Chronic Traumatic Encephalopathy: A Review of Clinical Diagnosis, Animal Models, Sex Differences, and A Revised Return-to-Play Protocol

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Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative disease marked by debilitating cognitive and behavioral symptoms. CTE is thought to be caused by traumatic brain injuries (TBIs), though it remains unclear how the frequency, duration and intensity of TBIs contribute to CTE vulnerability. It is estimated that as many as 4M sports-related TBIs may occur annually in the US, though mild TBIs are often underreported and/or undiagnosed. As participation in athletics is arguably a voluntary and controllable risk factor for TBI, it is important to identify and understand factors that might affect an athlete’s likelihood of developing CTE. This review summarizes CTE symptomology and pathology, reviews relevant findings from animal models of TBI/CTE, discusses clinical criteria and emerging technologies used for diagnosis, reviews the extent to which sex differences may contribute to TBI severity and/or recovery and, finally, presents a data-driven protocol for return-to-play procedures for student athletes in contact sports.


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**Introduction**

Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative disease associated with debilitating cognitive, behavioral and/or mood symptoms (McKee et al., 2009, 2013, 2015; Kiernan et al., 2015) thought to be caused by repetitive traumatic brain injuries (TBIs). TBIs can occur following blows or bumps to the head, or in motion from a position of rest such as impact acceleration injury (e.g. car accident), IAI, and can lead to brain injuries that alter normal cognitive and behavioral function. TBIs can range from mild (mTBI) to severe, based on the severity and persistence of symptoms following the injury (Langlois et al., 2006; Blennow et al., 2012; CDC 2016d; Mayer et al., 2017). Since the early 2000s, the rates of TBI-related emergency department visits and hospitalizations have increased dramatically (CDC 2016c).

A common cause of TBI, particularly amongst children and adolescents, is participation in contact sports (CDC 2016c). Contact sports typically include American football, ice hockey, boxing, and wrestling, though TBIs are also frequently reported in basketball, lacrosse, rugby, soccer, and cheerleading (Carter et al., 2014; Moor et al., 2015). It is estimated that as many as 4M sports-
related TBIs may occur annually in the US, though mTBIs are often underreported and/or undiagnosed (National Institutes of Health 1998; Barzarian et al., 2005; Langlois et al., 2006; CDC, 2016b). The underreporting of mTBIs amongst athletes may be due, in part, to limited knowledge regarding CTE, an overreliance upon self-report that is vulnerable to social pressure and response biases), and a lack of available and/or accurate objective diagnostic tools. In a recent study by Mez and colleagues (2017), 99% of former NFL players, and 87% of football players of all ages were diagnosed with CTE post-mortem, leading to a large push to format a revised return-to-play protocol as well as improved mTBI diagnoses. In 2012, over 300,000 children and adolescents were treated by US emergency departments for a sport- or recreation-related TBL, a rate that had doubled over the past decade (CDC 2016b). Amongst children aged 5-14, bicycles and sports-related injuries account for over 25% of mTBIs (Bazarain et al., 2005). Information on TBIs and their prevention and treatment has been developed for young athletes, coaches and parents (CDC 2016a, 2016b).

It is important to identify and understand how TBIs affect an athlete’s likelihood of developing CTE. Sports-related TBIs constitute a substantial proportion of TBIs in children (Bazarain et al., 2005) but, as participation in athletics is voluntary, it is arguably a controllable risk factor for CTE. The fact that mTBIs are common, often underdiagnosed, and may contribute importantly to the trajectory of CTE underscores the need for accurate and rapid diagnosis.

However, it remains unclear how the frequency, duration and intensity of engagement in contact sports contributes to the occurrence of TBI or, more pressingly, the likelihood of developing CTE. While the exact incidence of CTE amongst athletes remains unclear (e.g., Maroon et al., 2015; Safinia et al., 2016), CTE was diagnosed neuropathologically in 177 of 202 deceased former football players and in 99% of the NFL players studied (Mez et al., 2017), suggesting that a history of contact sports and/or level of play contributes to the development of CTE. A review of clinical studies suggested that boxers’ CTE symptoms are often more severe, suggesting the frequency and type of impact may contribute to symptom severity (Safinia et al., 2016). However, the majority of postmortem analyses have come from retired football players and, due in part to sample size, less is known about CTE in athletes in other sports (e.g., Branch 2016).

While most animal and human research has focused on males, emerging evidence suggests differences in TBI outcome between males and females. While there is limited information on the incidence of TBI/CTE in females, existing evidence suggests that females may have worse post-TBI outcomes than males (Farace & Alves, 2000; Stone et al., 2016), but that estrogens may have neuroprotective qualities when given post-TBI (e.g. Gaston et al., 2012; Klose et al., 2007; Wise et al., 2011).

This review will summarize CTE symptomology and diagnostic criteria, review animal models of TBI and their potential contribution to CTE neuropathology, and discuss outstanding questions and areas for potential future research. Finally, a data-driven protocol for return-to-play procedures for athletes in contact sports will be presented.

