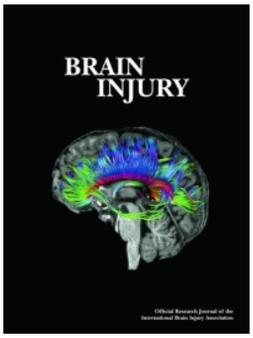

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Staying in the game: a pilot study examining the efficacy of protective headgear in an animal model of mild traumatic brain injury (mTBI)

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ABSTRACT

Primary Objective: Rugby is one of the few contact sports that do not mandate protective headgear, possibly because studies have shown poor efficacy for protection related to concussion pathology with existing headguards.

Research Design: Following innovative material technology utilization to produce headgear believed to have protective capabilities, this study examined the effects of a soft-shell headgear constructed from a novel viscoelastic material, on both behaviour and serum biomarkers after high and average impact force mild traumatic brain injuries (mTBI).

Methods and Procedures: Seventy-five male Sprague Dawley rats were divided into five groups: control, average - 37G impact, with and without headgear, and high - 106G impact, with and without headgear. Rats were sacrificed at 3 or 48 hours and serum samples were analyzed for levels of *TNF-α, NEF-L*, and *GFAP*. Animals sacrificed at 48 hours also underwent testing for balance and motor coordination, and exploratory/locomotor behaviour.

Main Outcomes and Results: The novel headgear offered significant protection against mTBI symptomology and biomarkers in the group that experienced an average impact force, but only moderated protection for the animals in the high impact group.

Conclusions: This innovative headgear may prevent some of the negative sequel associated with concussion pathology.

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Introduction

Not unlike other contact sports, rugby players are at high risk for concussion due to the physical nature of the game and high number of collisions that regularly occur between players. Unlike football and hockey where players are mandated to wear protective gear, rugby players typically have little, if any, protective equipment. Although players are allowed to wear headgear compromised of soft thin materials, which can be no thicker than 1 cm (1), the majority do not. When surveyed, players often insisted that the protective equipment available was not worth the expensive costs, as it was not efficacious at preventing injury (1), while others felt it was uncomfortable and interfered with communication on the field (2). Despite being mandated in many sports, the effectiveness of headgear in preventing sports-related concussions is questionable; they have been effective for high velocity sports such as skiing and snowboarding but for contact sports they have had few preventative effects (3,4). Indeed, headgear has been shown to be more effective at preventing skull fracture, facial injuries, or oral-dental damage than concussion (3,4). The inability to demonstrate that headgear offers protection for contact sports could be associated with the innate variable nature of the impact forces associated with these sports, and until recently, a lack of valid modeling platforms.

Although there are multiple collisions between players within rugby, American football, or ice hockey, the majority have been identified as mild impacts, ranging from between 20–30 G (5). A detailed study of rugby players found that over the course of 379 player match hours, there were 20,687 impacts that were over 10 G (6). Interestingly, of those impacts, only a small proportion, roughly 1%, registered at over 95 G, a force that would be considered severe (6). It is significant to note, that although 95 G has been defined as the concussion threshold in many sports, players can develop sports-related concussion from both high and low-impact hits (5,7–9). While concussion lacks a true diagnostic marker, it is generally defined as a blow to the head, upper body or neck, which may acutely cause loss of consciousness, nausea, headaches, and dizziness (10). The majority of individuals that experience concussion recover within 7–10 days of the injury, but many experience changes in motor function, anxiety, memory, executive function, social interaction, and effects that persist and are often categorized as post-concussion syndrome (PCS) (11). In addition to the often debilitating effects of PCS, recent evidence suggests that players who experience multiple and frequent concussions are at risk of developing chronic traumatic encephalopathy or other neurodegenerative disorders (12–15).

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Owing to the growing literature illustrating the long-term neurological deficits associated with repetitive brain injury, even injuries classified as mild (12), there is an imminent need to establish strategies to reduce, if not prevent insults like these to the brain. If an effective headguard can be generated, rugby offers a unique opportunity to introduce protective headgear that could substantially reduce concussion incidence and, in theory, much of the associated long-term neurodegeneration. Therefore, the goal of this pilot study was to determine the effectiveness of soft-shelled headgear for the prevention both of behavioural symptoms and physiological changes associated with concussion. The terms mild traumatic brain injury (mTBI) and concussion are often used interchangeably (16), especially within the context of sports. However, given that concussion is generally diagnosed based on self-report (4), which is not possible in a rodent model, the term mTBI will be used hereinafter. Headgear efficacy was tested at G force impacts believed to represent average and high impact forces. This experiment utilized male Sprague Dawley rats (age matched to young adulthood, 25 years), which were divided into five groups of animals: No impact (control), 37 G impact with and without protective headgear, and 106 G impact with and without protective headgear. Animals were sacrificed at either 3 hours or 48 hours post-injury, with a subset undergoing beam-walking (measuring balance and motor dysfunction) and open field (measuring anxiety-like behaviour) testing. Serum samples were taken from all animals and tested for *TNF-α, GFAP*, and *NEF-L*. These peripheral markers were selected because they have been thoroughly tested in the concussion literature providing a strong comparative foundation and they span many aspects of brain injury including cytokines characteristic of a pro-inflammatory states *TNF-α* (17–19)), and biomarkers of axonal injury such as glial fibrillary acid protein (*GFAP*) and neurofilament light *(NEF-L*) (20,21).

