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Characterizing the effects of repetitive head trauma in female soccer athletes for prevention of mild traumatic brain injury

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CHARACTERIZING THE EFFECTS OF REPETITIVE HEAD TRAUMA IN FEMALE SOCCER ATHLETES FOR PREVENTION OF MILD TRAUMATIC BRAIN INJURY

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First, I dedicate this work to my husband who has always supported my dreams and has always been proud of my accomplishments. Second, I dedicate this work to my parents who have instilled in me the importance of reaching my full potential and who have sacrificed so much to get me where I am today. I hope they know how thankful I am for everything they have done. Finally, I dedicate this work to my son Santiago. Everything I do is for him.

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LIST OF ABBREVIATIONS

MRI	Magnetic resonance imaging
FMRI	Functional magnetic resonance imaging
mTBI	Mild traumatic brain injury
TBI	Traumatic Brain Injury
CVR	Cerebrovascular reactivity
RS	Resting state
CBF	Cerebral blood flow
DMN	Default mode network
PTA	Peak translational acceleration
nCPTA	Normalized cumulative peak translational acceleration
PAA	Peak angular acceleration
RCE	Relative Cumulative Exposure
GM	Gray matter
WM	White matter
CSF	Cerebrospinal fluid
DMN	Default mode network
VIS	Visual network
SM	Somatomotor network

FP	Frontoparietal network
DA	Dorsal attention network
VA	Ventral attention network
L	Limbic network
SUB	Subcortical network
CER	Cerebellar network
SPL	Shortest path length
MFPT	Mean first passage time
ROI	Region of interest
RSN	Resting state network

ABSTRACT

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As participation in women's soccer continues to grow and the longevity of female athletes' careers continues to increase, prevention of mTBI in women's soccer has become a major concern for female athletes as the long-term risks associated with a history of mTBI are well documented. Among women's sports, soccer exhibits the highest concussion rates, on par with those of men's football at the collegiate level. Head impact monitoring technology has revealed that "concussive hits" occurring directly before symptomatic injury are not predictive of mTBI, suggesting that the cumulative effect of repetitive head impacts experienced by collision sport athletes should be assessed. Neuroimaging biomarkers have proven to be valuable in detecting brain changes that occur before neurocognitive symptoms in collision sport athletes. Quantifying the relationship between changes in these biomarkers and the cumulative mechanical load experienced by female soccer athletes may prove valuable in developing measures to prevent mTBI.

This work pairs functional magnetic resonance imaging with head impact monitoring to assess changes in cerebrovascular reactivity and resting state functional connectivity in female soccer athletes and to test whether the observed changes can be attributed to the cumulative mechanical load experienced by female athletes participating in high school soccer both transiently over a season and chronically over several years of play. Marked cerebrovascular reactivity changes over a season of play were observed in female soccer athletes, relative both to non-collision sport control measures and pre-season measures. These changes persisted 4-5 months after the season ended and recovered by 8 months after the season. Segregation of the total soccer cohort into cumulative loading groups revealed that population-level cerebrovascular reactivity changes were driven by athletes experiencing high cumulative loads in short periods of time and that focusing on impacts 50g or higher helped increase identification of individuals with cerebrovascular reactivity decreases using head impact data. Resting state functional connectivity assessments revealed hypoconnectivity in soccer athletes as compared to non-collision sport control athletes. This hypoconnectivity was more pronounced in between network connections as opposed to within network connections. However, resting state functional connectivity was not altered over a season of play in soccer athletes, suggesting that the observed differences between soccer athletes and control athletes were due to long term exposure to chronic exposure to mild repetitive head trauma over several years of play.

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CHAPTER 1. INTRODUCTION

1.1 Specific Aims

As participation in women's soccer continues to grow and the longevity of female athletes' careers continues to increase, prevention and care for mTBI in women's soccer has become a major concern for female athletes as the long-term risks associated with a history of mTBI, including Alzheimer's disease and dementia, Parkinsonian syndromes, and depression (Guskiewicz et al., 2005; Guskiewicz, Marshall, et al., 2007; Jordan & Bailes, 2000) are well documented. Among women's sports, soccer reports the largest numbers of mTBI annually (Zuckerman et al., 2015) and has among the highest mTBI rates (Gessel, Fields, Collins, Dick, & Comstock, 2007), with rates slightly exceeding men's football at the collegiate level (Gessel et al., 2007). Head impact monitoring technology has revealed that "concussive hits" occurring directly before symptomatic injury are not predictive of mTBI, suggesting that the cumulative effect of repetitive head impacts experienced by collision sport athletes should be assessed. Neuroimaging biomarkers have additionally detected brain changes in collision sport athletes not exhibiting symptoms associated with concussion (Abbas et al., 2015; Bailes, Petraglia, Omalu, Nauman, & Talavage, 2013; Bazarian, Zhu, Blyth, Borrino, & Zhong, 2012; Marchi et al., 2013; McAllister et al., 2014; Poole et al., 2014; Poole et al., 2015; Robinson et al., 2015; Shenk et al., 2015; Svaldi et al., 2015; Talavage et al., 2014). These studies strongly suggest that the

effects of repetitive head trauma begin before the presence of overt neurocognitive symptoms and may accumulate to cause symptomatic injury.

If symptomatic mTBI is an injury caused by cumulative exposure to repetitive head trauma, then understanding the timescale of brain changes related to repetitive head trauma is key in developing measures to prevent both mTBI and long term sequelae associated with mTBI. However, no studies to date have conducted comprehensive longitudinal assessments, both acute and chronic, of changes in key neuroimaging biomarkers of mTBI and their relationship to cumulative exposure in asymptomatic collision sport athletes. Identification of biomarkers exhibiting acute changes in mTBI will prove useful in creating guidelines for moderating loading in games and practices over a season. Identification of biomarkers exhibiting more chronic changes will prove useful in assessing how repetitive head trauma affects the brain over a career of play and making recommendations related to appropriate lengths of collision sport careers.

One neuroimaging biomarker of particular interest in mTBI is cerebrovascular reactivity (CVR) to CO_2. CVR is a compensatory mechanism, wherein blood vessels dilate/constrict in response to hypercapnia/hypocapnia to act as a crucial regulator of cerebral blood flow (CBF). CVR is known to be impaired acutely following both severe TBI (Lewis, Czosnyka, & J.D., 2014) and mTBI (Becelewski & Perzchala, 2003; Chan, Evans, Rosen, Song, & Kwong, 2015; Len et al., 2013; Mutch et al., 2014) and has previously been shown to be a robust, independent predictor of injury outcome following TBI (Lewis et al., 2014). Additionally, CVR impairment following mTBI has been found

to be a major contributor to acute post-injury decreases in CBF (Len & Neary, 2011), believed to be the cause both of neurocognitive symptoms (Giza & Hovda, 2001) and the increased risk for further injury, observed following mTBI (Golding, Steenberg, et al., 1999). As such, CVR changes likely precede neurocognitive symptoms observed following brain injury, and may serve as an ideal biomarker for identifying early trauma-related brain changes associated with mild repetitive head trauma—both in an acute and chronic sense.

Another biomarker of interest in the assessment of repetitive head trauma is resting state (RS) functional connectivity, or the brain's functional organization when the brain is not performing a specific task. Using various RS functional connectivity approaches, several stable resting state subnetworks (RSN) have been identified (Yeo, Krienen, Chee, & Buckner, 2014). Whole brain studies have reported global decreases in connectivity, in terms of either number or strength of connections (Bharath et al., 2015; Nakamura, Hillary, & Biswal, 2009; Stevens et al., 2012), both acutely (36h <) and subacutely (3 months) post symptomatic mTBI with recovery to control levels at 6 months post injury (Bharath et al., 2015; Messe et al., 2013) when mTBI patients are compared to healthy controls. Subnetwork specific studies have shown more varied results; however, this could be influenced by differences in the scale in which networks are defined. Several studies have reported an overall decrease in connections within the default mode network (DMN), but an increase in connections between the DMN and other networks (Bharath et al., 2015; B. D. Johnson, Neuberger, Gay, Hallett, & Slobounov, 2014; Mayer et al., 2015; Messe et al., 2013; Nakamura et al., 2009; Stevens et al., 2012). In contrast to

these studies, Nathan et al. and Bharath et al reported increased connectivity within the DMN. Abbas et al. found decreases in DMN connectivity in asymptomatic football athletes over a season of play but increased DMN connectivity when these athletes were compared to non-collision sport controls (Abbas et al., 2015). Both increases and decreases in connectivity in other RSNs (Bharath et al., 2015; Slobounov et al., 2011; Stevens et al., 2012) have also been observed. However, due to a lack of standardization in the regions and the scale that define of other networks (Yeo et al., 2014), it is hard to compare and contrast results. Despite this, it is clear that mTBI is, at least acutely and sub-acutely, associated with global decreases RS functional connectivity, though the effects on specific networks is unclear and may be heterogeneous.

Because neuronal activity, as indirectly measured using FMRI, is fundamentally modulated by local vascular physiology (Davis, Kwong, Weisskoff, & Rosen, 1998; Hoge et al., 1999), it is important to discuss functional connectivity changes observed using FMRI following mTBI in the context of any observed neurovascular changes. BOLD signal measured by FMRI is an indirect measure of neuronal activity and depends inherently on the coupling between increased neuronal metabolism in response to increased neuronal activity and CBF physiology (Davis et al., 1998; Hoge et al., 1999),. Though not believed to have direct neuronal effects, CVR has been shown to play a significant role in determining the extent of CBF changes in response to CMRO2 changes which in part govern the BOLD response (Maggio, Salinet, Robinson, & Panerai, 2014). In fact, CVR has been shown to substantially modulate BOLD signal measures obtained

using task based FMRI (Liu et al., 2013) and functional connectivity measures obtained using RS-FMRI (Golestani, Kwinta, Strother, Khatamian, & Chen, 2016).

In this work, we seek to assess the relationship of CVR changes and RS functional connectivity changes with repetitive head trauma in women's soccer. We will accomplish this objective through the following aims:

Aim 1: Test for alterations in CVR and RS functional connectivity in female soccer athletes, both acutely and chronically. Additionally, compare timescale of changes in CVR to timescale of changes in RS functional connectivity to assess whether CVR alterations affect RS functional connectivity measures.

Aim 2: Assess whether any changes exhibited in either biomarker are related to metrics of cumulative exposure to head trauma from soccer in the appropriate timescale for that biomarker.

If ***Aims 1 and 2*** are completed satisfactorily, we will know whether CVR and RS functional connectivity changes are present prior to symptomatic injury and associated with repetitive head trauma. Furthermore, we will have an understanding of the timescales in which these changes occur. This will allow us to assess whether CVR changes present in female soccer athletes affect RS functional connectivity measures. This will also allow us to assess the relationship of changes in these biomarkers to metrics of cumulative exposure in the appropriate timescale. Using this knowledge, we will be able to propose uses of these biomarkers in the development of guidelines aimed at preventing mTBI and associated long-term sequelae.

CHAPTER 2. BACKGROUND AND SIGNIFICANCE

Likely because of repetitive head trauma in the sport (Lipton et al., 2013), women's sports, soccer reports the largest numbers of mTBI annually (Zuckerman et al., 2015) and has among the highest mTBI rates (Gessel et al., 2007) among women's sports. Though research is now showing that the repetitive nature of head trauma in collision sports has detrimental effects on the brain (Bazarian et al., 2012; Lipton et al., 2013; Poole et al., 2014), the relationship between cumulative head impact exposure and detrimental brain changes is still not well understood.

2.1 mTBI Definition and Mechanisms of Injury

The most commonly used definition of mTBI was established at the first International Conference on MTBI in Sport as "a complex pathophysiological process affecting the brain, induced by traumatic biomechical forces" where "clinical symptoms largely reflect a functional disturbance rather than structural injury" resulting in a "set of clinical symptoms that may or may not involve loss of consciousness" (Aubry et al., 2002; McCrory et al., 2005). mTBI is the result of diffuse micro-injury to the brain due to linear and rotational acceleration of the brain following trauma (impact) to the head. Linear acceleration from an impact creates a pressure gradient between the coup and contra coup sites of injury leading to dynamic stresses in the brain that can induce shearing (Gurdjian, Lissner, & Lattimer, 1953; Ommaya & Gennarelli, 1974). Additionally, rotational

acceleration has been shown to cause diffuse injury at zones where tissue properties change (Gennarelli, 1983). Studies have shown that longer and lower magnitude rotational accelerations produce diffuse injury, while shorter, higher magnitude accelerations produce subdural hematomas (Gennarelli, 1983; Gurdjian et al., 1953). This is in agreement with higher rates of diffuse injury (such as mTBI) as opposed to focal injury reported in collision sports.

2.2 Mechanisms of injury in mTBI

Various processes contribute to the pathophysiological cascade of injury following mTBI. Animal models suggest widespread microshearing of neuronal and axonal membranes as the primary initiator for the cascade of injury following mTBI (Spain et al., 2010). Axonal and neuronal membranes are compromised resulting in unregulated flux of ions disrupting the cerebral microenvironment and causing impairments in neuronal signaling (Barkhoudarian, Hovda, & Giza, 2011). This triggers a chain of events including the release of excitatory neurotransmitters such as glutamate which binds to N-methyl-D-aspartate receptors resulting in further membrane depolarization (Nilsson, Hillered, Ponten, & Ungerstedt, 1990), a subsequent efflux of potassium and influx of calcium, and finally neuronal suppression (Barkhoudarian et al., 2011; Giza & Hovda, 2001). In an effort to restore ionic balance, membrane pumps increase glucose consumption depleting energy stores and resulting in an energy crisis where glucose driven cerebral blood flow (CBF) becomes uncoupled with neuronal activity (Barkhoudarian et al., 2011; Blennow, Hardy, & Zetterberg, 2012; Giza & Hovda, 2001). This decoupling, occurring during a period of increased neurometabolic demand, is

believed to be a major contributor to the neurocognitive symptoms of mTBI as well as increased risk for subsequent mTBI following initial injury (Giza & Hovda, 2001; Golding, Robertson, & Bryan, 1999; Len & Neary, 2011).

2.3 Long Term Risks Associated with mTBI

Research has established a clear link between a history of mTBI and chronic neurocognitive deficits, neurodegenerative diseases, and psychiatric illness making it clear that mTBI prevention is of major importance. In a study on retired high school, college, and professional football players, players with history of mTBI reported a higher frequency of headaches, movement disorders, hearing or balance disorders, and complaints of memory changes and speech difficulties (Jordan & Bailes, 2000). In a study of retired professional football players, revealed significant association between recurrent mTBI and self-reported memory loss, clinically diagnosed Mild Cognitive Impairment, and Alzheimer's disease (Guskiewicz et al., 2005). The study also reported a higher incidence of Alzheimer's Disease in retired professional football players below the age of 70, relative to the general population, suggesting that people with repetitive exposure to mild head trauma are at higher risk for early onset of this disease (Guskiewicz et al., 2005). A follow up study by the same group revealed a significant correlation between mTBI diagnosis and depression revealing that players with a history of mTBI were three times more likely to be diagnosed with depression (Guskiewicz, Marshall, et al., 2007). A history of mTBI has also been identified as an environmental risk factor in the sporadic (no genetic risk factors) form of fronto-temporal dementia

(Rosso et al., 2003), which accounts for 20% of dementias in individuals under the age of 65 (Snowden, Neary, & Mann, 2002).

More alarmingly, autopsy studies have revealed neuropathology, now termed chronic traumatic encephalopathy (CTE), in the brains of collision sport athletes. This pathology was first described in boxers as "Punch Drunk" in 1928 as slight gait abnormalities and/or slight slurring of speech both progressing to become serious dementia and parkinsonian syndrome (Martland, 1928). In 1973, Corsellis et al. termed the pathology "Dementia Pugilistica" after finding severe atrophy, lesions (typically perivascular), depigmentation of the substantia nigra, loss of nerve cells, neurofibrillary tangles in the absence of senile plaques, and in some cases cavum septum pellucidi in the brains of 15 boxers known to be "Punch Drunk" (Corsellis, Bruton, & Freeman-Browne, 1973). Omalu et *al*. coined the term CTE after finding neurodegeneration similar to Dementia Pugilistica in an autopsy study performed on a retired professional, a lineman known to have severe neurocognitive deficits. Many cases of CTE have now been reported including cases of CTE in football players, boxers, hockey players, soccer players, a professional wrestler, abuse victims, chronic head bangers, and a circus clown involved in "dwarf throwing" (Geddes, Vowels, Nicoll, & Revesz, 1999; McKee et al., 2013). Though many cases have been identified, it is difficult to estimate the prevalence of CTE among the general collision sport athlete population as majority of brains diagnosed with CTE were donated to science because of known neurologic or psychiatric problems prior to death.