Clinical Symptoms & Neuropathology of CTE

Most subjects diagnosed with CTE do not share a consistent neuropsychological profile, complicating efforts to identify and standardize clinical presentation and disease progression. Clinically, CTE can present as memory disturbances, behavioral and personality changes, motor problems and/or impulse control deficits that can begin years or decades after the last reported brain trauma (McKee et al., 2015). However, clinical diagnosis can be difficult because there can be substantial overlap between symptoms of CTE and other common neurological diseases (e.g., Hanlon et al., 2017).

Recent efforts to standardize diagnostic criteria for CTE (McKee et al., 2016) have emphasized neuropathological changes to the brain (McKee et al., 2009; Saulle & Greenwald...
2012; McKee et al., 2015). Indeed, CTE severity is staged from stage I to IV based on the presence, location and severity of various pathological changes (McKee et al., 2015). First, consistent evidence suggests that a dysregulation of tau protein (tauopathy) is associated with CTE (McKee, 2009). Although misfolded tau protein is implicated in Alzheimer’s disease (AD), tau pathology of CTE differs significantly from that of AD in that it is distributed primarily in the amygdala, hippocampus, midbrain, brainstem, and thalamus (Barrio, 2015; McKee, 2009; Stein et al., 2014) while patients with CTE show increased tauopathies in frontal, temporal, and medial temporal lobes of the brain, with the severity of the tauopathy correlating to the stage of the disease (McKee et al., 2015). The misfolding of the protein tau inhibits neuronal signaling by blocking off cells. This inhibition leads to apoptosis in neurons whose signaling no longer leads to downstream effects (Tepper et al., 2014). While there is some overlap in location between AD and CTE, the density of neurofibrillary tangles caused by tau are usually much denser in the case of CTE (McKee 2009). Furthermore, unlike AD, beta-amyloid (Aβ) protein aggregates, a consistent biomarker for AD, occur in less than half of CTE cases (McKee 2009).

Amyloid precursor protein (APP), the precursor for Aβ, contributes to axonal transport in neurons, and its aggregation is associated with neuronal apoptosis due to the decreased ability to send and receive signals from other neurons (Loo et al., 1993; Blumbergs et al., 1994). The presence of APP is a reliable marker for neuronal injury after TBI (e.g., Lewen et al., 1995; Itoh et al., 2009) and Aβ levels have also been investigated as a potential biomarker for TBI (Tsitsopolous & Marklund, 2013).

Significant atrophy of the frontal and temporal lobes is also present in late stages of CTE and corresponds to significant reductions in brain weight (Stein et al., 2014). Advanced (stage IV) CTE is also associated with widespread myelin loss, especially in the hippocampus and substantia nigra (Povlishock & Katz, 2005; Erb & Povlishock, 1991; McKee et al., 2015; Stein et al., 2014). Notably, these precursors to neurodegeneration found with repetitive TBIs in earlier stage CTE, and the atrophy of the frontal and temporal lobes in late stage CTE have not been found in those without a history of head trauma, even in age-matched controls, and is therefore not a normal part of the aging process (McKee et al., 2015). An outstanding question is how the quantity and severity of repetitive TBIs leads to this type of neurodegeneration.

Case Studies

While definitive CTE diagnosis requires postmortem anatomical analyses and/or in vivo advanced imaging technology (Sundman, Doraiswamy, & Morey, 2015), early reports and case studies relied on cognitive and behavioral symptomology present in groups at high risk of TBI, such as professional athletes in contact sports. CTE is not a new phenomenon; in fact, in the early 20th century, CTE was known as “punch-drunk syndrome” or “dementia pugilistica” due to its prevalence in the brains of former professional boxers. Such early case studies not only offer important insights into the trajectory of the disease but also point to research questions that can be pursued experimentally in animal models.

In a hallmark CTE case study, a 50-year-old retired NFL offensive linemen presented clinically, after a 12-year latency period, with cognitive impairment, mood disorder, and Parkinsonism (Omalu et al., 2005). Postmortem analyses identified neuropathological changes of CTE, including Aβ plaques and neurofibrillary tangles distinct from AD. Notably, the subject started playing football in high school, and played for three years in college, and 17 seasons in the NFL, suggesting a high likelihood of multiple mTBIs sustained over the course of his career, though none were reported (Omalu et al., 2005). Indeed, amongst retired NFL players diagnosed with CTE, the average length of exposure to repetitive TBIs is ~15 years and the average number of diagnosed mTBIs during those years is 20.3 (Eierud et al., 2014; Stein, Alvarez, & McKee, 2015). The underreporting of mTBIs amongst such athletes is likely due to,
in part, to limited knowledge regarding CTE, an overreliance upon self-report (which is vulnerable to social pressure and response biases), and a lack of available and/or accurate objective diagnostic tools.