Methods

Animals and concussion protocol

The experiments conducted were approved by the University of Calgary Animal Care Committee and followed Canadian Council of Animal Care guidelines. A total of seventy-five male Sprague Dawley rats were obtained from Charles Rivers Laboratories and maintained under vivarium conditions (12:12 light:dark cycle at 21°C with access to water and food *ad libitum*). Animals were handled daily for 14 days prior to experimentation to ensure they were familiar with the lab personnel and the results from the study were not influenced by the stress associated with transportation (22). On postnatal day 75 (P75) animals were randomized to one of the five categories: control (no hit) (n = 15); 37 G average impact, with (n = 15) and without protective headgear (n = 15); and 106 G severe impact, with (n = 15) and without protective headgear (n = 15).

The mTBI model used in this study was a lateral impact device (LI) developed and previously described by our lab (23). This model is particularly adept at generating sports-related concussions because it acts upon a freely moving animal, and allows for the acceleration, deceleration and rotational forces characteristic of sports-induced brain injury (24,25). Animals were lightly anesthetized using isoflurane (Pharmaceutical Partners of Canada) and placed in the prone position on a Teflon® board with low friction. The left side of the head was placed directly opposite the impactor. A 50 g weight was released through a thrust barrel using pneumatic pressure to strike the side of the rat's head, propelling it into a 180° horizontal rotation. The speed of the projectile was varied to alter the severity of the impact. The severity of the impact was calculated with the formula $G_{force} = \mathbf{a}/9.81\ m/s^2$ and velocity determined as $Vf^2 = Vo^2 + 2 \cdot \mathbf{a} \cdot m$. There were two types of injuries 1) the average impact: average speed of 6.0725 ± .101 m/s to produce an average G force of 37; 2) the severe impact: average speed of 10.2186 ± .227 m/s to produce an average G force of 106. A collection sponge was placed on the right side of the Teflon board to protect from further injury, however all animals came to rest before impacting the protective sponge. After impact or control injury, topical lidocaine (AstraZeneca) was applied to the rat's head and it was placed on its back in a clean and warm cage to recover. The ***time-to-right*** (time for each animal to flip from the supine position to a prone or walking position) was measured for each animal immediately after the injury, and is believed to represent the loss of consciousness that is often associated with concussion.

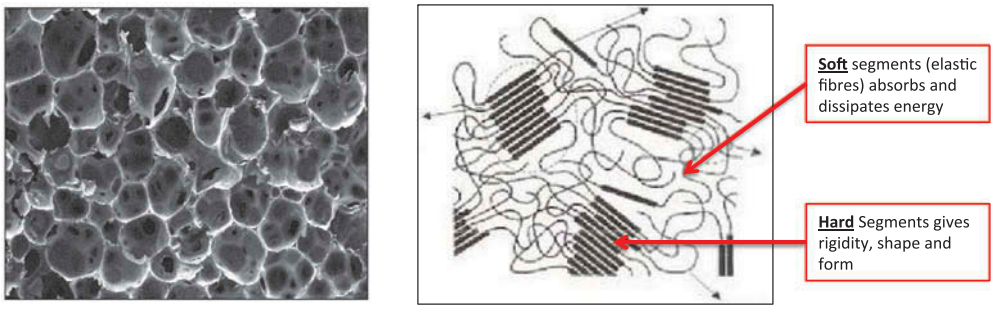
Protective 'headguards' material

Protective 'headguards' were cut from viscoelastic open-cell foam to the size of 30 × 13 × 0.6 mm, and were placed adjacent to the rat's head. The headguard was not fixed to the rat's head, as this is impractical given the orientation of the head to the spine, but the placement of the headguard was such that the headguard was in contact with the head for the duration of the impact. The novel protective equipment was supplied by Contego Sports Ltd, and is comprised of an impact absorbing proprietary foam technology. The viscoelastic material of the headgear was designed to dissipate the impact energy at the localized site of impact, in an effort to diminish intracranial pressure differentials, thus reducing the incidence and severity of tissue damage associated with coup and contre-coup sites. In other words, the headgear was believed to reduce the effects of the acceleration and rotational forces on the brain, the damaging aspects of concussion. The viscoelastic material has properties of both a liquid and elastic solid; the viscous material absorbs impact energy by dissipating it over a large area and the elastic material absorbs impact energy into its microstructure, which is then released over time as heat. The resulting combination functions much as a bullet-proof vest which is designed to disperse the round's energy and deform the slug to minimize blunt force trauma. See Figure 1 for micro-structural and morphological illustrations of the protective foam technology.