2.4 Relationship between mTBI and Head Impacts in Collision Sports

While prevention of mTBI requires an understanding of the associated contribution of head impacts experienced by collision sport athletes, the focus of research in this area has traditionally been on characterization of the "concussive" impact(s) directly preceding diagnosis of mTBI. This finding is in contrast to head impact telemetry evidence arguing against the expectation that exposure to a specific level of impact will directly produce mTBI (Broglio et al., 2011; Guskiewicz, Mihalik, et al., 2007). In fact, injuries are identified following fewer than half of the blows exceeding commonly-espoused "threshold" levels (McCaffrey, Mihalik, Crowell, Shields, & Guskiewicz, 2007; Mihalik, Bell, Marshall, & Guskiewicz, 2007; Schnebel, Gwin, Anderson, & Gatlin, 2007). Further, changes observed in post-mTBI clinical measures have not exhibited correlation with the linear or rotational acceleration of the "concussive impact" (Guskiewicz, Mihalik, et al., 2007). Rather, studies conducted across a range of competition levels, from high school to professional, have demonstrated that a broad range of impact magnitudes are proximally associated with initial observation of mTBI (Broglio et al., 2011; Guskiewicz, Mihalik, et al., 2007). Ultimately, this past research demonstrates that the simple classification of "concussive" (i.e., symptom-inducing) and "subconcussive" (i.e., not producing symptoms) blows is inadequate and possibly misguided. Instead, mTBI research should focus more on the cumulative effects of the mild, repetitive head trauma experienced on a regular basis by the majority of collision sport athletes.

2.5 The Role of Neuroimaging in mTBI Research

Neuroimaging biomarkers offer significant potential to elucidate the relationship between experienced head impacts and mTBI. Such biomarkers can be used to detect changes in neurophysiology that are attributable to repetitive head trauma in collision sports, even in the absence of symptoms. Several studies using different modes of MRI, including MR spectroscopy (Poole et al., 2014; Poole et al., 2015), functional MRI (FMRI) (Abbas et al., 2015; E. L. Breedlove et al., 2012; Breedlove Morigaki et al., 2014; Robinson et al., 2015; Shenk et al., 2015; Svaldi et al., 2015; Talavage et al., 2014), and diffusion-weighted imaging (DWI) (Bazarian et al., 2012; Lipton et al., 2013; Marchi et al., 2013; McAllister et al., 2014) have quantified structural and neurophysiologic changes in the brains of asymptomatic collision sport athletes. Findings from these studies suggest that neurocognitive symptoms are a downstream effect of accumulated microstructural and metabolic injury to the brain, beginning prior to the presence of overt neurocognitive symptoms.

Neuroimaing studies have additionally found correlations between neurophysiologic and structural changes in the brain and various cumulative measures of head impact exposure. Several studies have found number of impacts sustained to be associated with functional (E. L. Breedlove et al., 2012; Robinson et al., 2015; Talavage et al., 2014), metabolic (Poole et al., 2015), and micro-structural (Lipton et al., 2013; McAllister et al., 2014) changes in the brains of asymptomatic collision sport athletes. High magnitude impacts (60g +), and accumulation there of, have also been found to be predictive of both neurometabolic (Poole et al., 2015) and micro-structural changes (McAllister et al., 2014)

in the brain. Finally, location and distribution of head impacts has been found to be predictive of neurohysiologic changes in the brain (E. L. Breedlove et al., 2012; Robinson et al., 2015). These studies support the hypothesis that regulations implemented to reduce sports related mTBI need to be geared towards reducing the cumulative loads that athletes receive both over a season and a career, suggesting that assessing the relationship between changes neuroimaging biomarkers of mTBI and head impact exposure could prove useful in developing such regulations.

2.6 mTBI in Women's Soccer

Several studies have shown more adverse effects as a result of mTBI in women as compared to men. Studies consistently report that female collision sport athletes report significantly higher rates of mTBI in sports where both men and women participate (Delaney, Lacroix, Leclerc, & Jonston, 2002; Fuller, Junge, & Dvorak, 2005; Gessel et al., 2007; Marar, McIlvain, Fields, & Comstock, 2012; Zuckerman et al., 2015). In addition to this, studies report that women experience more severe symptoms (Broshek et al., 2005) and take longer to recover (Fuller et al., 2005) than males. As such, women may be more at risk for long-term effects associated with a history of mTBI.

Among women's sports, soccer has among the highest reported rates for mTBI (Gessel et al., 2007; Marar et al., 2012; Zuckerman et al., 2015), with rates slightly exceeding men's football at the collegiate level (Gessel et al., 2007), and accounts for the greatest number of mTBIs in women per year and second greatest number of mTBIs per year across genders (Zuckerman et al., 2015). As participation in women's soccer continues to grow

at all age levels and as opportunities for continuation of participation expand (e.g., increasing numbers of women's professional leagues) (Morris, 2015), it is clear that the development of enhanced approaches to mTBI prevention and care in women's soccer is increasingly important.

CHAPTER 3. GAPS AND PROPOSED WORK

3.1 Gaps Addressed

The work that will be proposed below addresses three major gaps in the field of mTBI. First, no studies have been performed assessing the relationship between head impacts in women's soccer and neuroimaging biomarkers of mTBI, despite numerous studies showing mTBI rates on women's soccer to be on par with those of other sports known to lead linked with long-term neurocognitive deficits. Second, though studies suggest that mTBI and associated sequalae may be an accumulated injury, few thorough longitudinal assessments of brain changes due to repetitive head trauma have been conducted on asymptomatic collision sport athlete populations. Finally, few studies have directly related metrics of cumulative head exposure to changes observed in the brains of asymptomatic collision sport populations.

3.2 Proposed Work

Neuroimaging, though useful in identifying subtle brain changes associated with mTBI, is not a viable tool for continuous monitoring of individuals to screen for risk of mTBI due to large costs associated with neuroimaging. Because of this, it is necessary to identify how cumulative exposure metrics are related to brain changes in athletes both

acutely and chronically. Identifying metrics that are predictive of brain changes, will allow for more informed development of regulations that can make collision sports safer.

The Purdue Neurotrauma Group is dedicated to predictive modeling of the relationship between mild, repetitive head trauma and mTBI. The group pairs multi-modal MRI with head impact monitoring to assess the effects of head impacts in collision sports over seasons of play. Athletes are monitored over several seasons of play in order to assess both acute and longitudinal changes associated with repetitive head trauma.

For this work, we paired serial monitoring of CVR and RS functional connectivity in asymptomatic female soccer athletes and female non-collision sport controls along with head impact monitoring over multiple seasons of play to test for the presence of brain changes in these biomarkers, identify the timescale of any changes present, and assess relationship of these changes to cumulative exposure in the appropriate timescale. CVR and RS connectivity are selected as the biomarkers of choice because both impaired following symptomatic mTBI and because of the possible effect of CVR alterations on accurate assessment of brain connectivity using the BOLD response.

We first established the stability of these biomarkers in controls and subsequently tested for changes in asymptomatic female soccer athletes relative to baseline measurements and to Control measures to assess the timescale on which changes in these biomarkers occur (***Aim1***). Finally, we tested whether any changes in these biomarkers were related to metrics of cumulative head impact exposure in the appropriate time scale (***Aim2***).

CHAPTER 4. AIM 1 – TEST FOR CVR ALTERATIONS IN FEMALE HIGHSCHOOL SOCCER PLAYERS

4.1 Aim1 – Imaging Data Collection

In the PNG study, all athletes underwent imaging using MRI during the course of a single competition season, including pre-season conditioning/training. Soccer athletes were imaged sessions before starting contact practices (*Pre)*, within the first half (approximately 5 weeks) of the contact season (*In1*), within the second half of the contact season (*In2),* 1-2 months after the end of the contact season (*Post1)*, and 3-4 months after the end of the contact season *(Post2)*. A subset of the soccer athletes were also scanned 8 months after the end of the season (*Post3)* during physical activity periods equivalent to the *Pre* scan, but now associated with preparation for the following year's high-school soccer season. Since aerobic exercise has been linked with increased CVR (Ainslie et al., 2008; Murrell et al., 2013), *Pre* scans were conducted such that soccer athletes were already physically active and the transition to *In1* was marked by an onset of contact practices. Comparable to the *Pre* to *In1* or *In1* to *In2* intervals, control athletes underwent two MRI scanning sessions (*Test, Re-Test)*, 4 to 6 weeks apart within their training/competition seasons, maintaining comparable levels of physical activity at both sessions.

Imaging was performed at the Purdue University MRI Facility (West Lafayette, IN), using a 3-T General Electric Signa HDx (Waukesha, WI) with a 16-channel brain array (Nova Medical; Wilmington, MA). CVR was measured using a hypercapnic breath hold challenge known to produce good repeatability in the human brain (Bright & Murphy, 2013; Kastrup, Gunnar, Neumann-Haefelin, & Moseley, 2001; Lipp, Murphy, Caseras, & Wise, 2015). For each imaging session, a single blocked breath-hold FMRI run (4 breath holds, 20s duration, separated by paced breathing, hold on the exhale) was acquired in each 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). PsychoPy (Peirce, 2007) was used to cue the task with instructions presented via a NordicNeuroLab fiber optic visual system. A respiration belt was used to monitor task compliance. For registration purposes across sessions and subjects, a T1-weighted anatomical scan was acquired using a 3D spoiled gradient echo sequence (TR/TE 5.758ms/2.032ms, flip angle=73°, 1mm isotropic resolution).

4.2 CVR Data Processing

The pipeline used to obtain a measure for CVR from the FMRI data is detailed in Figure4.1. A standard regression analyses was performed using AFNI (Cox, 1996). A processing stream adapted from *afni_proc.py*, including slice timing correction, motion correction, spatial smoothing, alignment to the structural scan, normalization to Talairach space, and conversion to percent signal change, was be used. Additionally, the FAST automated segmentation tool in FSL (Jenkinson et al., 2012; Smith, 2002) was be used to create a gray matter (GM) mask for each subject. FMRI analyses were conducted at both

the whole brain level (GM only) and at the gyrus level (GM only) in order to test for localization of changes present in the brain. For gyrus level analyses, the brain was parcellated into 70 regions of interest (ROI, 35 in each of the left and right hemispheres) obtained from probability maps in the DKD Desai Atlas in AFNI (Desikan et al., 2006).

For each subject-session, the mean FMRI time series across all voxels in a given region of interest was calculated. This series was regressed against a task time series calculated from the session-specific respiratory belt time series. To calculate the task time series, the onset time and duration of each hold were calculated from the respiration belt time series after low pass filtering to remove noise. The β-weight of the breath hold regressor was used as the CVR metric. Due to imperfect task compliance, hold durations often varied by several seconds (11s – 21s soccer athletes, 11s – 19s control athletes) within a given session. Because of this, the breath hold regressor was duration modulated.

Given that hemodynamic latency in respiratory challenges is known to vary across subjects and also across the brain (Birn, Smith, Jones, & Bandettini, 2008; Bright, Bulte, Jezzard, & Duyn, 2009), latency optimization was performed to obtain the best fit of the breath-hold regressor with the FMRI time-series. Breath-hold regressors were shifted with respect to the FMRI data over one hold/respiration cycle (0-40s), in 1.5s increments (27 total steps). The optimal latency calculated for each athlete/ROI at the *Pre/Test* scan was used for all follow-up sessions after initial confirmation that latency did not vary significantly between *Pre/Test* and follow-up scans. For control athletes and soccer athletes studied thus far, optimal latencies ranged from 6–12 seconds in the whole brain

regression. No statistical difference was found in latencies between control athletes and soccer athletes (Control Average: 10.4s ± 2s, Soccer Average: 10.3s ± 1.9s).

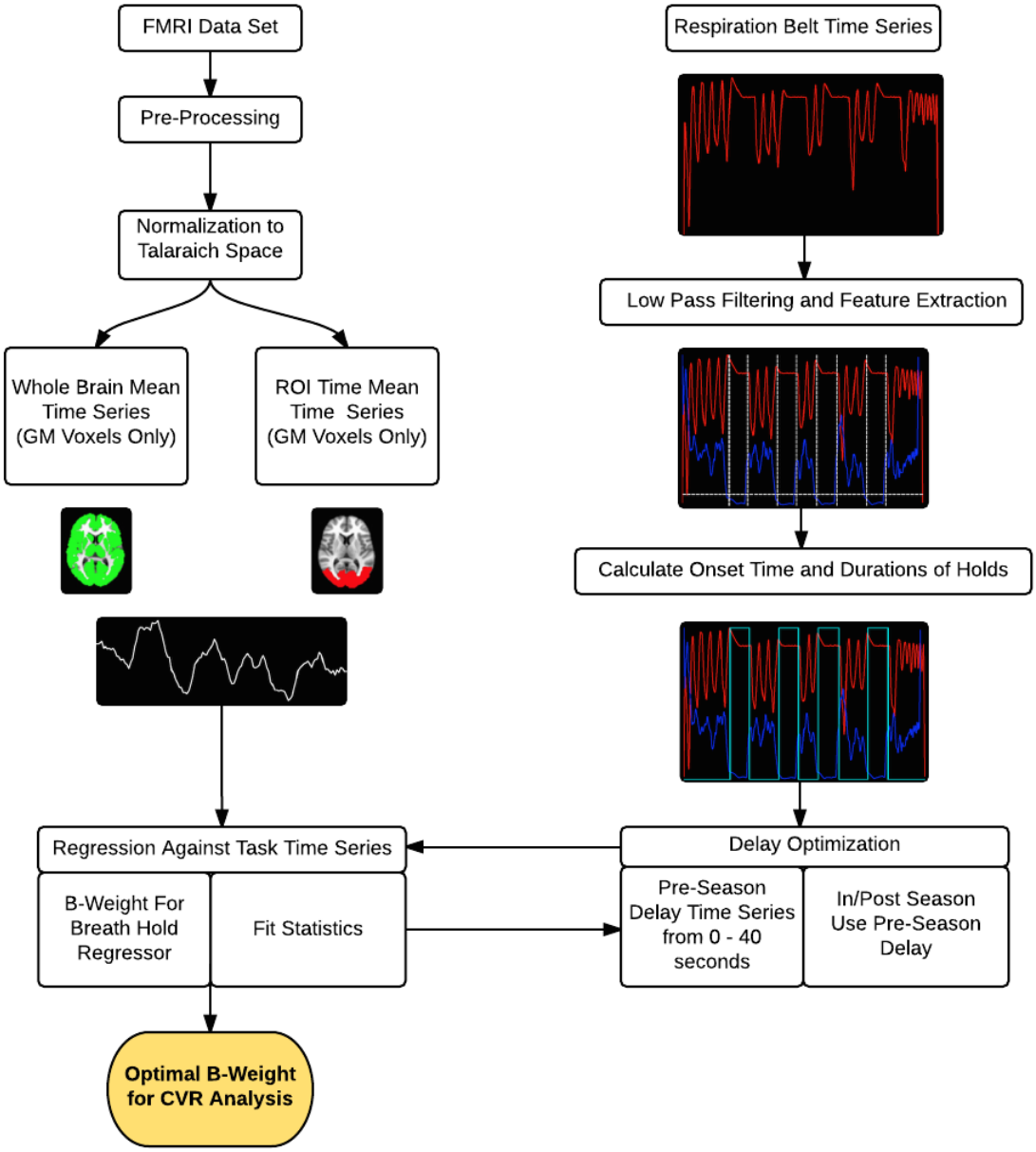

Figure 4.1 Processing Pipeline used to obtain the final β-weight as a measure of CVR from the FMRI and respiratory belt data for each subject-session.

4.3 Initial CVR Analysis

To identify population-level changes from *Pre/Test* in CVR due to measurement noise and/or exposure to repetitive head acceleration events, distributions of fractional change from *Pre/Test* in both athlete populations (soccer and control) were compared at each session to zero, using a Wilcoxon signed rank test followed by false discovery rate (FDR) correction. For the whole brain analyses, the FDR correction were performed across sessions to test for an overall effect of session. Gyrus-level analyses were conducted under the assumption that CVR changes could still be locally significant even when not effecting globally detectable change. Therefore, for the ROI analyses, FDR correction was performed across ROIs and we present below only those findings that survive a correction for multiple comparisons at the $p < 0.05$ level.

In control athletes, fractional change from *Test* was not found to be significantly different from zero at the *Re-Test* session for either the whole brain analysis or in any of the gyrus-level ROIs. Therefore, β-weights for the control *Test* and *Re-Test* sessions were averaged, on a within-subject basis, to reduce noise in the estimate of the population distribution (Figure4.2).

To test for potential group differences in CVR due to exposure to repetitive head acceleration events, the β-weights in soccer athletes were compared directly with those in control athletes at each session. Pairwise comparisons were conducted between the averaged control athlete β-weight distribution and the soccer athlete (all groups) β-weight

distribution at each session using a Wilcoxon rank sum test followed by FDR correction, as above.