However, a history of professional sports is not necessary for the development of CTE. Mez and colleagues (2016) reported on a 25-year-old college football player whose early presentation of CTE-linked clinical symptoms and behavioral problems suggested that CTE-associated symptomology and neurodegeneration can start at an early age. The patient’s cognitive and affective function declined rapidly and precipitously. At age 23, he became verbally and physically abusive towards his partner; parallel impairments in learning and executive function were identified using standardized neuropsychological tests. The speed of decline was thought to be attributed, in part, to the early age at which he sustained his first mTBI (at the age of 8) and/or the total number sustained (>10). While this symptomatology is consistent with clinical pathology of CTE (see Difficulties in CTE diagnosis and prevention section, below), it is rare that cognitive decline would be so advanced given this subject’s age (McKee et al., 2015). His neuropathology report showed widespread tauopathy and brain atrophy consistent with late-stage CTE. While clinical presentation of CTE occurs ~14.5 years after retirement, that timeframe was shortened substantially in this subject (Stein, Alvarez, & McKee, 2015). The early onset of neurodegeneration and clinical presentation, as well as the short latency to disease onset, suggest that early-life mTBIs, the total number sustained, or most likely, a combination of the two may have profound implications for neurotrauma (Mez et al., 2016). This case study has important implications for understanding the effect of number of allotted concussions, as well as the age at which young children should be allowed to play such high-impact sports.

Difficulties in CTE Diagnosis and Prevention

Multiple TBIs/mTBIs are thought to contribute to the development of CTE, and the early identification and treatment of mTBIs has become a medical priority. While severe forms of TBI can be diagnosed using an fMRI or CT scan, mTBIs do not show up on either (Sundman, Doraiswamy, & Morey, 2015). Current diagnostic techniques thus rely heavily on self-reported symptoms and neurocognitive testing completed by the patient on paper or via computer (Sundman, Doraiswamy, & Morey, 2015). The prevalence of mTBIs and their potential neurodegenerative implications underscores a need for better diagnostic tools for on-field application, as well as less reliance on self-report.

At present, it is unclear whether certain individuals are more susceptible to mTBI/TBI and/or CTE and, if so, what factors contribute to vulnerable versus resilient phenotypes. Given the role of apolipoprotein E (ApoE) in breaking down Aß, some research attention has focused on ApoE alleles (e.g., ε4 genotype; Liberman et al., 2002; Shadli et al., 2011), though no consistent difference in TBI symptom severity or duration of recovery has been found. Biomarkers based on biological sources of variability could be useful in identifying vulnerable populations, in order to better inform TBI diagnosis and treatment.

Researchers are still working to develop a valid CTE model that will show cognitive and neuropathological changes similar to mTBI-induced injuries in humans. Rodent models of mTBI have been developed that mimic CTE-related neuropathology, including an upregulation of precursors to Aß and hyperphosphorylated tau (Turner et al., 2015). However, CTE itself is difficult to model in rodents given the chronic, polysymptomatic nature of CTE neuropathology, and the difficulty in replicating complex cognitive deficits in rodent models. However, these mTBI studies shed light on the effect of mTBI incidence, frequency, and severity on cognitive and neuropathological changes, as detailed
below. With the style of play of current American football and other contact sports, it is plausible that, on average, athletes may have had years of exposure to repetitive head traumas compared to an individual not in contact sports. As of now, the total number of hits, concussive or subconcussive, to the head, as well as the severity and interval between them, needed to trigger this neurodegeneration is unknown.

Furthermore, while this disease has been found repeatedly in male athletes and military veterans, far less is known about CTE in women due, in part, to lack of an adequate sample of women who have participated in in vivo diagnostic imaging or donated their brains for postmortem analyses (e.g., Branch 2016). To date, no case studies have explored how CTE manifests in women, and whether there are sex differences in CTE vulnerability, progression and prognosis. As detailed later (see “Sex Differences and the Role of Sex Steroid Hormones,” below), women may be more susceptible to TBIs and tend to fare worse post-TBI (e.g., Farace & Alves 2000). Further research into sex differences, as well as the incidence of CTE in women, is important.

**Animal Models of mTBI**

Research on CTE in humans has been limited to correlational studies involving postmortem pathology, clinical symptoms identified via neuropsychiatric evaluation, retrospective next-of-kin reporting and/or self-report. These studies are subject to considerable selection bias, and are complicated by relatively indistinct diagnostic criteria (Hanlon et al., 2017). While causal studies cannot be done in humans for obvious ethical reasons, experimental studies conducted in animals can provide causal information about the types of injuries that lead to TBI pathology potentially contributing to CTE. Historically, a widely used technique for modeling TBI in rodents is the fluid percussion model that mimics the coup-contrecoup seen in humans (Lyeth 2016); however, this model is invasive (requiring craniotomy) and thus lacks face validity as an mTBI model. The following discussion focuses on less invasive, skull-intact models used to explore how varying the intensity of impact, inter-injury time, and/or number of injuries sustained contributes to the behavioral and neuropathological symptoms characterizing TBI and CTE.

