a change in mechanical loading of the brain, caused by the onset of sub-concussive blows, has at least a transient effect on cerebrovasculature observed during *in-season1*. The changes at the *post-season* could perhaps be due to the participation of these athletes in additional collision sports (e.g., wrestling).

Unlike S4 football athletes, in S5, no significant CVR deviation from *pre-season* was observed during any part of the contact season. This suggests that the athletes did not go through sub-concussive blows sufficient enough to cause significant CVR alterations. The results are in sync with the HIT system data as the cumulative hit count and the cumulative hits per week values of the S4 athletes in the top 50% of the pool were found significantly higher than the cumulative hit count and cumulative hits per week values of the athletes in S5 respectively; making S5 athletes less vulnerable to head trauma. The significant increase in CVR during *post-season2* indicates a neurological repair mechanism overcompensating for the effect of the repetitive head trauma.

The football athletes in S6 participated in less contact practices as compared to S4 athletes and hence, the results showing no significant CVR changes at any of the follow-up sessions are as per the expectation.

The comparison of ΔB-weights (*in-season1 – reference* and *in-season2 - reference*) of the football athletes with the ΔB-weights of controls (follow-up – *baseline*) suggests that the athletes having higher exposure to the head blows experience reduction in CVR following the onset of the season (first six weeks) which starts recovering in the latter part of the contact season (second six weeks). This shows that it takes time for the brain to get adapted to the head impacts and hence, an abrupt increase in head impacts can cause CVR impairment.

The comparison of B-weights during *pre-season* revealed that the *baseline* of football players is higher than the *baseline* of controls which can be due to the head impacts the athletes have got exposed to during their whole football career. The adaptive mechanism had to shift the *baseline* upwards to compensate for the head impacts the athletes might take in the future. The significant upward shift in the case of S4 athletes suggests that S4 athletes might have been going through a lot of hit exposure during their football career as compared to the S5 and S6 athletes.

5.2. HIT system/xPatch (Head Impacts) Data based fMRI Data Analysis Results

The almost significant ($p < 0.08$) decrease in CVR at *in-season1* for S4 top 50% athletes having high cumulative hit count and not for any other group suggests that higher accumulation of hits can have worse effect on CVR. In addition to that, there is a threshold (unknown) on cumulative hits above which the CVR becomes impaired. The non-significant decrease (p-value is not less than 0.05) also suggests that it is not just the accumulation of hits that matters and that cumulative hits can't explain the reduction in CVR completely. There are other parameters like frequency of hitting, number of days of practice per week which can also affect CVR and should be studied.

When S4 athletes belonging to the top 50% of the pool based on cumulative hits per week values were studied, they showed a significant decrease in CVR at *in-season1* ($p < 0.03$). This suggests that higher frequency of hitting can lead to higher reduction in CVR. It also suggests that frequency of hitting affects CVR more than the cumulative hits. No

significant decrease in CVR at *in-season1* for S6 athletes belonging to the top 50% of the pool suggests that there is a threshold (could be 38 hits per week) on cumulative hits per week above which CVR becomes impaired.

5.3. Overall

Impairments in CVR have previously been observed after concussion [9-10, 32]. The link between impaired CVR and secondary injury [8] coupled with past evidence of increased risk for secondary injury subsequent to concussion [33] and the results of this study raise the concern that athletes may be at risk of incurring symptomatic injury when the brain is trying to adapt to the abrupt increase in the mechanical loading. It may be beneficial to make transitions in mechanical loading on athletes' brains more gradual/smooth (with gaps in between practice sessions big enough to recover from the recent history of head impacts) to reduce the risk of serious injury. Football teams typically switch from limited collision levels prior to the season to two practices per day, at least one of which includes contact. The brain may not be able to adjust to this transient change happening due to high exposure to the head impacts quickly enough, leaving players at increased risk for injury.

The results also question the belief that the brains of the athletes are healthy until they are diagnosed with a symptomatic injury. The presence of subconcussive mTBI in asymptomatic football players reveals neurophysiologic impairment in the absence of clinical symptoms. Hence, the presence of subconcussive mTBI should be studied in order to get the correct information about the health of the brain.

6. CONCLUSSION

Significant changes in CVR were observed in football athletes who were exposed to high number of head impacts in short period of time, relative to the football athletes who were not exposed that much and the non-collision controls, during the initial part of the football season. This suggests that abrupt increase in exposure to collision events produce neurophysiologic changes in the brain that (at least transiently) place athletes at greater risk for neurological injury.

6.1. Future Work

Along with cumulative hits and frequency of hits, the effect of other parameters like linear acceleration and rotational acceleration (xPatch) associated with the head impacts, recovery time period in-between two consecutive contact practices etc. could be studied in order to evaluate their effects on CVR alterations. Different mathematical modelling (Machine Learning) techniques could be deployed to map all the parameters to the CVR changes so that risk of getting concussed can be predicted and the high school athletes can be kept away from participating in more contact practices. Larger pool of high school athletes could be studied during each competition season so that more robust conclusions could be made from the results.

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