Additionally, to assess whether soccer athletes exhibit a meaningfully different distribution of CVR measures (β-weights), the number of soccer athlete measures below the 50th (median) and 25th percentiles of the control athlete population were computed and compared to the corresponding chance distributions. Chance distributions were modeled based on a Bernoulli trial design, assuming the null hypothesis that soccer and control athletes belong to a common underlying distribution. A Binomial test ($\alpha = 0.05$) was used to accept or reject the null hypothesis.

4.4 Initial CVR Results

Participant Demographics: Initially twenty-six female high school athletes participated in this study. *Soccer athletes:* 14 athletes (ages 15-17; mean 15.9) were members of high school junior varsity or varsity soccer teams, representing two high schools (7 per team). All fourteen athletes were scanned according to the study paradigm above (*Pre, In1, In2, Post1, Post2)*. Additionally, seven of the 14 soccer athletes were also scanned 8 months after the end of the season (*Post3). Control athletes:* 12 athletes (ages 15-18; mean 15.9) participated only in non-collision high school sport junior varsity or varsity teams (6 basketball; 3 track & field; 2 cross-country; 1 each softball, gymnastics and swimming).

Participants were not excluded from the study due to a history of mTBI. Four control subjects reported a prior history of mTBI (#mTBI = 1: $n = 3$; #mTBI = 3: $n = 1$) and three

soccer subjects reported a prior history of mTBI (#mTBI = 1: n = 1; #mTBI = 2: n = 1; #mTBI = 3: n = 1). No included participant was diagnosed with mTBI during the course of the study and no included participant's symptom score was flagged relative to baseline, as assessed using ImPACT—see K. M. Breedlove et al. (2014) for assessment protocols.

Soccer Athlete vs. Control Analysis: No between-group effect was found in whole brain β-weight distributions, between soccer athletes and controls at any session, for any loading group (Figure 4.2A *Total* Group). Likewise, after correction for multiple comparisons, no ROI exhibited significant group differences in β-weight between soccer athletes and controls at any session.

However, while no between-group effect was observed in the Wilcoxon Rank Sum Test, the distribution of whole brain β-weights of the soccer athlete group, did exhibit statistically significant shifts over the course of the season and post-season periods relative to the control group. The number of soccer athletes with CVR measures below the control median increased from 1 of 14 athletes at *Pre* to 7 of 14 athletes at *In1* and continued to increase through *Post2* (Table 4.1 *Total* Group). At each in-season and post-season session, the majority of soccer athletes exhibiting CVR measures below the median of the control athletes were also below the 25th percentile. The distribution of CVR measures in the soccer athlete cohort was significantly different from the chance distribution of controls at *Pre, In1*, *Post1*, and *Post2*. At *Pre*, the soccer athlete cohort skewed high relative to the median of the control population. At the *In1, Post1,* and *Post2*

time points, the soccer athlete cohort skewed low relative to the 25th percentile of the control population, with a significant deviation observed below the median at *Post2*, as well.

Table 4.1 Number of soccer athletes (Soccer Total: n=14; Soccer HighLoad: n=7; Soccer LowLoad: n=7) at each session, with CVR measures below the 50th and 25th percentiles of the control distribution (n=12: within-subject average Test and Re-Test)

Session Count of Soccer Athletes Below Control CVR Distribution Percentiles												
	Pre		***In1***		***In2***		***Post1***		***Post2***		***Post3***†	
Soccer Athlete Group	**50th %ile**	**25th %ile**	**50th %ile**	**25th %ile**	**50th %ile**	**25th %ile**	**50th %ile**	**25th %ile**	**50th %ile**	**25th %ile**	**50th %ile**	**25th %ile**
Total (n=14)	***1*****	1	7	***7*****	9	6	8	***8*****	***11*****	***9*****	2	2
HighLoad (n=7)	***0*****	0	4	4	6	4	5	***5*****	6	***5*****	*N/A*	*N/A*
LowLoad (n=7)	1	1	3	3	3	2	3	3	5	4	*N/A*	*N/A*

**Observed soccer athlete distribution is statistically significantly different ($p < 0.05$; *Binomial Test*) from noncollision-sport control athletes

Within-Soccer Athlete Analysis: Significant differences were observed within the soccer athletes when comparing CVR measures observed at within-season (*In1*, *In2*) or after-season (*Post1*, *Post2*, *Post3*) sessions against the pre-contact practices (*Pre*) assessment, both for the whole-brain and ROI-specific analyses. Soccer athletes, in the aggregate,

exhibited within-group changes in whole brain CVR measures (Figure 4.2B) that were significantly different from zero, relative to *Pre*, at *In2* ($p_{fdr} < 0.01$), *Post1* ($p_{fdr} < 0.05$), and *Post2* ($p_{fdr} < 0.01$). Of note, CVR appears to have returned to *Pre* levels in the seven soccer athletes evaluated at *Post3*.

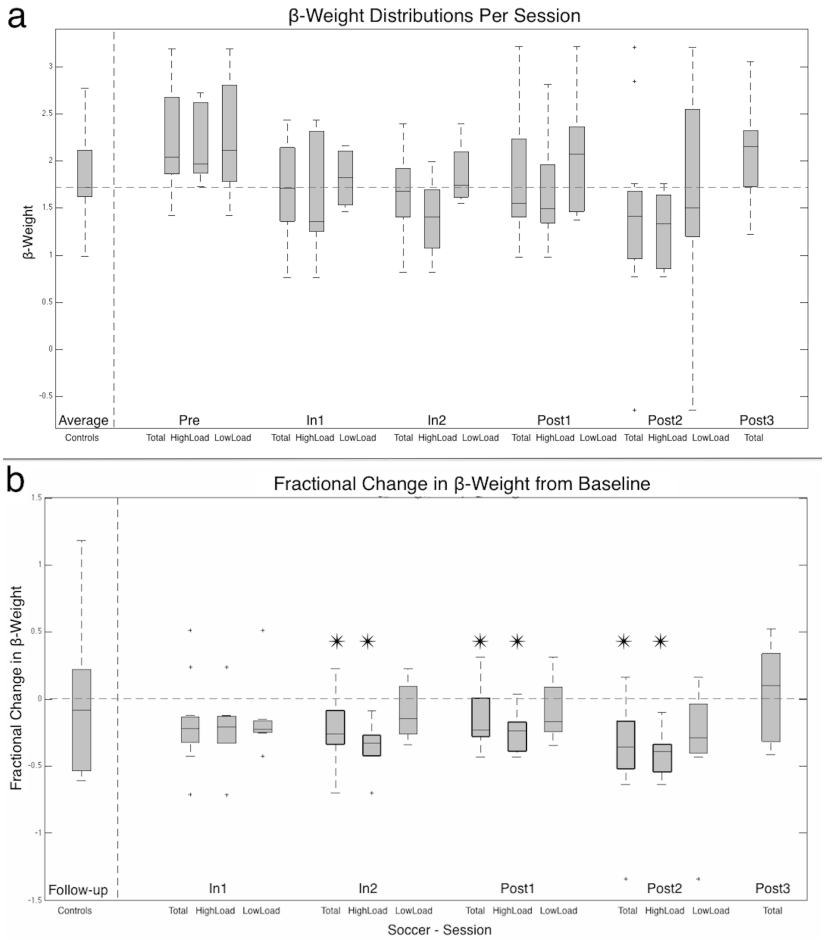

Figure 4.2 Distributions of β-weights and changes thereof for soccer athletes and non-collision-sport control athlete peers. (A) β-weight distributions for controls ($n = 12$, within-subject averages of *Test* and *Re-Test*) and soccer athletes at each assessment session. Note that at no session are raw CVR measures significantly different between soccer and control athletes. (B) Percent change in β-weight from *Pre/Test* for controls ($n = 12$) and soccer athletes at each follow-up assessment session. Session-specific distributions of fractional change from *Pre/Test* found to deviate significantly ($p_{fdr} < 0.05$, Wilcoxon Signed Rank Test) from zero (the null hypothesis) are indicated by an asterisk above the distribution and bolding around the box.

In the gyrus-level analysis, the number of ROIs in the *Total* soccer group exhibiting statistically significant deviations (relative to *Pre/Test*) at each session corroborated well with whole brain results. Deviant ROIs were observed for the *Total* soccer athlete group at all sessions excluding *Post3.* There does not appear to have been any lateralization of these effects (Table 4.2 *Total* Group). Conversely, control athletes did not exhibit any significantly changed ROIs, at any session (Table 4.2 *Total* Group).

The numbers of sessions in which each of the 70 ROIs were deviant from *Pre/Test* are presented for both the *Total* soccer cohort (Figure 4.3, *Total* Group). While the majority of the cortical surface was deviant relative to *Pre* in at least one session, perhaps reflecting heterogeneity in mTBI across subjects, frontotemporal surfaces of the brain were persistently affected (*Total*: *In1-Post2*).

Table 4.2 Number of regions of interest (out of 70) at each session exhibiting fractional change from *Pre/Test* significantly different from zero ($p_{fdr} < 0.05$, Wilcoxon Signed Rank Test) for each athlete grouping (see Table 1 for details). Note that the *HighLoad* and *LowLoad* groups are session specific—i.e., an athlete need not remain in the *High/LowLoad* group for all three groupings.

	Number of Deviant ROIs in Left				**Number of Deviant ROIs in Right**			
	Soccer			**Control**	**Soccer**			**Control**
Session	*Total*	*High Load*	*Low Load*		*Total*	*High Load*	*Low Load*	
In1 /	9	0	0	0	7	0	0	0
In2	24	25	0	*N/A*	21	21	0	*N/A*
Post1	13	17	0	*N/A*	16	17	0	*N/A*
Post2	26	21	0	*N/A*	28	26	0	*N/A*
Post3	0	*N/A*	*N/A*	*N/A*	0	*N/A*	*N/A*	*N/A*

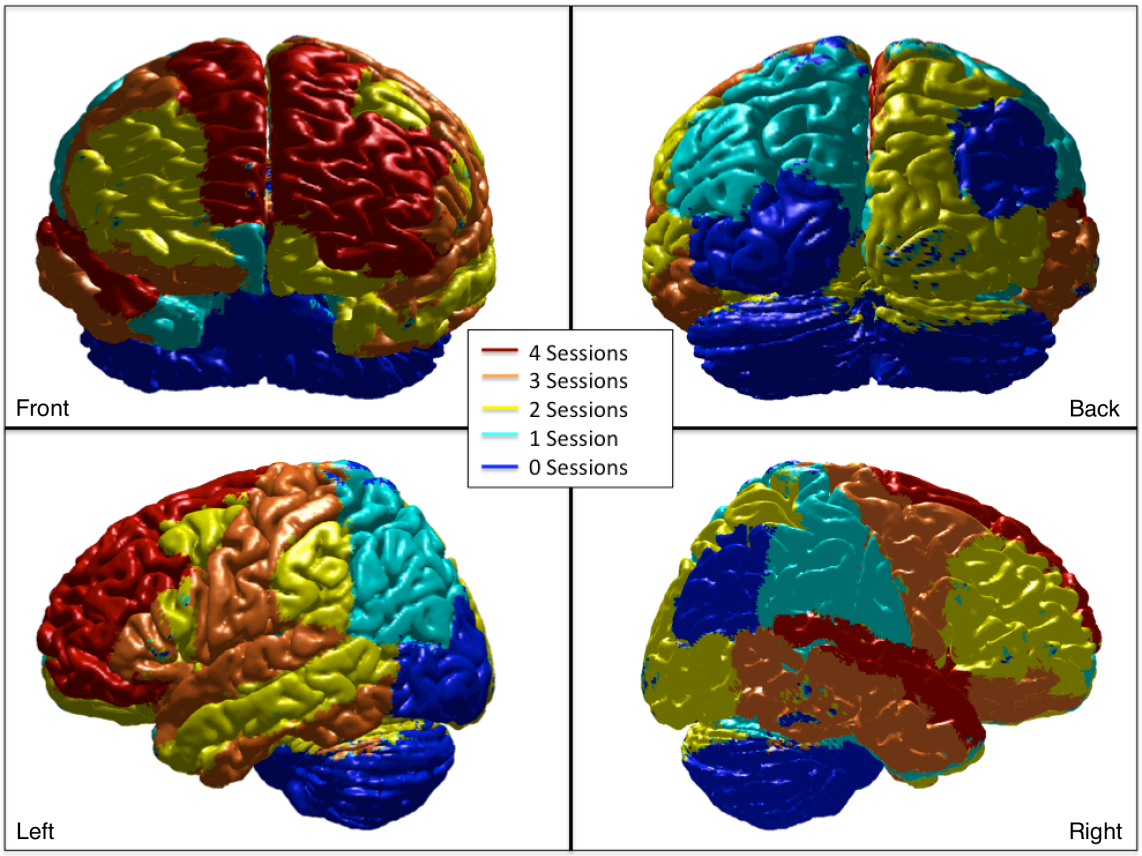

Figure 4.3 Graphical depiction on a rendered brain surface (four views) of the cumulative number of sessions (out of 5 possible—*In1*, *In2*, *Post1*, *Post2*, *Post3*) during which each of 70 regions of interest (Desikan et al., 2006) in the *Total* cohort of soccer athletes (n = 14) exhibited a percentage change in β-weight from *Pre* significantly different from zero ($p_{fdr} < 0.05$, Wilcoxon Signed Rank Test).

4.5 Impact CVR Results

Interpretation of CVR Changes as Evidence of Brain Injury: Findings suggest that CVR decreases observed in this study are indicative of developing injury to the brain, preceding neurocognitive symptoms. Decreases in CVR and CBF (not directly assessed here) have also previously been reported sub-acutely (Becelewski & Perzchala, 2003; Golding, Steenberg, et al., 1999; Len et al., 2013; Maugans, Farley, Altaye, Leach, & Cecil, 2012) and chronically following sports- and recreation-related mTBI (Chan et al., 2015; Maugans et al., 2012; Mutch et al., 2014; Wang et al., 2015). Therefore, the CVR

changes seen in this study are both associated with the cumulative exposure to head collision events in soccer, and consistent with previous outcomes of symptomatic mTBI.

Regional deviations from *Pre,* observed most persistently in frontotemporal regions (Figures 3 and 4 and Online Resources 3 and 4), are also consistent with regional alterations in CVR (Chan et al., 2015) and CBF (Wang et al., 2015) reported following symptomatic mTBI. Brain regions exhibiting the most persistent CVR changes closely matched dorsolateral frontal regions found to be decreased in a case study of CVR in a woman assessed 2 months and 1 year after mTBI (Chan et al., 2015) and further encompassed frontotemporal regions found in a pediatric population to exhibit decreases in CBF subsequent to mTBI (Wang et al., 2015). These areas are expected to experience the most directed acceleration-related forces during heading events, and are known to be especially susceptible to head trauma (De Kruijk, Twijnstra, & Leffers, 2001).

Regional findings are of additional importance because the frontal cortex has been implicated in dementia. CVR decreases in the forebrain cortex have been reported in patients with Alzheimer's Disease (Gao et al., 2013; Yezhuvath et al., 2012). Additionally, head trauma has been identified as an environmental risk factor in the sporadic (i.e., no genetic risk factors) form of frontotemporal dementia (Rosso et al., 2003), which accounts for 20% of dementias in individuals under the age of 65 (Snowden et al., 2002).

The finding of reduced CVR in asymptomatic female soccer athletes exposed to significant head acceleration events is also consistent with previous work suggesting mild repetitive head trauma can produce neurophysiologic changes even in the absence of neurocognitive symptoms (Marchi et al., 2013; McAllister et al., 2014; Poole et al., 2014; Poole et al., 2015; Svaldi et al., 2015; Talavage et al., 2014). Further, CVR decreases from *Pre* found here in asymptomatic soccer athletes are consistent with findings in asymptomatic high school football athletes, in whom decreased CVR was observed during the first six weeks of the competition season (Svaldi et al., 2015).

CVR Changes Support Prolonged Recovery Period: The temporal behavior of deviations observed in this study is consistent with that observed in other studies of asymptomatic and symptomatic mTBI populations, suggesting that the brain requires an extended period to recover from repetitive head trauma. Specifically, the temporal behavior of CVR deviations observed in this study is similar to the temporal behavior associated with deviations of neurometabolism (as measured by magnetic resonance spectroscopy, MRS) and functional connectivity (as measured with rs-FMRI) in studies of asymptomatic football athletes. In a study quantifying neurometabolic changes during the football season, Poole *et al.* (2014) observed decreases in metabolic concentrations of total creatine (precentral gyrus, rostral middle frontal gyrus), glutamate (precentral gyrus), and inositol (rostral middle frontal gyrus). These deviations relative to *Pre* began during the first half of the contact season and persisted through the end of the season. The present study also observed CVR deviations from *Pre* in both of these regions, beginning in the second half of the contact season and persisting at least 3-4 months after the end of the

season. Changes in brain connectivity during the contact season have also been observed in asymptomatic football athletes by Abbas *et al.* (2015), who reported decreases in connectivity relative to *Pre*, during months of increased head acceleration event exposure, that were still present five months after the contact season—a finding directly comparable to those in the present work.