To explore the role of impact intensity, Fujita, Wei, & Povlishock (2012) varied the number of impacts, intensity, and inter-injury interval time in groups of male rats. The researchers varied the number of impacts (1-3), intensity of impact (three levels, 0.5, 0.75, and 1.0m, of IAI – an injury when the head is set in motion from a position of rest and a great model for mTBI) and the interval between injuries (1.5, 3, 5, and 10 hours). Interestingly, regardless of inter-injury time and number of impacts, only rats with IAI levels of 1.0m differed significantly from control mice in in vivo and post-mortem analysis, suggesting that the intensity of the impact must be at a level between 0.75 and 1.0m IAI to have any impact on neuropathological changes. As might be expected, rats subjected to higher intensity and shorter inter-injury time fared the worst, meaning that the group experiencing two impacts of 1m IAI with a three-hour time interval had the greatest neurological changes (Fujita, Wei, & Povlishock, 2012). The neuropathological changes found included significant decreases in reactivity to acetylcholine, a neurotransmitter essential for learning and memory processing, as well as axonal change as evidenced by numerous APP-immunoreactive profiles (swelling, large bulbs) at 3 hours (in vivo) and 4 hours (post-sacrifice) post injury, respectively. This study therefore suggests that, while no neuropathological changes were identified after one injury, full pathological and physiological changes can manifest when multiple injuries occur in rapid succession.

While this research was not focused on the long-term effects of these injuries, it does suggest that pathological changes can occur with traumas sustained in short succession. This is important for understanding factors contributing to CTE onset in that it identifies measurable pathological changes that occur almost immediately after multiple impact injuries.
While the hope would be that these injuries can be overcome when given time to heal, the lack thereof may contribute to the individual differences in the manifestation and progression of CTE. These findings also support the concept of second impact syndrome, or SIS -- a condition wherein an individual sustains two mTBIs in short succession. This is an especially important consideration for individuals who are reluctant to report, or do not know whether they have sustained, an mTBI, and may re-enter a situation where they could sustain a second impact relatively quickly, leading to substantial brain swelling that can cause coma and death (Fujita, Wei, & Povlishock 2012). When caught early, however, the patient still experiences intense neurological trauma in a short period of time, potentially increasing their likelihood of developing CTE later.

While prominent, acute neuropathology has been identified in various animal models (Turner et al., 2015), the persistence and severity of these brain changes over time are not well understood. To monitor the long-term physiological and behavioral effects of mTBI in mice, Luo and colleagues (2014) exposed mice to varying numbers of impacts (0-5) with an interval of 24 hours between each. Importantly, the stereotaxic impact device used to deliver impacts allowed free movement of the mice's heads following injury, replicating the impact received in humans. Researchers found an increase in astrogliosis, an increase in the number of astrocytes suggestive of neuronal injury and neuronal apoptosis, with each subsequent hit of 1 to 3, with no further increase after three hits.

Two months post-injury, mice were trained on a radial arm water maze task requiring spatial learning and memory. The multiple mTBI mice showed impairment in the learning and retention phases of the maze task, while the single mTBI mice were impaired during only the learning phase, as compared to both the control (non-mTBI) mice and their own pre-TBI performance. Three months post-injury, the mice were trained on a fear-learning paradigm, and completed an elevated-zero maze test, to measure anxiety (Luo et al., 2014). This measure is important as up to 70% of patients with mTBI complain of increased generalized anxiety (Malkesman et al., 2013). All mTBI mice displayed stronger fear conditioning than the control group, but performed the elevated-zero maze at baseline levels, suggesting that hippocampal-amygdala circuits underlying fear-mediated learning, but not unconditioned anxiety, may be impaired following mTBI. After six months, the mTBI mice showed significant neuropathological changes, although, again, the single-impact mice were not significantly different than the control groups. Together, these findings suggest that repetitive impacts in short intervals (here, 24 hours) can cause long-term cognitive deficits likely associated with persistent neuropathology as seen in CTE.

However, emerging evidence suggests that even a single mTBI event in mice may be linked to cognitive deficits at 6, 12, and 18 months post-injury. In a study by Mouzon and colleagues (2014), mice were split into four groups, a single injury group with a single sham control group, and a repetitive injury group and a repetitive sham control group, wherein the injury mice received intercranial impacts of 5 m/s at 1 mm depth. The researchers used behavioral measures, such as limited periods of apnea and an intact righting reflex following injury, to confirm mild rather than severe TBI. The repetitive TBI group sustained 5 injuries with an inter-injury interval of 48 hours. At 6, 12, and 18 months post-injury, cognitive function, anxiety levels, and locomotion were tested using Barnes Maze, elevated-plus maze, and rotarod respectively (for an in-depth analysis of these tests in TBI, see Malkesman et al., 2013); neuropathological changes were also assessed at the 18 month point. Mouzon and colleagues (2014) found that Barnes Maze performance, which tests a subject’s ability to remember spatial cues to an escape hole, was impaired, suggesting poorer spatial learning and memory, in both single and repetitive TBI groups, with the repetitive TBI group performing worse than the single TBI and all sham groups. Both TBI groups spent more time in the open arms of the elevated plus-maze than sham groups, indicating reduced anxiety, which is maladaptive for a rat in an open field. Locomotion levels did not differ between...
groups, suggesting that gross motor function remained intact regardless of TBI presence and level. Finally, neuropathological changes showed thinning of the corpus callosum, but not the hippocampus, in both TBI groups, with more prominent pathology in the repetitive TBI group, as shown by staining done 12 months post injury. Authors speculated that white matter pathology may contribute to behavioral deficits measured at long post-injury time points (Mouzon et al., 2014). Notably, the authors used mild rather than severe TBI to more closely mimic circumstances seen frequently in athletes participating in contact sports. This work offers insight into how a single or repetitive mTBI can change neuropathological and neuropsychological outcomes similar to those characterizing CTE. This research is timely and important, given that human research can only monitor what has already happened.