Despite resolution of neurocognitive symptoms in adolescents occurring on average 10-14 days subsequent to the diagnosis of mTBI (Lovell et al., 2003; Sim, Terryberry-Spohr, & Wilson, 2008), neurophysiologic changes present several months after symptomatic mTBI have also been reported. Maugans *et al.* (2012) reported significant decreases in CBF of adolescents, relative to matched healthy controls, that persisted as long as 30 days after mTBI. Wang et al. (2015), also studying adolescents relative to matched controls, reported decreased CBF in bilateral frontotemporal regions for 3-12 months after participants suffered mTBI. Though long term CVR studies have not been conducted on adolescents, CVR decreases, relative to matched controls have also been observed 2-12 months post injury in adult populations (Chan et al., 2015; Mutch et al., 2014). Additionally, in a study of working memory in controls and mTBI subjects, McAllister et al. (1999) observed continued activation differences one month after injury in dorsofrontal and lateral parietal regions. Therefore, consistent timelines of neurophysiologic changes due to exposure to repetitive head accelerations across different biomarkers of mTBI—even if obtained from different sports—continue to support the argument that the changes observed in this study are indicative of injury, from which it that takes several months to recover.

CHAPTER 5. AIM2 - RELATING CVR CHANGES TO CUMULATIVE EXPOSURE METRICS

5.1 Head Acceleration Event Monitoring

Head acceleration events experienced by soccer athletes will be monitored using an xPatch (X2 Biosystems; Seattle, WA) throughout the entire contact season including games and practices—cf. McCuen et al. (2015). Sensors will be placed on the head with an adhesive patch behind the right ear. The sensors monitor (1 kHz sampling rate) three axes of translational acceleration and three axes of angular velocity. Acceleration events are recorded when translational accelerations exceeded a 10 g threshold. Following each practice and game, the data will be downloaded using the Head Impact Monitoring System software (X2 Biosystems). Features within the software will provide peak translational acceleration (PTA) and peak angular acceleration (PAA) measurements for each detected event. To focus the analysis on head accelerations likely resulting from direct impacts to the head or the body, and not from accelerations due to hard stops or cuts (10-20 g), only acceleration events surpassing a 20 g threshold will be included (McCuen et al., 2015). Note that non-collision sport athletes will not be monitored for head acceleration events during the period between *Test* and *Re-Test* scan

5.2 Head Acceleration Event Evaluation

For each soccer athlete, three metrics will be computed at each imaging session: (1) the (to-date) cumulative number of head acceleration events; (2) the (to-date) cumulative PTA, cPTA; and (3) the (to-date) cumulative PAA, cPAA. The cPTA and cPAA measures for a given athlete at a given session will thus represent weighted versions of the count of impacts, to-date. Specifically, for the *i*-th soccer athlete, athlete-specific cPTA (Eqn 5.1) and cPAA (Eqn 5.2) measures will be calculated for the *j*-th session (*In1-2*, *Post1-3*) by summing the PTA or PAA of each of the N_j head acceleration events experienced by the corresponding athlete from the beginning of the season until either the day of the assessment (*In1, In2*) or through the end of the season (*Post1, Post2, Post3)*.

$$cPTA_{(Session\ j)}^{(Athlete\ i)} = \sum_{k=1}^{N_j} PTA_k^i \tag{5.1}$$

$$cPAA_{(Session\ j)}^{(Athlete\ i)} = \sum_{k=1}^{N_j} PAA_k^i \tag{5.2}$$

To incorporate cPTA, and cPAA into a single measure, representative of the *i*-th soccer athlete's cumulative exposure at the *j*-th session, relative to the cumulative exposure of the ensemble of soccer athletes at the *j*-th session, a unitless measure of relative cumulative exposure (*RCE,* Eqn 5.3) will be calculated for each athlete at each imaging session.

$$RCE_{(Session\ j)}^{(Athlete\ i)} = \frac{cPTA_j^i}{\underset{i}{median}(cPTA_j^i)} + \frac{cPAA_j^i}{\underset{i}{median}(cPAA_j^i)} \tag{5.3}$$

Due to large variance in *RCE* among the high school cohort studied thus far, soccer athletes will be divided into two equal-sized groups, reflecting the upper (*HighLoad*) and lower (*LowLoad*) halves of distribution of *RCE*. The grouping process described above will be repeated at each follow-up session to best capture effects of *RCE* throughout the season. Statistical analyses for each group were conducted as explained in CHAPTER 4**.**

5.3 Cumulative Load Grouping Results

Participant Grouping: Of the 14 soccer athletes studied thus far, 4 remained in the *HighLoad* group at every follow-up session and 4 remained in the *LowLoad* group at every follow-up session. The remaining 6 athletes fluctuated between the two groups as the season progressed. Because the *HighLoad* groups and *LowLoad* groups differed at each session, they are hereafter referred to by a *Session-Group* label (e.g. *In1-HighLoad*,). The lack of competition-related exposure after the end of the season permits a single *Post* designation for the *Post1-Post2* groupings (i.e., *Post-HighLoad* and *Post-LowLoad*). Note that, because of the small sample size, athletes who returned for the *Post3* session were not divided into upper- and lower-half groupings. However, of the seven athletes who returned for the *Post3* session, 3 were from the *Post-HighLoad* and 4 *Acceleration Events*

Head Acceleration Events: After division of the soccer athletes into loading groups based on RCE at each session, athletes in the *HighLoad* groups exhibited a significantly higher number of hits, and associated cPTA and cPAA, than did athletes in the *LowLoad* groups (Figure5.1). Soccer athletes, as a whole, experienced 1991 total events throughout the

season. Athletes comprising the *Post-HighLoad* group accounted for 1402 events (70.4%) and athletes comprising the *Post-LowLoad* group accounted for 589 events (29.6%).

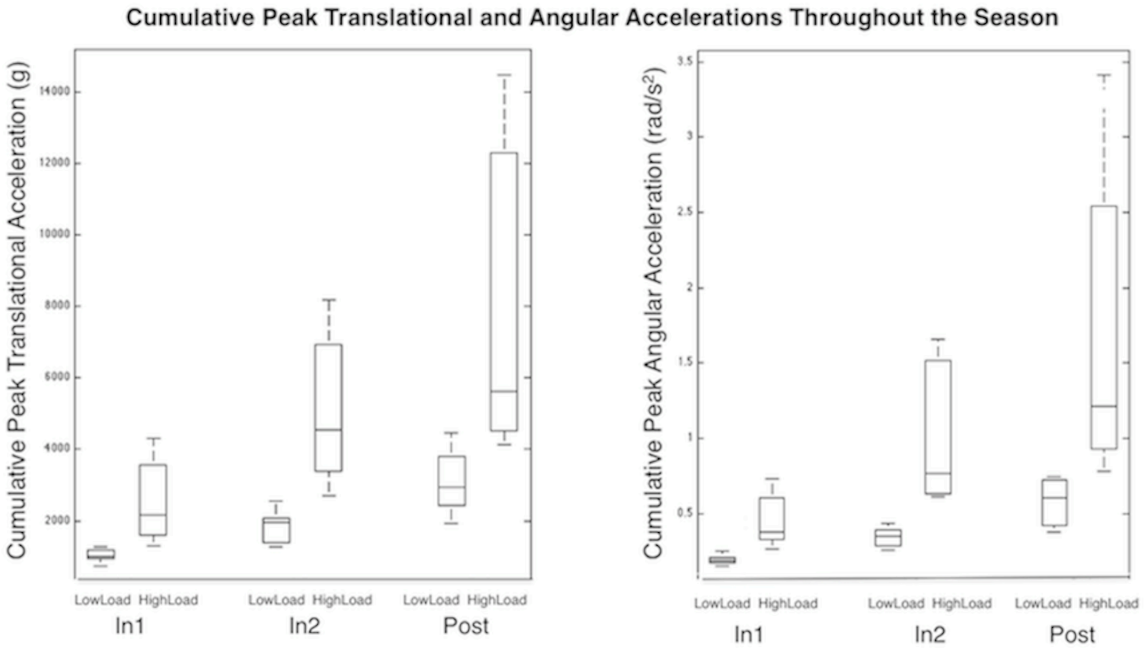

Figure 5.1 Box and whisker plots of cumulative peak translational acceleration (cPTA) and cumulative peak angular acceleration (cPAA) distributions for soccer athletes at each assessment session. Boundaries of boxes represent the 25th and 75th percentiles and the line inside the boxes indicates the median of the distribution. Assessment sessions *In1* and *In2* comprise measurements during first and second half of the competition season; and *Post* represents groupings based on end of season cumulative totals. Athletes are grouped by individual rank above (*HighLoad*; $n = 7$) or below (*LowLoad*; $n = 7$) the median Relative Cumulative Exposure (*RCE*; see text). Athletes in the *HighLoad* group were observed to have experienced significantly greater cPTA and cPAA than athletes in the *LowLoad* group at each session ($p_{fdr} < 0.05$, unpaired *t*-test). Note that the *HighLoad* and *LowLoad* groups are session specific—i.e., an athlete need not remain in the *HighLoad or LowLoad* group for all three assessment periods.

5.4 CVR Changes in Cumulative Loading Groups over a Season

Soccer Athlete vs. Control Analysis: Like the *Total* soccer cohort, no between-group effect was observed in the Wilcoxon Rank Sum Test (Figure4.2 A) but the distribution of whole brain β-weights of the *HighLoad* soccer athlete groups, did exhibit statistically

significant shifts over the course of the season and post-season periods relative to the control group (Table4.1). Notably, most soccer athletes exhibiting CVR measures below the control athlete population median at each session were in the *HighLoad* group. The distribution of CVR measures in the *HighLoad* soccer athlete groups was significantly different from the chance distribution of controls at *Post1*, and *Post2* again skewing markedly below the 25th percentile of the control athlete cohort.

Within-Soccer Athlete Analysis: Athletes in the *HighLoad* groups exhibit similar changes to the *Total* soccer group (Figure3B), with *In2-HighLoad* ($p_{fdr} < 0.04$), and *Post-HighLoad* (at *Post1*: $p_{fdr} < 0.05$; at *Post2*: $p_{fdr} < 0.04$) presenting with significant CVR decreases relative to *Pre.* Athletes in the *LowLoad* groups did not deviate significantly from *Pre* at any follow-up session (Figure4.2 B).

In the gyrus-level analysis (Figure5), only the *HighLoad* soccer groups had ROIs exhibiting statistically significant deviations relative to *Pre/Test* (Figure 5 and Supplementary Table2). Similarly to the *Total* soccer cohort, frontotemporal surfaces of the brain were most persistently affected (*HighLoad*: *In2-Post2*).

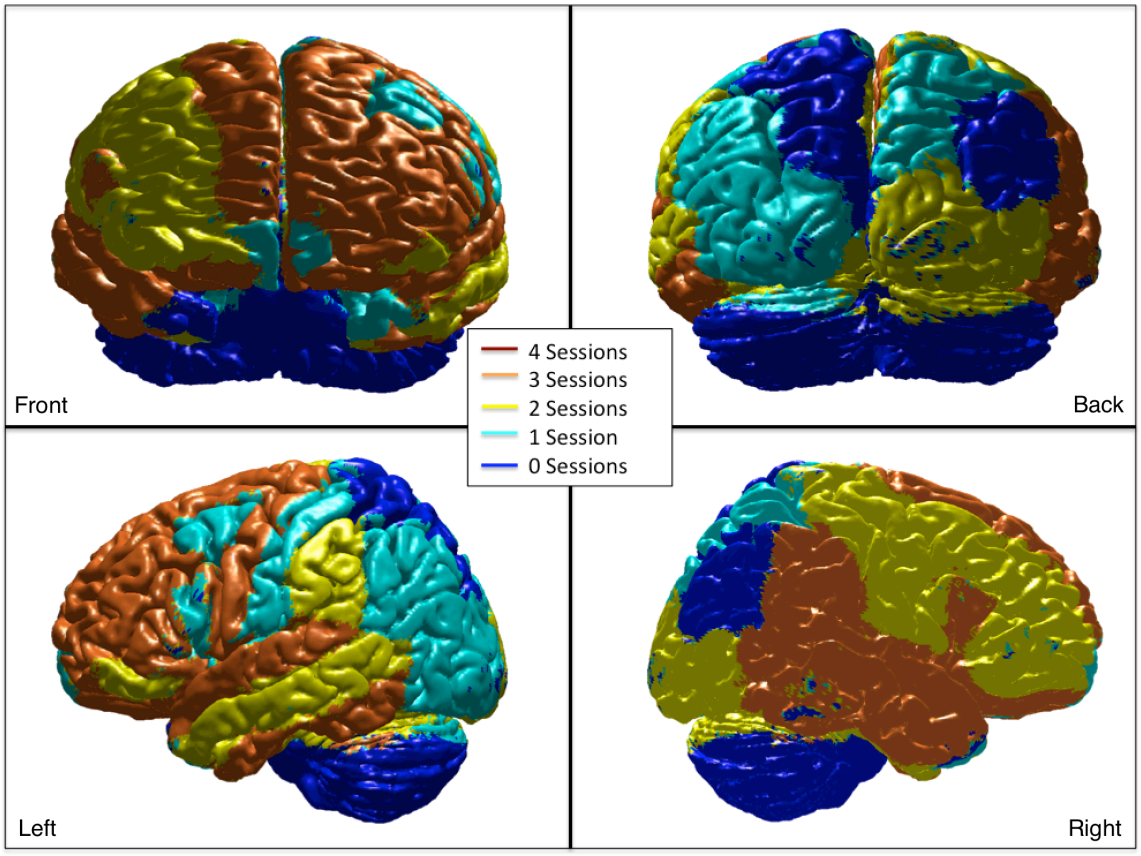

Figure 5.2 Graphical depiction on a rendered brain surface (four views) of the cumulative number of sessions (out of 5 possible—*In1*, *In2*, *Post1*, *Post2*, *Post3*) during which each of 70 regions of interest (Desikan et al., 2006) in the *HighLoad* cohorts of soccer athletes (n = 7) exhibited a percentage change in β-weight from *Pre* significantly different from zero ($p_{fdr} < 0.05$, Wilcoxon Signed Rank Test)

5.5 Impact of CVR Grouping Results

Stability of CVR measurements in the control population facilitates interpretation of significant CVR differences from *Pre* in the soccer athlete population within the context of exposure to head acceleration events. Observation of no significant changes from *Test* in global or regional CVR for control athletes supports the *a priori* expected stability of the measurement (Birn et al., 2008; Bright & Murphy, 2013; Kastrup et al., 2001; Lipp et al., 2015).

Changes observed in the *Total* soccer cohort proved to be associated with cumulative exposure, supporting the hypothesis that CVR changes in collision sport athletes are a function of cumulative loading. CVR measures for both the *Total* and *HighLoad* soccer athlete groups exhibited significant whole brain and regional CVR decreases, relative to *Pre*, that persisted at least 3-4 months after exposure ceased, and recovered by 8 months after the end of the contact season. In contrast, soccer athletes in the *LowLoad* groups did not experience significant CVR changes, relative to *Pre*, at any follow-up session, in any ROI, further supporting the stated hypothesis.

The results of this study support the prior hypothesis that accumulation of head acceleration events continually increments neurophysiologic alterations, which may ultimately exceed a minimum threshold beyond which statistically-detectable changes will be observed for modest to small sample sizes of athletes. The relationship between cumulative exposure and cerebrovascular alterations is not binary as evidenced by the contribution of athletes in *LowLoad* groups to CVR alterations observed in *the Total* soccer group, despite the lack of observation of significant changes in the *LowLoad* groups. Specifically, CVR changes observed in the *Total* soccer groups were always more significant than in the *HighLoad* soccer group, suggesting that the contribution of the *LowLoad* athletes was something other than neutral or noise. Additionally, though no significant whole brain changes were seen at *In1*—at which point athletes had received the least cumulative loading—significant ROI-level deviations were persistently observed in the *Total* soccer group from *In1* through *Post2*, implying that "sub-threshold" changes in CVR may have begun during the first half of the competition season. One

interpretation of these findings is that a nonlinear relationship exists between cumulative exposure and the associated neurophysiologic alterations with a threshold of these alterations above which risk for injury increases.

Results from CVR comparisons of control athletes and soccer athletes are consistent with the asymptomatic nature of the studied soccer population. While higher levels of mechanical loading were significantly coupled to CVR changes, the CVR distribution for the aggregate population of soccer athletes did not significantly differ from controls at any session. As such, this population would not—solely on the basis of CVR assessment—be expected to exhibit sufficiently deviant brain function as to readily be diagnosed with a symptomatic mTBI.