Future research should explore the duration and severity of TBI-related changes, via longitudinal studies using animal models. Tracking neuropathological changes over time may also provide insight into why some individuals develop CTE and others remain resilient. Given that TBI can cause CTE, increased emphasis on characterizing overt CTE-like symptoms expressed by animals that are analogous to the human condition (e.g., emotionality) would improve the face and construct validity of these models. Finally, females are underexplored in biomedical research (see “Sex Differences”, below) and can be explored in systematic, objective ways using animal models.

Sex Differences and the Role of Sex Steroid Hormones

Despite the increased focus on the short- and long-term impacts of mTBIs, the majority of existing literature focuses on males, consistent with other biomedical disciplines (Beery & Zucker, 2011). Understanding sex differences in the occurrence and progression of TBI-related neuropathology is crucial given that these phenomena are not exclusive to male physiology and that increasing numbers of females are at elevated risk for CTE via growing involvement in contact sports. Many human studies suggest that females tend to fare worse than males following mTBI (Farace & Alves, 2000), and female athletes take longer to return to play than men (Stone et al., 2016), suggesting underlying sex differences in diagnosis, severity of symptoms, and/or recovery. It may be inferred that CTE prevalence is also different in men versus women. However, differences in return-to-play time may also be due, in part, to the different societal pressures placed on men and women athletes. A combination of hormonal, physiological, and anatomical differences between men and women have been proposed to underlie differences in mTBI prevalence and outcome (Dick 2009), but the study of these factors is complicated by the heterogeneity and variability of experiences contributing to TBI and CTE in humans. Intriguingly, female gonadal steroids, namely estrogen and progesterone, can alter neuronal morphology and physiology (e.g., McEwen 2002; Wooley 2007), and estradiol in particular may mediate neuroprotective effects in the adult brain (e.g., Wise et al., 2001), potentially implicating gonadal hormones in short- and long-term TBI outcome.

Findings from a recent human observational study investigating the relationship between sex and mTBI outcome lend support to the idea of underlying differences between sexes. Bazarian and colleagues (2010) followed 1425 mTBI patients for 3 months after their appearance in the emergency department for mTBI; of that sample, ~45% were female. Of this population, females were more likely to have higher post-concussion syndrome (PCS) scores than age-matched males at the 3-month follow-up. Interestingly, these differences in PCS scores pertained to females of childbearing age, but not postmenopausal women, suggesting that gonadal hormones may be contributing to mTBI outcomes. No sex differences emerged in time taken to return to work or to normal activities, which authors speculated could be due to sex differences in the accuracy and veracity of these voluntary self-report measures. Rather, in both sexes, an abnormal CT scan at time of mTBI
and receipt of analgesics in the ED were more predictive of the number of workdays missed (Bazarian et al., 2010).

Importantly, this study focused on individuals who reported to the emergency room following an mTBI. However, many people do not know that they may have sustained an mTBI, choose to not report it, or seek alternative (non-ED) help following an mTBI, so may not represent the mTBI population of males and females as a whole. Also, while authors did not collect data on circulating gonadal hormones, it is possible that estrogen levels could also contribute to sex differences (see below); an interesting follow-up study could compare these data to data in prepubescent girls who have lower estrogen levels.

Work in animal models suggests that estrogen may be neuroprotective in male rats when administered soon after sustaining a TBI. Gatson and colleagues (2012) showed that estrone, a form of estrogen most predominantly found in postmenopausal women, may also have neuroprotective effects. In this experiment, male rats either received an estrone or vehicle injection 30 minutes after sustaining a severe TBI, using an open head impact procedure, or sham surgery. Pain was monitored and managed post-TBI, increasing the validity of this model, and the rats were sacrificed 72 hours post injury. The estrone-treated rats had smaller brain lesions and more intact neurons compared to the vehicle-treated rats, who had substantial damage as well as significant apoptosis to the cortex and corpus callosum. Cortical Aβ production in response to TBI was also significantly reduced in the estrone-treated rats, as compared to the vehicle-treated rats, and was equivalent to levels in rats that did not experience TBI, suggesting that estrone may have prevented Aβ production induced by TBI. Components of the ERK signaling pathway, including the neuronal growth factor BDNF, were also up-regulated in the cortex of estrone- versus vehicle-treated rats, suggesting that these neuroprotective effects may be attributable to BDNF-mediated changes in neuronal growth, survival, and/or synapse formation following TBI, potentially decreasing the apoptosis known to follow TBI. This is especially important in cases of repeated TBI that is most often seen in CTE, leading to the large atrophy and cell death seen in later stages. The aggregation of Aβ precursor protein (APP) has been associated with cognitive deficits (Gatson et al., 2012) and the in vivo removal of Aβ was recently shown to improve performance on a series of memory tasks (Leinenga & Götzt, 2015), suggesting that pharmacotherapies targeted at reducing Aβ aggregation could be useful in TBI recovery, potentially decreases the likelihood of long-term neuropathological changes and developing CTE.