While there were no significant group differences in β-weight distributions between controls and soccer athletes, the ensemble of CVR measurements for soccer athletes primarily in *HighLoad* groups did progress from being more elevated at *Pre* than expected by chance relative to the control median, to being increasingly lower at each follow-up session than expected by chance relative to the control median. Given that the within-season changes associated with this drop are comparable to those observed in symptomatic mTBI, athletes who reached the lowest quartile of the "normal" CVR measurement spectrum may be at greater risk for developing symptomatic injury. Additionally, elevated CVR measures at *Pre* relative to controls—a trend that visually emerges again at *Post3*—may be indicative of a neuroprotective or compensatory effect,

likely mediated by the nitric oxide pathway (Zhang et al., 2002), occurring in response to repetitive head trauma.

The current results suggest that limiting the cumulative mechanical loading athletes sustain throughout a season and allowing for adequate rest time after the season may be beneficial in maintaining brain health. Such actions may contribute to reducing the incidence of mTBI in female soccer athletes. Quantification of a threshold related to cumulative loading above which, risk for injury increases will be pivotal in instituting general head impact regulations that are best suited to protect female soccer athletes from the long-term risks associated with repetitive head impacts.

CHAPTER 6. AIM2 - EFFECT OF MINIMUM PTA THRESHOLD ON PREDICTING CVR CHANGES IN LOADING GROUPS

Extending the findings detailed in CHAPTER 5 (Svaldi et al., 2016), we add data from an additional season of play and assess the effect of varying the minimum peak translational acceleration (PTA) threshold used to calculate cumulative load on identifying individuals with CVR decreases. We hypothesize that increasing the minimum PTA threshold in calculation of cumulative loading will better identify individuals exhibiting CVR decreases.

6.1 Methods for Evaluation of Effect of Minimum PTA Threshold

High School Participants: Female participants included 22 soccer athletes (ages 15-17; mean 15.9) and 12 non-collision sport control athletes (ages 15-18; mean 15.9). Control athlete data were collected over one season of play. Soccer athlete data were collected over two seasons of play, with 7 of the 22 athletes participating in both seasons. As our previous work showed that CVR in these 7 athletes returned to baseline levels, data from consecutive seasons were treated as separate observations yielding a total of 29 athlete-season observations. No included participant was diagnosed with mTBI during the course of the study.

Imaging Protocol Schedule: Athletes were imaged as described in CHAPTER 4. However, as survey data revealed that most athletes were participating in club soccer during the period of the *Post2* scan, data from this session will not be included in this analysis. The *Post1* scan will be referenced as the *Post* scan for this section.

Head Acceleration Event Analysis: Because cPTA and cPAA measurements were found to be highly correlated, we focus only on cPTA as this is the more accurate of the two measurements (McCuen et al., 2015). For each soccer athlete, the (to-date) cumulative PTA (CPTA) (Eqn. 5.1) was calculated at each imaging session. To test the contribution of various levels of acceleration events to changes in CVR, the CPTA was calculated using four minimum PTA thresholds: 20g, 30g, 50g, and 70g (approximately corresponding to the 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile for all impacts recorded over the two seasons). Because imaging sessions (*In1, In2, Post*) were conducted over periods of six weeks and several athletes experienced comparable CPTA despite their scans being several weeks apart (Figure6.1), CPTA was normalized by the number of days over which the impacts were accrued for each *subject-session* to give a final measure of normalized CPTA (nCPTA). For in-season imaging sessions (*In1-2)* this normalizing factor was the number of days from the start of the season until the scan date. For the *Post* session the normalizing factor was the number of days over which the contact season extended (start to end). At each imaging session, Soccer athletes were divided into three groups, reflecting the upper (*High, n = 10*), middle (*Mid, n = 9*), and lower (*Low, n = 10*) thirds of the nCPTA distribution.

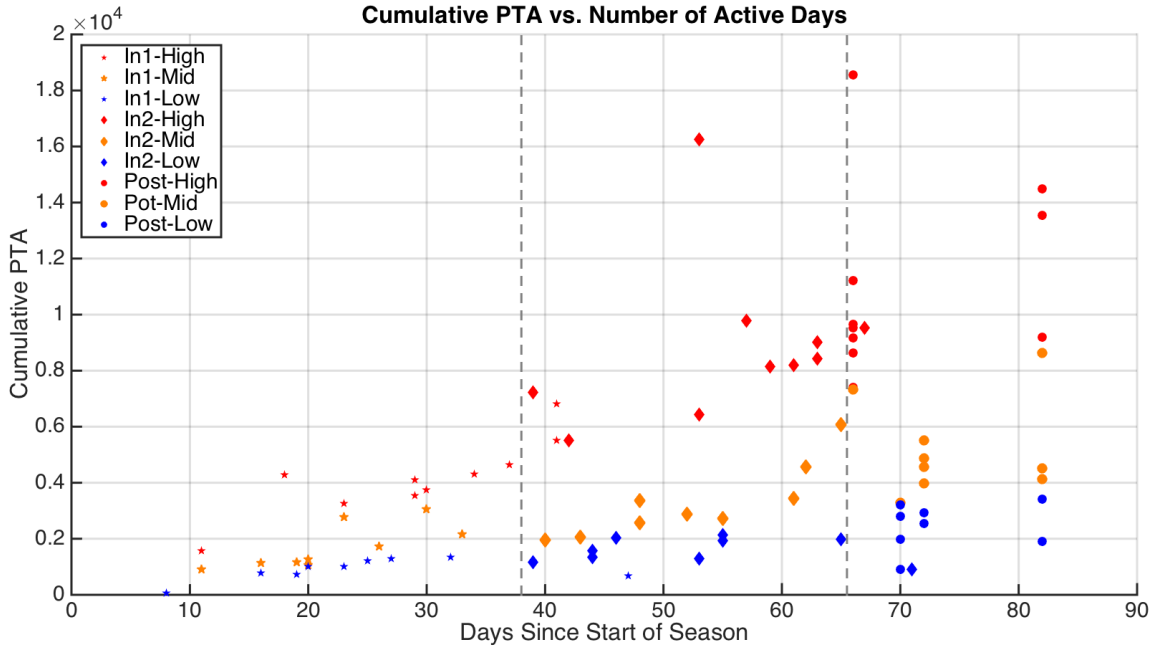

Figure 6.1 Scatter plot of cumulative peak translational acceleration (CPTA) versus the number of days since start of the season for loading groupsat each session, for a minimum PTA threshold of 20g. Normalized CPTA groups—calculated using CPTA normalized by number of days since the start of the season (nCPTA)— groups are coded using colors while measurements belonging to different sessions are coded using different marker types, as explained in the legend. Circled pairs of observations are examples of athletes experiencing comparable cumulative loads (<= 100g apart) at their imaging sessions, despite imaging sessions being over 10 days apart.

Analysis of Cerebrovascular Reactivity: Groups evaluated at each session for changes in CVR as a function of exposure included: (1) the *Total* cohort of soccer athletes; (2) *High, Mid*, and *Low* loading subgroups of soccer athletes for each minimum PTA threshold, and the (3) control athlete cohort. To assess whether distributions of GM fractional change in β-weight from *Pre/Test* differed significantly from zero for any group, 10,000 bootstrapped random samples of size n = 9, corresponding to the sample size of the smallest loading group, were generated for each group in order to estimate a 95% confidence interval on the median of each group (Figure6.2). This bootstrapping scheme was repeated 20 times. Distributions were deemed significantly different from zero if

zero was outside of the 95% confidence interval for 6 of the 20 trials as this is more than can be expected by chance (Binomial Test, $\alpha = 0.05$).

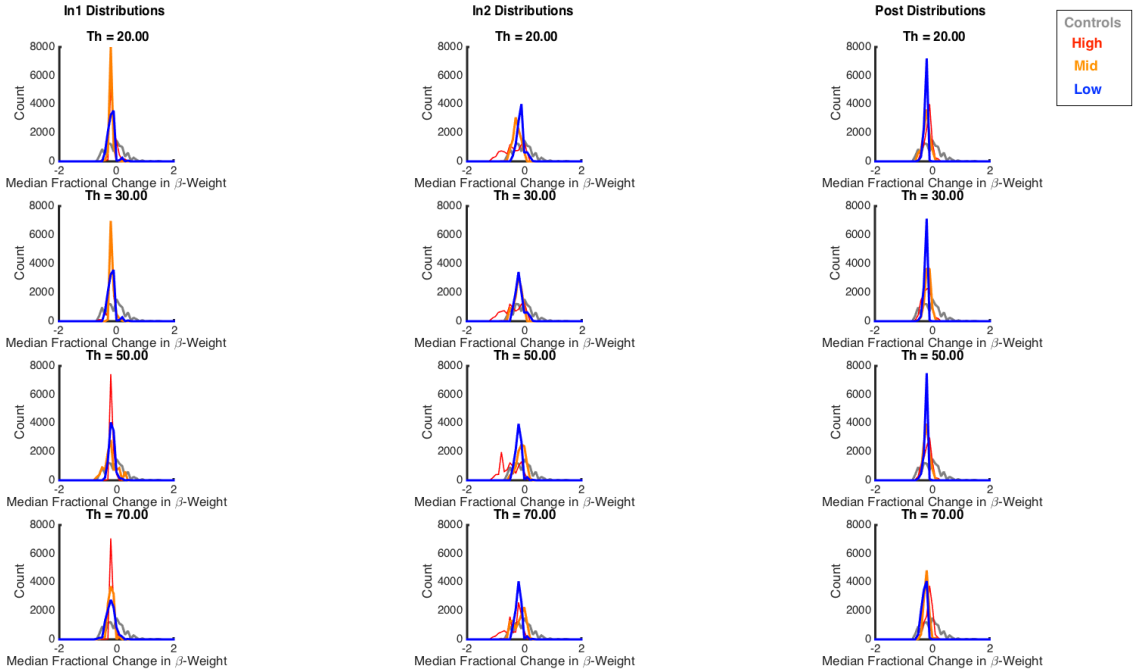

Figure 6.2 Histograms of the medians calculated from 10,000 bootstrapped random samples for all loading groups for each minimum PTA threshold at each assessment session.

Because grouping based on *nCPTA* at the *In2* session consistently identified soccer athletes exhibiting the greatest CVR decreases from *Pre* across thresholds, a linear fit (Eqn. 6.1) was used to model the relationship between *nCPTA* and fractional change in β-Weight from *Pre* at *In2* ($\Delta\beta_{Pre}$) for each threshold.

$$\Delta\beta_{Pre} = x_0 + x_1 * nCPTA \qquad (6.3)$$

To robustly assess the fits, a sensitivity analysis was conducted whereby each data point ($nCPTA$, $\Delta\beta_{Pre}$)was iteratively removed from the sample and the regression was repeated. For each iteration, the R^2 was calculated and an *F-test* ($\alpha = 0.05$) was conducted to compare the resulting model to a constant model. To assess the model for

each threshold, the mean of each coefficient (x_0, x_1), the mean R^2, and mean *p-value* of all iterations was calculated for each threshold. To assess whether the fit for any given for each threshold significantly explained more variance, a repeated measures ANOVA *(α = 0.05)*, followed by pairwise t-tests *(α = 0.05)*, was conducted on the distribution of R^2 values for each threshold.

6.2 Results of Evaluating Minimum PTA Threshold

Control athletes did not exhibit a significant difference in fractional change in β-weight from *Test* at their follow-up session (Figure6.3). The *Total* cohort of soccer athletes also did not exhibit significant decreases in β-weight from *Pre* at *In1-2* (Figure2), although there is a visible negative trend below zero at these follow-up sessions. The *Total* cohort did exhibit significant CVR decreases from pre at the *Post* session.

In the first half of the season, grouping the athletes by nCPTA and raising the minimum PTA threshold did not consistently attribute CVR decreases to any given loading group (Figure6.3). Despite only the *Mid* and *High* loading groups exhibiting significant differences from *Pre,* each of these groups only exhibited significant decreases from *Pre* for one of the four minimum PTA thresholds. The *High* loading group exhibited a significant difference from *Pre* only when the minimum impact threshold was 50g while the *Mid* loading group evidenced a significant decrease in CVR from *Pre* only for the 20g minimum impact threshold. The *Low* loading group never exhibited a significant decrease from *Pre*, despite the distributions of fractional CVR change from baseline appearing to skew below zero at the *In1* imaging session.

Consistent with the original hypothesis, grouping athletes by nCPTA in the second half of the season showed led to only the *Mid* and *High* loading groups exhibiting significant decreases in CVR from *Pre* (Figure6.3). Significant CVR decreases were concentrated in the *Mid* groups for minimum thresholds of 20g and 30g. When the minimum impact threshold was raised to 50g, significant CVR decreases were concentrated in the *High* loading group, a result which still held at 70g.

At the *Post* session, all loading groups exhibited significant decreases from *Pre* for at least one of the four minimum impact thresholds (Figure6.3). The *High* loading group exhibited significant decreases from *Pre* for the 30g minimum impact threshold while the *Mid* loading group exhibited significant decreases from *Pre* for the 70g minimum impact threshold. The *Low* group exhibited significant decreases from *Pre* for all impact thresholds.

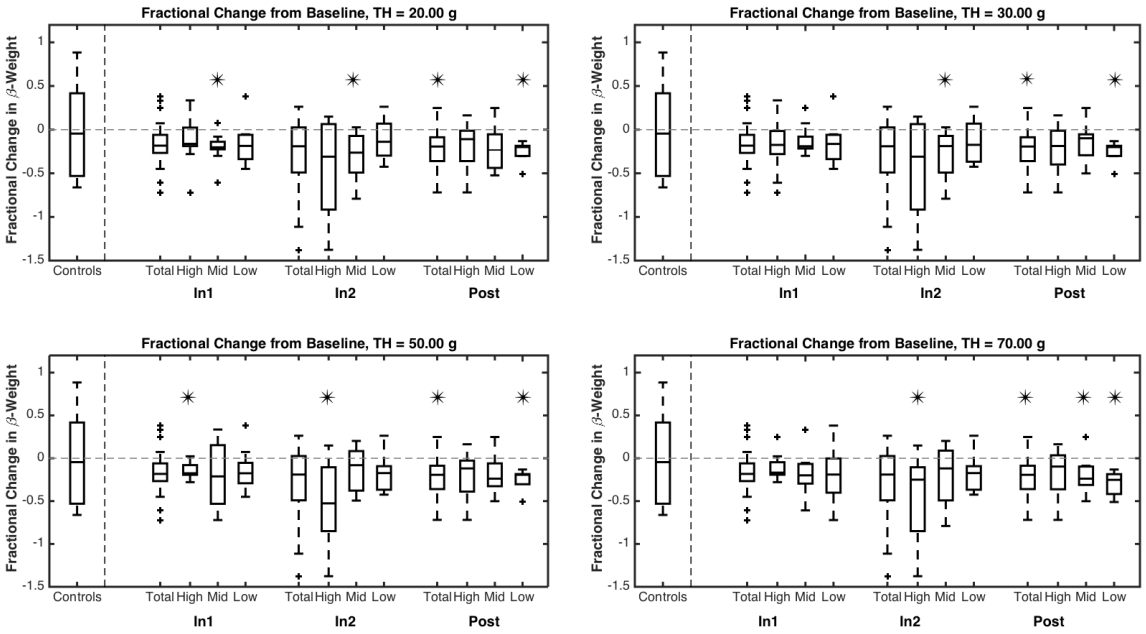

Figure 6.3 Box and whisker plots of Fractional Change in β-weight from *Pre/Test* for loading groups (*Total* n = 29, *High* n = 10, *Mid* and *Low* n = 9) assessed using nCPTA. Distributions exhibiting significant deviations from zero (*95%* CI on median generated using 10,000 bootstrap random samples, binomial test $p < 0.05$) are designated by asterisks. The midline of the box represents the median of the distribution and the top and bottom edges represent the first and second quartiles, respectively; outliers are denoted with plus signs.

Regardless of threshold value, all linear fits showed a significant negative relationship between *nCPTA* and *In2* fractional change in β-weight from *Pre*. ANOVA and pair-wise tests revealed that there was a significant effect of threshold on the variance explained by each fit. The variance explained by each fit was significantly different from the variance explained by every other fit, with the 50g threshold explaining the most variance of the four thresholds (21.9%).

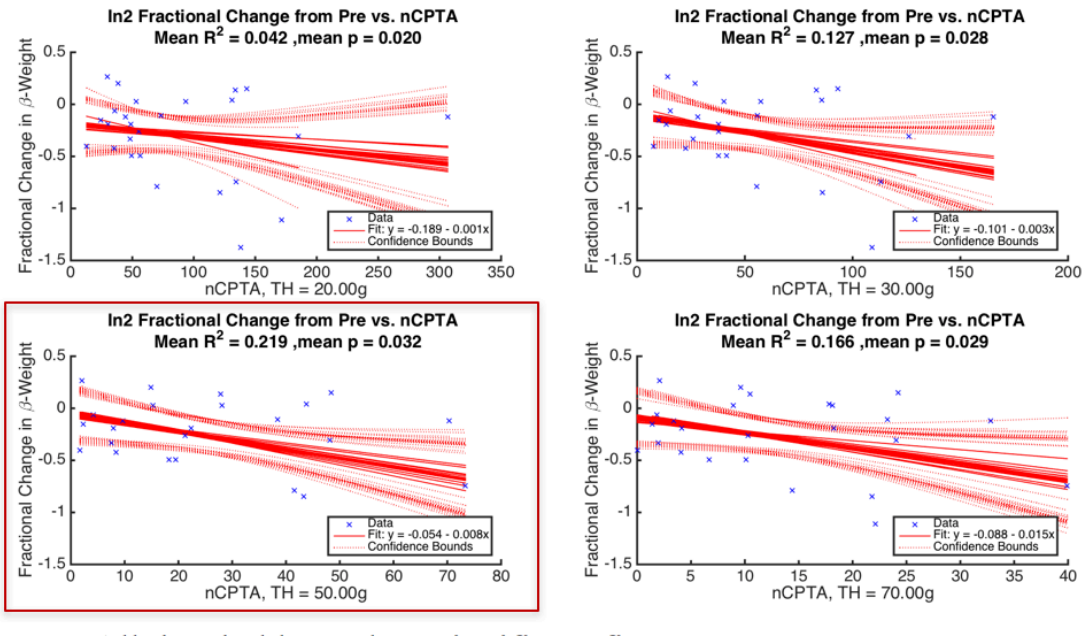

Figure 6.4 Linear fits between *nCPTA* and *In2* fractional change in *β-weight* from *Pre*. Each data point was iteratively removed and the model was re-fit, producing a total of 29 fits for each threshold. The mean R^2 and mean *p-values* (F-Test against constant model, $\alpha = 0.05$) were calculated for each threshold. Variance explained was significantly different between all fits (repeated measures ANOVA, paired t-test $\alpha = 0.05$). Variance explained was maximized for a threshold of 50g.

6.3 Impact of Evaluating Minimum PTA Threshold

Addition of a second season of data continued to reveal subacute CVR decreases in soccer athletes as a result of participation in soccer. This decrease became more pronounced as cumulative loading increased over the season, and was most pronounced in soccer athletes receiving the greatest cumulative loads. Increasing the minimum PTA threshold used to calculate groupings based on nCPTA led to robust concentration of individuals with CVR decreases within the *High* loading groups in the second half of the season. Furthermore, in the second half of the season linear fits used to model the relationship between cerebrovascular reactivity and cumulative load over a season showed a significant negative relationship. The variance explained by the model was

maximized at 50g, suggesting that limiting accumulation of impacts exceeding a PTA threshold around 50g may help reduce neurovascular changes associated with mTBI in a female high school soccer demographic. Finally, CVR decreases at the *Post* imaging session—conducted after one to two months of recovery from the contact season—support the notion that appreciable recovery time is necessary for the brain to return to normal following a season of contact-sport participation.

CHAPTER 7. AIM 1 – TEST FOR RS FUNCTIONAL CONNECTIVITY ALTERATIONS IN FEMALE HIGHSCHOOL SOCCER PLAYERS

For this work, RS functional connectivity over a season of play in female soccer athletes as well as non-collision sport control athletes was assessed. Functional connectivity and organization were assessed using a graph theoretical paradigm (Bullmore & Sporns, 2009). This paradigm models the brain's functional organization as a complex network, referred to as a connectome, of regions that are functionally coupled. Edges in this network are measures of functional coupling between regions, typically measures of statistical dependence between the FMRI time series between two regions such correlations, coherence, or mutual information. This paradigm was chosen because it allows for both whole brain characterization of the brain's functional organization in terms of graph organizational properties such as integration and segregation and also allows for data driven identification of important subnetworks.

High School Participants: Data from thirty athletes was used in this portion of the study. *Soccer athletes:* 17 athletes (ages 15-17; mean 15.9) were members of high school junior varsity or varsity soccer teams, representing two high schools (8 team 1, 9 team 2). All seventeen athletes were scanned according to the study paradigm above (*Pre, In1, In2, Post1, Post2)*. Additionally, 8 of the 17 soccer athletes were also scanned 8 months after the end of the season (*Post3). Control athletes:* 13 athletes (ages 15-18; mean 15.9)

participated only in non-collision high school sport junior varsity or varsity teams (7 basketball; 3 track & field; 2 cross-country; 1 each softball, gymnastics and swimming).

7.1 RS Imaging Protocol

Scans were conducted as per the imaging schedule described in CHAPTER 4. For each subject-session, RS data was acquired on the same day as CVR data also at the Purdue University MRI Facility (West Lafayette, IN), using a 3-T General Electric Signa HDx (Waukesha, WI) with a 16-channel brain array (Nova Medical; Wilmington, MA). A single resting state functional FMRI run was acquired using gradient-echo echo-planar sequence (length 9 min 48 sec; TR/TE = 2000/26 msec; 20cm FOV; 64x64 matrix; 34 slices; 3.8 mm thickness; 117 volumes. This run was acquired immediately following two functional working memory scans. The same T1-weighted anatomical scan acquired using a 3D spoiled gradient echo sequence (TR/TE 5.758ms/2.032ms, flip angle=73°, 1mm isotropic resolution) was used for registration purposes.

7.2 RS Functional Connectivity Processing

Preprocessing: Data processing was performed using FSL using standard fMRI processing guidelines (Amico et al., 2016) including slice timing correction, motion correction, intensity normalization to mode 1000, demeaning and linear detrending. Motion was regressed out using 12 regressors consisting of translations [x y z] and rotations [roll pitch yaw] and their corresponding derivatives. Tissue noise was regressed out using 3 regressors (mean whole brain time series, mean WM time series, mean CSF time series). A procedure to censor high motion volumes from the regression was

implemented based on frame displacement, changes in signal intensity between volumes, and the standard deviation of the BOLD signal of within brain voxels at each TR, as detailed in (Amico et al., 2016). Athletes with more than 50% of volumes censored were discarded from the study. Subsequently, a first-order Butterworth bandpass filter [0.001 Hz 0.8 Hz] was applied to the resulting RS functional volumes in the forward and reverse directions. Finally, the first five principal components of the WM and CSF masks were regressed out of the GM mask. To extract tissue masks for tissue related regressors, T1 anatomicals were denoised, bias field corrected, and then segmented using the FAST algorithm in FSL. These segmentations were then warped into the functional space using the FLIRT algorithm in FSL (6dof).

Graph Theoretical Methods: The pipeline used to construct connectomes from the processed RS FMRI data can be seen in Figure7.2. Connectomes were constructed in the functional space of each subject-session using a parcellation of 278 functional regions (Shen, Tokoglu, Papademetris, & Constable, 2013) in the MNI 152 space. This parcellation was chosen because it is a functional parcellation specifically designed for graph theoretical analyses of brain organization and was created with eighty healthy subjects. The parcellation was firt warped from the MNI space into the subject-session specific T1 space using both linear and non-linear transformations in FSL. (FLIRT, FNIRT) and then into the FMRI space using the previously calculated transform. FSL boundary-based-registration was additionally applied. Functional connectivity matrices were derived by calculating the Pearson correlation coefficient (Eqn. 7.1) between mean time-series of all regions creating connectomes of size 278 x 278.

$$r_{ij} = \frac{\sum_{k=1}^{n}(T_{ik}-\overline{T_i})(T_{jk}-\overline{T_j})}{\sqrt{\sum_{k=1}^{n}(T_{ik}-\overline{T_i})^2}\sqrt{\sum_{k=1}^{n}(T_{jk}-\overline{T_j})^2}} \quad (7.1)$$

In 7.1, T_i and T_j are the mean time-series for ROIs *i* and *j* respectively, n is the total number of time-points in the time-series for ROIs *i* or *j*, r_{ij} is the Pearson correlation coefficient between ROIs *i* and *j*, and finally, $\overline{T_i}$ and $\overline{T_j}$ are the sample means for the *i*-th, and *j*-th ROI time-series.

A Fisher's transform was then applied to all r_{ij} (7.2) to give final transformed correlation coefficients $z_{ij.}$

$$z_{ij} = \frac{1}{2}\ln\left(\frac{1+r_{ij}}{1-r_{ij}}\right)$$

Self-connections were subsequently removed from the resulting connectomes by setting the diagonal of the connectome matrix to zero. Anti-correlations were removed by setting all negative elements of the connectome to zero. Finally, connectomes were ordered according to 7 resting state subnetworks, visual (VIS), somato-motor (SM), dorsal attention (DA), ventral attention (VA), limbic (L), fronto-parietal (FP), and default mode network (DMN), described in (Yeo et al., 2014) with the additional two, subcortical (SBU) and cerebellar (CER), described in (Amico et al., 2016) (Figure7.1). These subnetworks were chosen because they were created using a data driven ICA approach and were shown to be consistent across 1000 healthy subjects. The final resulting connectomes are weighted, undirected networks representing the functional organization at a given *subject-session* (Figure 7.1).

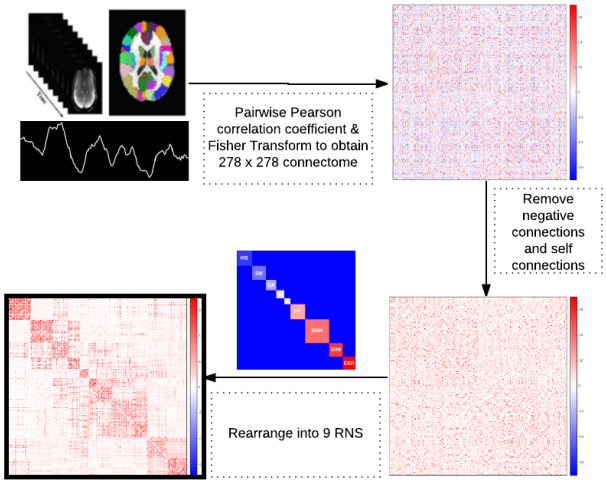

Figure 7.1 Pipeline for construction of connectomes from RS FMRI data for each subject-session. Pairwise pearson correlation coefficients were computed between mean time series of all ROIs (Shen et al., 2013) resulting in connectomes of size 278x278. Self connections and negative connections were removed. Finally connectomes were rearranged according to 9 RSNs (Amico et al., 2016; Yeo et al., 2014). Final bolded connectomes contain weights w_{ij} associated with all edges in the network.

These networks are comprised of $n = 278$ nodes corresponding to the regions in the Shen parcellation which comprise N the set of all nodes in the network. The connections between each of these regions comprise L the set of all edges in the network, where each element w_{ij} in the final connectome represents the connectome weight associated with edge (i,j) in the connectome.

7.3 Graph Theoretical Analysis

Soccer athlete and control athlete connectomes were assessed for differences in three categories: *(1)* measures of basic global network connectivity, *(2)* measures of global network integration, *(3)* measures of network segregation.

Measures of Basic Network Connectivity*:* To assess global changes in RS functional connectivity in both soccer athletes and controls two basic network measures, degree and strength, were calculated for connectomes at each *subject-session (Bullmore & Sporns, 2009)*. Degree of a network is defined as the mean degree per node, where degree per node, k_i (Eqn. 7.3), is the number of edges connected to a given node.

$$k_i = \sum_{i,j \in N} a_{ij} \quad (7.3)$$

$$a_{ij} = 1 \; when \; w_{ij} \; is \; positive \; and \; zero \; otherwise$$

Strength of a network is defined as the mean strength per node, where strength per node, s_i (Eqn. 7.4), is defined as the sum of all weights associated with edges connected to the node.

$$s_i = \sum_{i,j \in N} w_{ij} \quad (7.4)$$

Measures of Global Integration: Additionally, to assess global network efficiency, measures of network integration were calculated for connectomes at each *subject-session* (Bullmore & Sporns, 2009*)*. Measures of network integration assessed included shortest path length and mean first passage time. Shortest path length, *SPL* (Eqn. 7.5), is defined as the average distance along the shortest paths, $d_{i,j}^{min}$ (Eqn. 7.6), between all possible node pairs *(i,j)*. The length of a given path, $d_{i,j}$ (Eqn. 7.7), between nodes *(i,j)* is defined as the sum of all the inverse of all weights associated with edges in the path.

$$SPL = \frac{\sum_{i,j \in N, i \neq j} d_{i,j}^{min}}{\frac{1}{2} n(n-1)} \quad (7.5)$$

$$d_{i,j}^{min} = \min\left(d_{ij}\right) \quad (7.6)$$

$$d_{i,j} = \sum_{u,v \in g_{i \to j}} \frac{1}{w_{u,v}} \quad (7.7)$$

$$where\ g_{i\to j}\ is\ a\ given\ set\ of edges\ between\ i\ and\ j$$

Mean first passage time (MFPT) of a network is defined as the mean of MFPT's between each node pair *(i,j)* in the network. MFPT (Eqn. 7.8) between a given node pair, *(i,j)*, is defined as the expected value of the passage time in a random path between the two nodes. Passage time, $t_{g_{i\to j}}$ (Eqn. 7.9), for a given path between two nodes is defined as the sum of all weights along the path between any two nodes *(i,j)*. MFPT for a given node is simulated using 100 random walks between each node pair *(i,j)* and calculating the average passage time in these 100 random paths.

$$MFPT_{i,j} = E\left[T_{G_{i\to j}}\right] \tag{7.8}$$

$$T_{G_{i\to j}}\ is\ the\ set\ of\ times\ associated\ with\ the\ set\ G_{i\to j}\ of\ paths\ between\ (i,j)$$

$$t_{g_{i\to j}} = \sum_{u,v\in g_{i\to j}} w_{u,v} \tag{7.9}$$

$$where\ g_{i\to j}\ is\ a\ given\ set\ of\ edges\ between\ i\ and\ j$$

Measures of Network Segregation: Finally, measures of network segregation were calculated for connectomes at each *subject-session (Bullmore & Sporns, 2009)*. Measures of segregation calculated included clustering coefficient, transitivity, and modularity. Clustering coefficient is defined as the mean of the clustering coefficients of each node. The weighted clustering coefficient (Barrat, Barthelemy, Pastor-Satorras, Vespignani, & Parisi, 2004), c_i (Eqn. 7.10), quantifies weights of connections that exist between the nearest neighbors of a node normalized by the weights of all possible connections of a given node.

$$c_i = \frac{\sum_{j,h\in N} \frac{w_{i,j}+w_{i,h}}{2} * a_{i,j} * a_{i,h} * a_{j,h}}{s_i * (k_i - 1)} \tag{7.10}$$

Transitivity of a network, *T* (Eqn. 7.11), is very similar to clustering coefficient, however normalization is performed over all possible connections in the network.

$$T = \frac{\sum_{i \in N} \sum_{j,h \in N} \frac{w_{i,j} + w_{j,h}}{2} * a_{i,j} * a_{i,h} * a_{j,h}}{\sum_{i \in N} s_i * (k_i - 1)} \quad (7.11)$$

Finally, computation of a modularity scores seek to assess the strength of the division of a network into modules. Modularity algorithms iteratively partition the network such that the number of edges within modules is optimized (Blondel, Guillaume, Lambiotte, & Lefebvre, 2008), thereby optimizing the modularity score *Q (Newman, 2004)* (Eqn 7.12) .

$$Q = \frac{1}{l^w} \sum_{i,j \in N} \left[w_{ij} - \frac{s_i * s_j}{l^w} \right] \delta_{m_i, m_j} \quad (7.12)$$

$$where\ m_i\ is\ the\ module\ containing\ node\ i, \delta_{m_i,m_j} = 1\ if\ m_i$$

$$= m_j\ and\ zero\ othewise$$

$$l^w = \sum_{i,j \in N} w_{ij}$$

For this work, the Louvain (Blondel et al., 2008) algorithm was used to calculate the optimized partition and associated modularity score, $Q_{Louvain}$, for the connectome. A modularity score, Q_{Yeo}, was also calculated for the Yeo and SUB, CER partition, which represents "average" human, functional brain network organization. Finally, the ratio (Eqn. 7.13) of Q_{yeo} to $Q_{Louvain}$ was computed to assess how the Yeo partition related to the "ideal" partition of the connectome.

$$Q_{Yeo} = \frac{Q_{Yeo}}{Q_{Louvain}} \quad (7.13)$$

To assess changes in the network measurements delineated above, within group comparisons and between group comparisons were performed on all network measurements for soccer athletes and control athletes at an $\alpha = 0.05$ significance level.

Because these network measurements have shown to follow a normal distribution across people, t-tests were used to conduct all statistical comparisons. Pairwise paired t-tests relative to *test* in Controls and *Pre* in soccer athletes were used for within group comparisons. Pairwise unpaired t-tests, relative to both the *test* control athlete session, were used to conduct between group comparisons, after confirming no significant difference in measurement between the *Test* and *Re-test* control sessions. Pairwise tests conducted across multiple sessions were corrected for multiple comparisons across sessions using FDR correction.