Unfortunately, cognitive changes were not assessed in this study, so the functional outcomes of estrone treatment remain unclear. It is important to note that while this study used a relatively severe rather than mild TBI model, and more research needs to be done in mTBI rather than severe TBI, estrone should impact mTBI-related damage similarly as it works in the same way regardless of severity of TBI, by decreasing neuroinflammation (López Rodriguez et al., 2011). It is also unclear how estrogen supplementation would affect female rodents, using a similar paradigm.

Limited yet compelling evidence suggests that TBI impacts the function of the anterior pituitary gland, which mediates the release of gonadal hormones. Klose and colleagues (2007) identified significantly lower levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as slightly lower estrogen levels, in pre-menopausal women with recent (<2 weeks) TBI, compared to healthy controls. These differences, however, were not seen in post-menopausal women, suggesting that circulating levels of gonadal hormones in reproductive-aged women may be impacted by TBI. Thus, rather than estrogen and/or progesterone lacking neuroprotective effects, it is possible that, after mTBI, the anterior pituitary gland cannot release the hormones normally, preventing females from experiencing potential neuroprotective effects of these endogenous hormones. Again, the efficacy of estrogen-based interventions in post-TBI females remains unclear and requires further research.

Taken together, mounting evidence suggests that estrogen’s neuroprotective effects may
make it a viable therapeutic option for mTBI/TBI patients, potentially reducing the long-term effects of mTBI/TBI and thus the likelihood of CTE. Additional research needs to be done to clarify the effect of estrogen in post-TBI females and extend this research to examine the neuropathological and cognitive effects of estrogen-based therapies in TBI patients.

**Early Diagnosis in Humans**

Crucial to understanding CTE and its progression is a clear, objective, and prompt diagnosis of mTBIs. The nature and significance of TBI-related neuropathology, both in the acute, post-trauma phase and at relatively extended time points, are still being revealed, making it difficult to identify consistent, useful biological targets. Having a clear, objective means of identifying even subclinical head traumas (e.g., mTBIs) for non-medical personnel available to the athletes would allow more careful monitoring and treatment, with the hope of reducing future risk of CTE.

Recent advances in diagnostic imaging may facilitate the early diagnosis of mTBI (e.g., Eierud et al., 2014). Diffusion tensor imaging (DTI) offers a means of examining the macroscopic arrangement of white matter, reflecting axonal bundles connecting brain regions (Assaf & Pasternak 2008). DTI is based on diffusion properties of water molecules, within and outside of these tracts, and can produce two- and three-dimensional images of white matter tracts in the living human brain; changes to the integrity of white matter may help inform early mTBI diagnosis (Assaf & Pasternak, 2008). Importantly, these white matter changes can be widespread and only partially determined from the area of impact, and the cognitive functioning effects was shown to depend on the key areas damaged (Kinnunen et al., 2011). DTI following mTBI shows increased mean and axial diffusivities and decreased fractional anisotropy (Kinnunen et al., 2011). Furthermore, in PCS patients, DTI has revealed white matter damage lasting up to 18 years following an mTBI, specifically in the corpus callosum and frontal lobes (Aoki et al., 2012), while other research has shown significant changes in neuroinflammation of white matter following just one season of contact sports, without mTBI diagnosis (Johnson et al., 2013). Tracking these changes in individuals following mTBI could help researchers understand the extent of the damage and how long it takes the brain to subsequently heal, thus informing if/when an athlete should return to play (see “Return-to-play protocols” section, below) and the extent of damage that leads to CTE.

*In vivo* imaging techniques also allow the identification of abnormal proteins, such as tau (Zimmer et al., 2014; Villemagne et al., 2015) and Aβ (e.g., Rowe & Villemagne 2013; Adlard et al., 2014). Such imaging techniques offer insight into the progression of diagnosed neurodegenerative diseases, such as AD, as well as their prodromal identification. Recent studies have applied these imaging techniques to identify tauopathy, Aβ aggregation, and fibrillary neuroaggregates in CTE (Mitsis et al., 2014; Barrio et al., 2015). These techniques may also inform the early diagnosis of TBI and allow real-time monitoring of disease progression (Sundman et al., 2015). Furthermore, radiotracers introduce the possibility of monitoring disease progression, as well as measuring the impact of potential pharmacotherapies targeting these neuropathologies. Radiotracers are used to monitor neurological diseases, and others, as they are able to semi-invasively enter the body and bind to, and thereby mark, specific proteins of interest. This is important for potentially monitoring neurological diseases such as CTE as one is able to determine the density of neurofibrillary tangles and Aβ plaques, which increase as the disease progresses (Israel et al., 2016). Therefore it is a direct measurement of disease progression.