Subnetwork Measures: To assess whether global changes in basic network connectivity were localized to a specific subnetwork and to help interpret modularity results, within sub-network strength and between sub-network strength were also calculated. Within network strength was defined as the mean of the within network strengths of each node in a given network. Within network strength for a given node is defined as the sum of the weights of all connections with nodes also within the given network (Eqn. 7.14).

$$s_{iA}^{within} = \sum_{j \in A} w_{i,j} \qquad (7.14)$$

Between network strength for a given node was defined as the mean of the between network strengths of each node. Between network strength for a given node is defined as the sum of the weights of all edges between node *i* in the given network and all nodes not within the given network (Eqn. 7.15).

$$s_{iA}^{between} = \sum_{j \notin A} w_{i,j} \qquad (7.15)$$

Statistical comparisons on sub-network strength and within sub-network strength were conducted in the same manner as those conducted on global network properties.

After verifying that there were no within group effects in within or between subnetwork connectivity, one mean connectome was calculated for each group (Figures7.1-3). This was done by taking averaging connectomes across all subjects and sessions (excluding *Post3* for soccer athletes) for each group (Soccer, Controls). Mean connectivity (w_{ij}) for within subnetwork connections and between subnetwork connections was computed for each RSN from these connectomes. Control strength measures were subtracted from soccer strength measures and the corresponding difference for each RSN was displayed on the MNI152 template to provide a visual representation of within network and between network connectivity differences in each RSN.

7.4 Graph Theoretical Results

Within group results: Controls showed no significant changes between the *Test* and *Re-test* sessions for any of the global measures of connectivity, global measures of integration, or global measures of segregation (Figure 7.2). There were also no significant differences in between or within subnetwork strengths between *Test* and *Re-test* sessions (Figure 7.3). This confirms the stability of functional organization in "healthy" population. Similarly, soccer athletes did not exhibit significant changes from the *Pre* session in any category of global network measures (Figure 7.2) at any follow-up session, either before or after multiple comparisons correction.

No within group differences in subnetwork measures (Figures 7.3 & 7.4) were found for soccer athletes at any follow-up session, either before or after multiple comparisons correction.

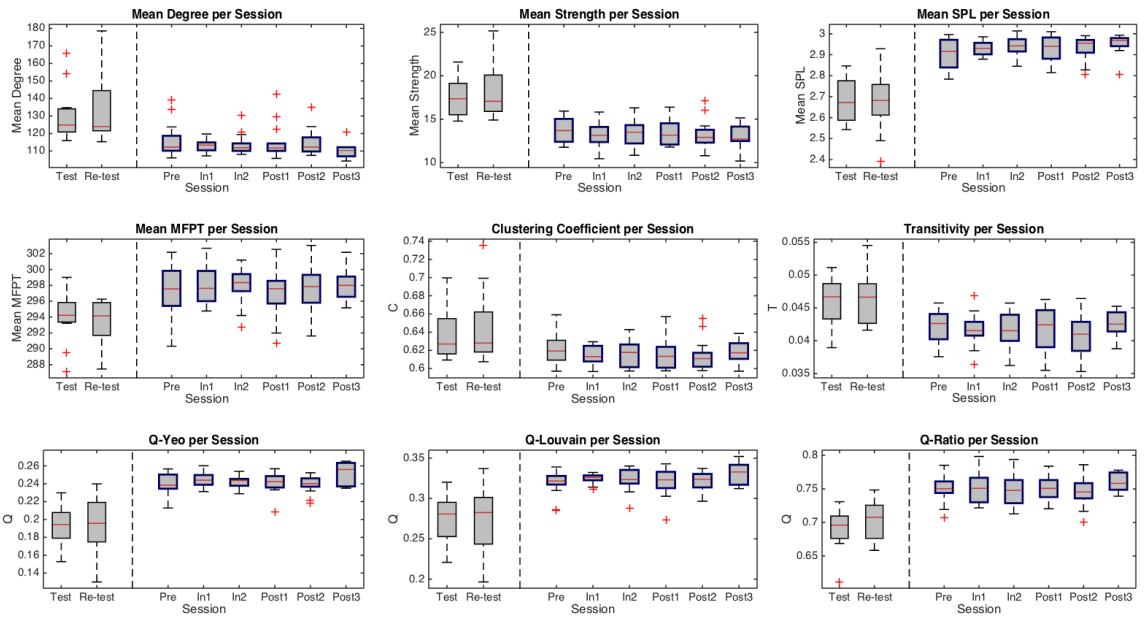

Figure 7.2 Distributions showing global network measures of basic connectivity (degree, strength), integration (SPL, MFP), and segregation (C, T, $Q_{Louvain}$, Q_{Yeo}, Q_{Ratio}) for 13 non-collision sport control athletes (*Test, Re-test)* and 17 soccer athletes *(Pre, In1-2, Post1-3)*. Note that only 8 of the 17 athletes underwent a *Post3* imaging session. Bold blue boxes indicate soccer athlete distributions significantly differing (unpaired t-test, $p_{fdr} < 0.05$) from Control *Test* session distributions. Neither control athletes nor soccer athletes exhibited significant deviations (paired t-test, $p_{fdr} < 0.05$) from corresponding baseline sessions (Controls *Test,* Soccer *Pre*) at any follow-up imaging session (Controls *Re-test*, Soccer *In1-2 & Post1-3*).

Between group results: Though soccer athletes did not exhibit significant changes in RS functional connectivity over the season, soccer athletes did exhibit stark differences, at all imaging sessions including *Post3*, in basic network measures, measures of network integration, and measures of network segregation as compared to controls (Figure 7.2). Comparisons of basic network measures revealed that soccer athlete brains exhibited significantly fewer connections (degree) as well as significantly weaker connections (strength) than control athlete brains. In terms of measures of integration, soccer athlete connectomes exhibited longer SPL and longer MFPT as compared to control athletes.

Comparisons of network segregation also revealed differences between soccer athlete brains and control athlete brains. Both clustering coefficient and transitivity were significantly lower in soccer athletes as compared to control athletes. However, soccer athlete connectomes exhibited significantly higher modularity scores, both for the ideal partition and the Yeo partition, as compared to soccer athletes. The modularity ratio, Q_{Ratio}, was significantly higher in soccer athletes as compared to control athletes.

Subnetwork comparisons showed that both between subnetwork connections and within subnetwork connections accounted for global differences in network strength between soccer athletes and control athletes, though between network connections were most affected. All nine soccer athlete subnetworks exhibited significantly weaker between network connections (Figure 7.3) as compared to control athlete brain subnetworks. In contrast, only five of nine soccer athlete brain subnetworks (VIS, DA, L, FP, and DMN; Figure 7.4) exhibited significantly weaker within network connections, at all imaging sessions, as compared to control brain subnetworks (Figure 7.3). As seen in Figure 7.5, the differences between control subnetwork connectivity and soccer subnetwork connectivity are more pronounced for between network connections than they are for within network connections.

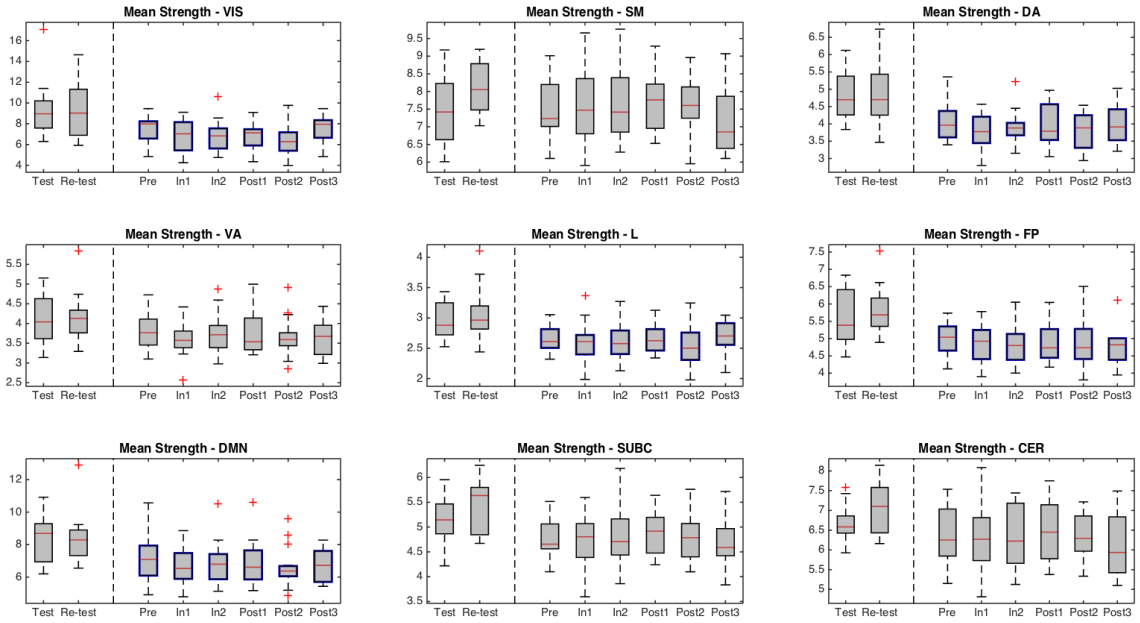

Figure 7.3 Distributions of within-network strength for 13 non-collision sport control athletes (*Test, Re-test)* and 17 soccer athletes *(Pre, In1-2, Post1-3)*. Note that only 8 of the 17 athletes underwent a *Post3* imaging session. Bold blue boxes indicate soccer athlete distributions significantly differing (unpaired t-test, $p_{fdr} < 0.05$) from Control *Test* session distributions. Neither control athletes nor soccer athletes exhibited significant deviations (paired t-test, $p_{fdr} < 0.05$) from corresponding baseline sessions (Controls *Test,* Soccer *Pre*) at any follow-up imaging session (Controls *Re-test*, Soccer *In1-2 & Post1-3*).

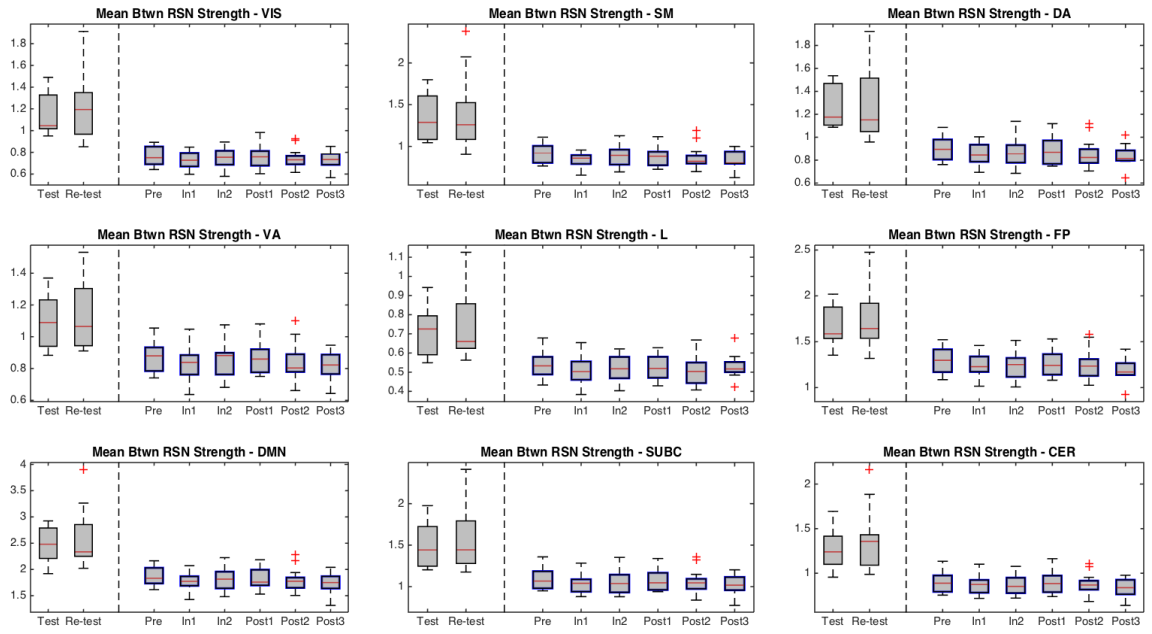

Figure 7.4 Distributions of between-network strength for 13 non-collision sport control athletes (*Test, Re-test)* and 17 soccer athletes *(Pre, In1-2, Post1-3)*. Only 8 of the 17 athletes underwent a *Post3* imaging session. Bold blue boxes indicate soccer athlete distributions significantly differing (unpaired t-test, $p_{fdr} < 0.05$) from Control *Test* session distributions. Neither control athletes nor soccer athletes exhibited significant deviations (paired t-test, $p_{fdr} < 0.05$) from corresponding baseline sessions (Controls *Test,* Soccer *Pre*) at any follow-up imaging session (Controls *Re-test*, Soccer *In1-2 & Post1-3*).

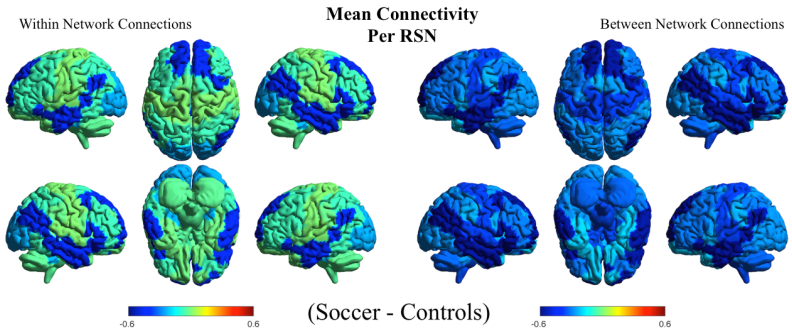

Figure 7.5 Difference between soccer athletes and control athletes in mean subnetwork connectivity (w_{ij}). (left) Difference in mean within subnetwork connectivity. (right) Difference in mean between network connectivity. Differences were quantified from mean connectomes for each group. Mean connectomes were calculated by averaging all connectomes, for each group, across athletes and sessions.

7.5 Impact RS Functional Connectivity Results

Evidence of Chronic Hypoconnectivity Between Networks in Soccer athletes: Lack of changes in any network measures across sessions in control athletes attests to the stability of FMRI measurement of resting state functional brain organization in healthy subjects. Overall, the findings in soccer athletes show reduced connectivity in comparison to control athletes which is already present before the start of the contact season and is sustained throughout the contact season. This reduced connectivity is more pronounced in between network connections causing soccer athlete brains to be less globally connected and more modular, despite decreased clustering. All three measures modularity measures ($Q_{Louvain}$, Q_{Yeo}, and Q_{Ratio}) were higher for soccer athletes than for control athletes. Since the Yeo subnetwork partition represents a typical brain organization in healthy individuals, increases in both Q_{Yeo} and $Q_{Louvain}$ in soccer athletes as compared to control athletes show that soccer athlete connetomes maintain typical network organization but reduce communication between networks.

Comparison of Results with Other Graph Theory Studies of mTBI: Our findings both agree with and differ from other studies of similar properties in mTBI populations. Findings of reduced global strength and degree in female soccer athlete brains as compared to controls are consistent with studies showing global decreases in connectivity in patients with mTBI as compared to healthy controls (Messe et al., 2013; Nakamura et al., 2009; Stevens et al., 2012). Furthermore, findings of reduced connectivity in the DMN is also consistent with several studies of RSFC in mTBI populations (B. Johnson et al., 2012; Messe et al., 2013; Stevens et al., 2012), although also conflicting with others (Abbas et al., 2015; B. D. Johnson et al., 2014). Only three other studies to date have assessed RS functional connectivity using graph theoretical methods. Two of these studies were conducted on mTBI populations 3 to 6 months post injury (Messe et al., 2013; Nakamura et al., 2009) and the third one was conducted on football athletes experiencing repetitive head trauma (Abbas Manuscript Submitted). In agreement with our study, one study reported reduced global strength and increased shortest path length in mTBI patients examined 3months post injury (Nakamura et al., 2009). In contrast to our study, the second study of mTBI patients failed to find significant differences in global connectivity at any time point (Messe et al., 2013) and the third study (Abbas, manuscript submitted) hyperconnectivity in football athletes relative to non-collision sport controls. Assessments of network segregation from these three studies all conflicted with our findings of decreased clustering and increased modularity. Both studies assessing clustering either failed to find significant differences in clustering coefficient in mTBI patients as compared to healthy controls (Nakamura et al., 2009) or found a significant increase in clustering coefficient in football athletes with a history of

concussion as compared to controls (Abbas, manuscript submitted). Both studies assessing modularity reported decreased modularity in mTBI patients 6 months post injury (Messe et al., 2013) and in asymptomatic football athletes as compared to healthy controls (Abbas, manuscript submitted). The variability in changes found in mTBI populations attests to the variability of mTBI injuries, themselves.