Given the estimated rates of underreporting of TBIs in sports (e.g., Barzarian et al., 2005; Langlois et al., 2006), developing an objective, standardized means of assessing mTBI is critical. Studying these changes across the average career of an athlete in contact sports could be extremely useful. As long-term
changes may occur without a single diagnosed mTBI in mTBI-vulnerable groups (McKee et al., 2015; Sundman et al., 2015), the emergence and severity of these changes could be monitored across the career of any athlete involved in contact sports or others at high risk for mTBI and CTE. Such technological advances in detection of TBI-related neuropathological changes are promising in the diagnosis and treatment of at-risk individuals, as well as playing a role in the decision of an individual or their doctor when or if to return following repetitive injuries. The monitoring of neuropathological changes in vivo can further the knowledge of the progression of CTE, potentially leading to better diagnoses and treatments.

Return-to-Play Protocols: Current Limitations and Potential Improvements

Recovery from mTBI includes being symptom-free, cognitively intact, and able to remain so during participation in normal daily routines, ranging from work to school to full contact sports. In athletics, following a hit or blow to an athlete’s head, standard protocols help to identify symptoms of mTBI and dictate whether the athlete is healthy enough to continue to play (McCrory et al., 2009; Giza et al., 2013; McCrory et al., 2013; CDC 2016a). TBI assessment typically consists of a graded symptom checklist (e.g., pupil size), some of which is self-reported by the athlete (e.g., headache, nausea), and neuropsychological assessment that is frequently conducted by coaching or athletic training staff or, in professional athletics, a medical professional. Depending on the type and severity of the athlete’s symptoms in this assessment, these so-called “return-to-play” protocols may advise sitting out for a portion of the ongoing game or the remainder of a season. Return-to-play protocols are present in most organized sports from amateur to professional levels.

There are three major issues with existing return-to-play protocols. First, these protocols are based largely on a set of clinical tools that, while established and valid metrics of certain cognitive functions (e.g., pegboard; verbal learning tests), only provide insight into a limited range of cognitive functions in a diagnostic (laboratory) environment. In this way, the reported absence of cognitive symptoms may not encompass all cognitive deficits or reflect “real-world” function in an accurate way. Indeed, early research suggested that the average time needed for mTBI-related cognitive symptoms to dissipate is approximately a week (McCrea et al., 2003; Bleiberg et al., 2004). However, in one study, nearly 50% of students with mTBI experienced symptom recurrence when they returned to play or returned to school (Carter et al., 2014), suggesting that neurological deficits may linger and/or be exacerbated by an early return to normal activities.

Second, this average recovery period is not sensitive to individual differences in symptom presentation or trajectory of recovery, and is often not adhered to. Students can be advised to take 20-40 days of physical and mental rest before returning to normal activities (Carter et al., 2014; Moor et al., 2015). Vulnerable individuals may require longer post-TBI recovery times, though researchers are still working to identify an accurate biomarker that would identify populations that might be slower to recover and allow the recovery time needed (see Liberman et al., 2002; Shadli et al., 2011).

Adherence to the return-to-play protocol itself, by the athlete as well as individuals assessing the athlete, can vary based on sport, level of play, and organizing body, and can be difficult to enforce. Students’ self-reported adherence to recommendations for physical/mental rest was high (Moor et al., 2015), but more objective reports suggest that ~15-50% of high school students fail to comply with return-to-play guidelines (e.g., Yard & Comstock 2009). Avoiding social activities, electronics, school, or work can have negative psychological, social, and/or economic implications, particularly amongst young people. This failure to comply may also be due, in part, to inadequate training, infrastructure,
and/or oversight on behalf of the athletic associations and schools (e.g., Olympia et al., 2016), which allow students to return to activities earlier than is advisable.

Third, it is likely that TBI-related neuropathological changes might persist past the point of overt cognitive symptoms (see previous section). However, it is difficult to assess such changes, given currently available technology and logistical considerations of imaging all suspected sports-related TBI cases soon after the impact/trauma occurred (e.g., expense, resource availability).

Given the current research on TBI and CTE, it is worth revisiting criteria common to return-to-play protocols to ensure that they are consistent with existing literature and to protect the long-term health and wellbeing of athletes in contact sports with high risk for TBIs. One of the most common criteria shared across current protocols (McCrory et al., 2009, Giza et al., 2013; McCrory et al., 2013; CDC 2016a) is a gradual return to daily activities (e.g., 7-30 days; Yard & Comstock 2009; Moor et al., 2015), such as school or work, so as not to exacerbate the symptoms of the mTBI. For many, this means having a slow entrance back into their routine with many breaks and resting periods. For students, accommodations in school might consist of extending deadlines, reducing assignments, or postponing exams (Olympia et al., 2016). For athletics, accommodations upon returning to play, such as no tackling in football for a certain amount of time, can vary widely.

As age is an important aspect of the return-to-play protocol (e.g., Stein & Spettell, 1995), we will focus on suggestions for a revised return-to-play protocol in student athletes (e.g., 11-19 years old).

(a) Limited postmortem analyses on young athletes seem to indicate that the age at which the first mTBI occurs may have serious implications for long-term neuropathology and CTE risk (Mez et al. 2016). Children’s brains are still developing throughout their mid-20s, and their physiology (e.g., muscle development, bone structure/thickness) differs from that of adults, potentially increasing their susceptibility to long-term changes following mTBI, such as CTE (Pullela et al., 2006; Huh et al., 2008; Mez et al., 2016). Females’ body structure and physiology (e.g., muscle mass) may also increase their risk for mTBI/TBI and, potentially, CTE, though this remains unclear due to limited research (see “Sex Differences” section, above). Therefore, it may be advisable to restrict full contact in sports to age 15, when a student can make a more deliberate, informed decision about the risks of TBI and CTE in consultation with parents, coaching staff, and medical personnel.

(b) Underreporting and missed diagnosis of mTBI/TBI has potentially serious consequences for repeated traumas upon return-to-play before healing from the first trauma is complete, yet diagnosis relies heavily upon self-report. Advances in imaging technologies would provide an objective measure of neurological damage that would supplement existing diagnostic tools, which are overly reliant upon self-reported symptoms and assessment by ancillary figures (in non-professional sports, typically coaching staff) that may vary in training, responsibility, and adherence to athletic association guidelines. Continued monitoring during recovery will increase the likelihood that return-to-school or return-to-play will occur when the individual meets objective criteria, reflecting minimal lasting changes to the brain, and even CTE. Such technology (e.g., DTI) is expensive and must be located in larger medical facilities, making it unwieldy for large-scale application. As these technologies improve and become more affordable and commonplace, however, they should be considered for inclusion in TBI diagnosis and return-to-play recommendations.

(c) Student athletes may benefit from both an extended “cognitive rest” period, including absence from school, and an extended period before which s/he can return to play. As return to contact sports is inherently riskier given the issues associated with multiple impacts/TBIs, the recommended timeframe for return-to-play should be even longer than that for return-to-school or other
normal activities (e.g., 20 days return-to-school and 40 days return-to-play). These periods may limit the time that cognitive deficits are experienced (e.g., McCrea et al., 2003; Bleiberg et al., 2004), the reemergence of cognitive deficits upon return to school/sports (Carter et al., 2014), and acute and/or persistent neurological damage (e.g., Huh et al., 2008; Pullela et al., 2006).

(d) Adequate training, infrastructure and oversight of ancillary figures, coaches, and school staff (e.g., Olympia et al., 2016), should help students to adhere to recommended rest periods and encourage a culture of responsibility. Improving mTBI/TBI diagnosis and enacting more conservative return-to-play protocols will hopefully reduce the risk of student athletes developing long-term neuropathological changes and CTE.

Conclusions

The presence of CTE-associated neuropathology identified in young athletes at the beginning stages of their careers (Omalu et al., 2011; Sundman et al., 2015) suggests that even a limited number of early brain injuries (mTBIs) may have potentially devastating and long-term effects. Impact-induced damage to basic neuronal processes at early ages may also have serious implications on the future of high-impact sports in young children, and needs to be studied more extensively in young populations in order to determine the true dangers. Furthermore, the comorbidity of CTE with other neurodegenerative diseases, such as AD, Parkinson’s, and amyotrophic lateral sclerosis (ALS), can lead to complications with diagnosis, or misdiagnoses, of these diseases (McKee et al., 2015). Additionally, individuals with no reported history of mTBIs in high-risk groups have been found to have CTE as well -- while this may be due to the issue of underreporting, it may also be proof that repetitive concussive hits that are noteworthy enough to report might not be necessary for CTE onset. Instead, even repetitive subconcussive hits might lead to similar neurodegenerative processes as concussive ones. In animal models, few subthreshold hits within a relatively brief timeframe impaired learning and memory as much as singular concussive hits in mice (c.f., Turner et al., 2015), suggesting that multiple subthreshold hits may cause a similar degree of damage as one bigger hit.

Use of new mTBI imaging techniques may lead to more definitive diagnoses of concussions and, potentially, insights into CTE progression and clinical prognosis (Johnson et al., 2013; Eierud et al., 2014; Mitsis et al., 2014; Barrio et al., 2015). Although expensive, imaging data of high-risk CTE individuals extended over a long period can help show the progression of the disease more explicitly and allow for better and earlier diagnoses, treatments, and understanding of the disease by the patient and those around them.

Further research should also focus on longitudinal studies of mTBI/TBI in animal models that track behavioral, cognitive and neuropathological changes at extended post-injury time points. More definitive research should be conducted on the potentially neuroprotective effects of estrogen, and its clinical applications.

Ultimately, improving our understanding of CTE will help individuals and healthcare professionals decide when is an appropriate time to refrain from participating in contact sports or other high-risk activities, in order to protect the cognitive and anatomical integrity of the human brain.

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