Conclusions: In conclusion, this work has demonstrated pronounced differences in functional connectivity, present prior to the start of the contact season and sustained throughout the contact season, in asymptomatic soccer athletes as compared to control athletes that both agree and contrast with similar studies conducted on other mTBI populations. This hyppoconnectivity is most pronounced in between network connections causing soccer athlete brains to be less integrated than control athlete brains. Alterations observed can presumably be attributed to participation in soccer as the two populations of athletes studied are otherwise comparable. However, the changes do not appear to be modulated by subacute exposure to repetitive head trauma over one season of play, suggesting these to be chronic changes attributable to multiple years of play without adequate recovery.

CHAPTER 8. AIM2 - RELATING DIFFERENCES IN RS FUNCTIONAL CONNECTIVITY TO LENGTH OF CAREER

8.1 Grouping RS Functional Connectivity by Years of Experience

Here we seek to test whether group differences in RS functional connectivity exhibited between soccer athletes and non-collision sport control athletes in CHAPTER 7 are related to the years of exposure to repetitive head impacts in soccer athletes. Because group differences were already present at the *Pre* scan, and were sustained throughout the season, only data from the *Pre* imaging session was used for this assessment. This allowed for inclusion of more athletes, as athletes needed only have participated in the *Pre* imaging session to be included in this portion of the study.

High School Participant Grouping: Data from 37 athletes was used in this portion of the study. *Soccer athletes:* 24 athletes (ages 15-17; mean 15.9) were members of high school junior varsity or varsity soccer teams, representing two high schools (8 team 1, 9 team 2). All soccer athletes underwent imaging during the *Pre* session as described in CHAPTER 4. *Control athletes:* 13 athletes (ages 15-18; mean 15.9) participated only in non-collision high school sport junior varsity or varsity teams (7 basketball; 3 track & field; 2 cross-country; 1 each softball, gymnastics and swimming). Soccer athletes were grouped according to number of years of High School soccer experience. Athletes with 3-4 years of experience (n = 12) were separated from athletes with 1-2 years of experience (n = 12).

Graph Theoretical Analysis: Following grouping, distributions of whole brain measures of basic network connectivity, network integration, and network segregation were generated for each soccer subgroup in the same manner as described in CHAPTER 7. Corresponding soccer subgroup (3-4 years, 1-2 years) distributions were compared to each other using unpaired ttests. Each soccer subgroup distribution was also compared to the corresponding *Test* control distribution again using an unpaired ttest. For this section, multiple comparisons correction was performed across measures for each subgroup using FDR correction.

8.2 RS Functional Connectivity Grouping Results

As seen in Figure 8.1, there were no differences in basic network measures, measures of integration, or measures of segregation between soccer athletes with 3-4 years of high school experience versus soccer athletes with 2-3 years of high school experience.

Adding soccer athletes to the *Pre* cohort of soccer athletes and dividing soccer athletes into subgroups based on years of experience did not change the relationship between soccer athlete connectivity measures and control athlete connectivity measures seen in CHAPTER 7. Both subgroups of soccer athletes (3-4 years, 2-3 years) showed significant differences in comparison to control athletes in all categories of network comparable to those observed in CHAPTER 7. Both groups continued to show global decreases in degree and strength causing decreased global integration as evidenced by increased SPL as well as increased MFPT. Both subgroups of soccer athletes showed decreased clustering and transitivity as compared to control athletes, but increased

modularity scores for the Yeo partition, the optimized Louvain partitions, and for the ratio of the modularity scores for the two partitions. Regardless of years of experience, soccer athlete brains were less globally integrated than non-collision sport control brains.

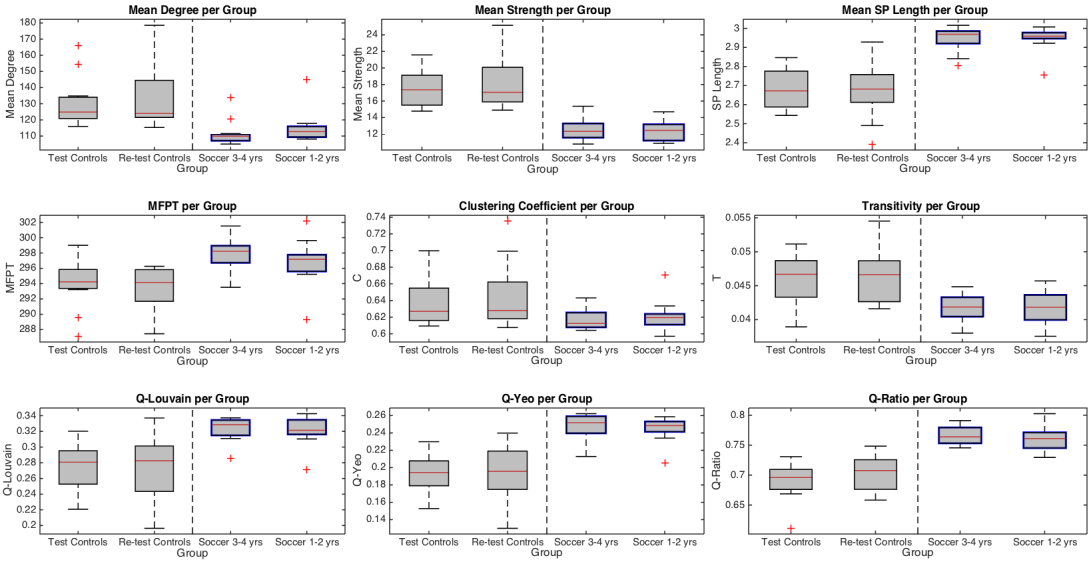

Figure 8.1 Distributions showing global network measures of basic connectivity (degree, strength), integration (SPL, MFPT), and segregation (C, T, $Q_{Louvain}$, Q_{Yeo}, Q_{Ratio}) for 13 non-collision sport control athletes (*Test, Re-test)* and 24 soccer athletes. Soccer athletes are grouped into two groups (*2-3 yrs, 3-4yrs*) based on years of experience at the high school level. Bold blue boxes indicate soccer athlete distributions significantly differing (unpaired t-test, $p_{fdr} < 0.05$) from Control *Test* session distributions. Soccer subgroups did not differ significantly from each other (unpaired t-test, $p_{fdr} < 0.05$) for any network measure.

8.3 Impact of RS Functional Connectivity Grouping Results

The results suggest that to comprehensively characterize the effects of repetitive head trauma and sports related mTBI, athletes should be assessed over several years of play, starting in early childhood. Grouping athletes by years of experience at the high school level did not shed light on the shift in network connectivity measures between soccer athletes and control athletes. There were no significant differences in any network measures between athletes with 1-2 years of high school experience versus athletes with 3-4 years of soccer experience. Furthermore, independent of years of experience, both subgroups of soccer athletes continued to show sustained significant differences from control athletes in all network measures that were comparable to those exhibited by the total cohort of soccer athletes in CHAPTER 7.

CHAPTER 9. OVERALL IMPACT

Prevention of mTBI in soccer has come to the forefront of the soccer community as the long term risks associated with mTBI are well established (Guskiewicz et al., 2005; Guskiewicz, Marshall, et al., 2007); studies report high rates of mTBI in soccer (Gessel et al., 2007; Marar et al., 2012; Zuckerman et al., 2015); and research reports that the repetitive nature of head trauma in collision sports has detrimental effects on the brain (Bazarian et al., 2012; Poole et al., 2015; Talavage et al., 2014). In response to a recent law suit (Strauss, 2015), USA Soccer Association has begun to regulate head impacts during training at youth levels. However, the effect of cumulative repetitive acceleration exposure on the brain is not well understood.

9.1 Evidence for Injury as a Result of a Cumulative Loading Over a Season

We present the first comprehensive evidence of subacute changes in the brains of asymptomatic athletes attributable to participation in a season of soccer. This evidence strongly suggests that mTBI should be viewed as an injury on a spectrum starting with subtle subconcussive brain changes and finally resulting in manifestation of neurocognitive symptoms associated with diagnosed mTBI. While assessments focusing on the relationship between "concussive" impacts and symptoms associated with mTBI have failed to find an explanation for the effects of head trauma on mTBI (McCaffrey et al., 2007; Mihalik et al., 2007; Schnebel et al., 2007), we have shown a significant negative relationship between cerebrovascular changes associated with mTBI and cumulative loading over a season.

Our results provide strong evidence that a comprehensive approach, rather than one that focuses on "concussive" impacts, must be taken to reduce the detrimental effects of repetitive head trauma. We have shown it is important to both limit the accumulation of high magnitude head acceleration events over a season and limit accumulation of these impacts in short periods of time. Specifically, for the sport of soccer, this could be implemented in the form of limiting the number of practices a week during which athletes can practice heading and by imposing a minimum on the number of days between sessions (games/practice) where heading is performed.

9.2 Evidence for Prolonged Recovery from Season of Play

In addition to showing detrimental changes in asymptomatic soccer athletes during the season, our results also highlight the importance of allowing for sufficient recovery time following a season of play. Consistent with symptomatic cases of mTBI, where cerebrovascular deficits have been reported several months to a year after resolution of mTBI symptoms (Chan et al., 2015; Maugans et al., 2012; Mutch et al., 2014; Wang et al., 2015), asymptomatic soccer athletes studied here also exhibited prolonged recovery to *Pre-season* CVR measures following a season of play. Cerebrovascular changes, beginning in the latter half of the season, were still present 1-2 months post-season and were more pronounced 4-5 months post-season when the majority of athletes were participating again in spring soccer suggesting that athletes may not have had enough time to recover from the fall season when they began spring soccer. If athletes are not allowed sufficient recovery time, they may perpetually at elevated risk for symptomatic mTBI.

9.3 Evidence for Chronic Changes as Result of Repetitive Head Trauma

Finally, consistent with reports of collision sport populations exhibiting increased risk for neurologic disorder relative to the general population (Baugh et al., 2012; Gavett, Stern, & McKee, 2011; Guskiewicz, Marshall, et al., 2007), we are the first to provide evidence of chronic shifts in functional brain connectivity in the brains of asymptomatic soccer athletes relative to non-collision sport control athletes. Soccer athletes exhibited global functional connectivity changes relative to control athletes that were already present at the *Pre* imaging session and were sustained one year following the initial *Pre* scan.

These results highlight the importance of longitudinally assessing the effects of chronic exposure to repetitive head trauma, suggesting that the effects of repetitive head trauma may begin early on in athlete's careers and may chronically compound over a career of play. This provides further support for the importance of instituting policies for monitoring and limiting athlete exposure to head impacts starting from a young age.

9.4 Overall Conclusions

In conclusion, we have provided evidence of both subacute and chronic changes in the brains of female high school soccer athletes attributable to participation in soccer. These results strongly advocate for the need to institute policies aimed at limiting cumulative exposure over a season in order to reduce rates of mTBI. To implement such policies, we believe it will be necessary to conduct a large scale comprehensive study to fully characterize the effects of repetitive head trauma across age groups. Though conducted on a small cohort of female soccer athletes, the study paradigm and methods presented here provide a scalable model for implementation of such a study.

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VITA

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Diana Otero Svaldi
Purdue University
Biomedical Engineering Doctoral Candidate

Education:

Purdue University, West Lafayette IN
PhD, Biomedical Engineering (August 2013-Present) --- Expected to graduate in December 2016 (GPA: 3.76)

Illinois Institute of Technology, Chicago IL
B.Sc., Biomedical Engineering, concentration in Image and Signal Processing (2009-2012, GPA 3.75)

Research & Professional Experience:

August 2013 – Present: Doctoral Fellow/Research Assistant, Purdue University

Project: Effects repetitive head trauma on female high-school soccer athletes using head impact monitoring and functional MRI

1. Used signal processing and multivariate statistical methods to *assess cerebrovascular reactivity* changes in female soccer athletes and non-collision sport female controls using a blocked breath hold fMRI task.
2. *Characterized head impacts* experienced in high school and collegiate women's soccer using X2 biosystem sensors. Characterization involved assessment of cumulative, average, and daily translational and rotational loads over a season of play. Comparisons of loads at various levels of play (collegiate vs. high school) as well as different styles of play (HS1 vs. HS2) were performed.
3. Applied *graph theoretical methods*, novel connectomics ICA based methods, and multivariate statistical methods to *assess resting state functional connectivity* changes in female soccer athletes and non-collision sport controls.
4. Served as *study coordinator* for two years. Duties involved subject recruiting, scheduling of subjects for scans, administration of sensors at practices and games, and scheduling of primary and secondary operators to conduct scans.

January 2013 – July 2013: Research and Development Intern, Indigo Biosystems

Project: Configure ASSAYS and develop quality assessments for ASCENT mass- spectrometry software using R-statistical programming language and JAVA.

August 2012 – December 2012: Under Graduate Researcher, Medical Imaging Research Center, Chicago IL

Project: As part of a longitudinal study aimed at finding early neuroimaging biomarkers of Alzheimer's disease, I used AFNI to perform manual modifications on high resolution, post-mortem MRI brain images.

May 2012 – July 2012: Summer Student Researcher, Helmholz Zentrum, Munich DE

Project: Implemented "heat kernel smoothing" technique in MATLAB on optical projection tomography volumes for the purpose of validation and testing of a mathematical model of gene expression.

January 2009 – May 2009: Chemistry Recitation Leader, Indiana University Purdue University Indianapolis, Indianapolis IN

Project: Led a weekly introductory chemistry recitation. Each week consisted of reviewing the basic concepts of the week's lectures, dividing students into small groups to work on problems, providing assistance to solve the problems, and finally leading exam review sessions.

Skills and Expertise:

Task Based and Resting State fMRI data preprocessing techniques
Network Modeling
Proficient in AFNI
Proficient in Matlab
Proficient in Linux Shell Scripting
Certified MRI Scanning Operator - GE HDx Signa 3T
Knowledge of FSL
Knowledge of R
Knowledge of JAVA & C programming
Bilingual in Spanish and English

Graduate Courses:

Complex Systems: Theory and Applications
Digital Image Processing
Biomedical Signal Processing
Introduction to Biomedical Imaging Systems
Measurement and Stimulation of the Nervous System
Biostatistics
Neural Mechanisms of Disease
Neuroanatomy

Awards and Honors:

Bilsland Dissertation Fellowship, Purdue University 2016
Stephen R. Ash Fellowship, Purdue Weldon School of Biomedical Engineering 2015
NSF Graduate Research Fellowship Honorable Mention, 2014 & 2015
Purdue Doctoral Fellowship, 2013-2015
Illinois Institute of Technology Women's Soccer Best Newcomer, 2009
Illinois Institute of Technology Athletics Soccer Scholarship, 2009-2012
Bepko Scholarship, Indiana University Purdue University Indianapolis, 2008

Extra Curricular Activities

West Lafayette HS Girls Soccer Coach
IIT Women's soccer Team (NAIA)

PUBLICATIONS

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Journal Publications

Svaldi, D. O., McCuen, E. C., Joshi, C., Robinson, M. E., Nho, Y., Hannemann, R., . . . Talavage, T. M. (2016). Cerebrovascular reactivity changes in asymptomatic female athletes attributable to high school soccer participation. *Brain Imaging Behav*. doi:10.1007/s11682-016-9509-6

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Shenk, T. E., Robinson, M. E., **Svaldi, D. O**., Abbas, K., Breedlove, K. M., Leverenz, L. J., . . . Talavage, T. M. (2015). FMRI of visual working memory in high school football players. *Dev Neuropsychol, 40*(2), 63-68. doi:10.1080/87565641.2015.1014088

Conference Publications

Svaldi D.O., McCuen E.C., Music J.P.E, Nho R.Y., Nauman E.A., Talavage T.M. PreventingnMild Traumatic Brain Injury in Women's Soccer: The Relationship Between Cerebrovascular Reactivity Changes and Daily Loading. *Society for Neuroscience, November 2016, San Diego CA.*

Svaldi D.O., Bari S., Abbas K.A., Jang I., Shenk T.S., Mao X, Leverenz LJ, Nauman E.A., Talavage T.M. Purdue Neurotrauma Group: Dedicated to Prevention of mTBI by Assessing the Effects of Repetitive Head Trauma in Collision Sports. *Integrative Neuroscience Center Symposium on Traumatic Brain Injury, May 2016, West Lafayette IN.*

Svaldi, D.O., Joshi C., Levernz L.J., Nauman E.A., Talavage T.M. Cerebrovascular Reactivity Alterations in High School Football Athletes. *Biomedical Engineering Society, October 2015, Tampa Bay FL.*

Svaldi D.O., McCuen E.C., Joshi C., Robinson M.E., Nauman E.A., Talavage T.M. Cerebrovasular Reactivity Alterations in Female High School Soccer Athletes. *Organization for Human Brain Mapping, June 2015, Honolulu HI.*