Behavioural testing

Beam-walking

Fourteen hours after the injury, using a protocol that is similar to the one described by Schallert (26), all animals in the 48 hr sacrifice cohort were tested in a beam walking paradigm designed to measure balance and motor coordination. Similar to the Schallert paradigm, rats were required to traverse a



Micro-structural level: Dissipates the impact energy through the cellular microstructure of the viscoelastic foams

Morphological-level: Dissipates the impact energy through the morphological microstructure (hard and soft segments) of the viscoelastic foams

Figure 1. Illustrative representation of the proprietary foam technology implemented as protective 'headguards' for the study. The illustrations demonstrate the microstructural and morphological aspects of the visoelastic foam that permits absorption and dissipation of the impact force.

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tapered beam (165 cm long), moving from the wide end to the narrow end. The rat's home-cage was placed at the end of the beam as an incentive for the rat to traverse the elevated beam. The tapered beam was suspended 1 meter in the air, but was equipped with 'safety ledges' (2 cm wide) that caught the rat's foot if it slipped while walking. Rats were given a single trial to learn the task, and 4 additional videotaped trials that were scored. Between each trial, the rat was permitted to remain in its home-cage for 1 min to reinforce the location of the target destination. The beam was cleaned between each testing session. Two research associates who were blinded to the experimental conditions scored the videos for the average number of hind-leg foot- slips (when the rat slipped and needed to use the safety ledges with the hind legs while traversing the beam), and the average time required to travel from the starting point to the home-cage.

Open field

Twenty-four hours post-injury, animals were tested in the open field arena as previously described by our lab (23). Rats were placed in the center of a circular open field (diameter 135 cm) and were permitted to explore the environment for 10 minutes. An overhead camera was used to track the rat's overall movement (distance travelled and speed of travel). The open field was cleaned with Virkon® between each testing session.

Sacrifice, brain collection, and serum biomarkers

Animals were anesthetized with isofluorane and sacrificed at either 3 or 48 hours post injury. These time-points were chosen in an attempt to capture events resulting from both classical inflammation and neurogenic inflammation, known to exert their effects from within minutes to days of the impact. Neurogenic inflammation is moderated through release of neuropeptides, such as substance P and it is thought that levels of neuropeptides in the serum during the first 4 h are critical in determining the severity of brain injury following trauma (27). Similarly, TNF-alpha has been demonstrated to peak at 3–9 h (28) in mTBI and over the course of 48 h following moderate-severe TBI (29,30). A similar temporal profile is seen in terms of microglial activation, astrogliosis and axonal damage (31). In addition, to peek times in inflammation, these two time points also correspond to time-periods after an impact that human subjects would be under observation.

Trunk blood was collected at the time of sacrifice in serum separator tubes (BD). Samples were clotted for 30 minutes at room temperature and then centrifuged at 1000 g for 15 minutes. Serum was aliquoted into 300 – 400 µl samples and stored at −20°C. ELISA kits were purchased for *TNF-α, NEF-L*, and *GFAP* (R & D Biosystems, MN, USA; Cusabio, Hubei, China; EMB Millipore, ON, Canada, respectively). ELISAs were performed according to the manufacturer's instructions for each specific biomarker analysis. All standards, positive and negative controls, and samples were run in triplicate, and measured with the BioTek Synergy H.T. plate reader and Gen5 2.00.18 software using a path length correction algorithm. All samples fell within normal range of the standard curve. At the time of sacrifice, whole brains were extracted, submersed in 4% paraformaldehyde, and stored at 4°C until processed.

Neuropathological examination

Whole brain tissue was cut into coronal sections at 7 µm in 3 mm intervals and processed to paraffin slides. For each brain, 10 serial sections were cut at 5 levels through the brain (each level being 2500 µm apart), in a coronal orientation. Brain sections were stained for H&E and immunohistochemistry, with antibodies against GFAP, phosphorylated tau, and amyloid precursor protein (Dako (z0334), Agilent Pathology Solutions; Invitrogen (MN1020) BioSource International; Chemicon (mab348) Merck Millipore, respectively). Slides were stained on a Bond III automatic stainer (Leica Biosystems). Briefly, this stainer brings the sections through the following stages: dewaxing, rehydration, antigen retrieval (using Bond Epitope Retrieval Solution 1, ER2, Leica Biosystems AR9961), blocking using normal serum, incubation in primary antibody, appropriate buffer washes, incubation in Bond Polymer Refine Detection system (Leica Biosystems DS9800) which is complexed with horseradish peroxidase, incubation with diaminobenzidine chromogen, counterstaining with haematoxylin, dehydration, clearing and coverslipping. All analysis of brain histology was carried out independently by co-authors MF and JM. Analysis was carried out, as is routine, by scanning slides at 100x and 200x magnification and examining detail at 400x and 600x.

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Statistical analysis

All statistical analyses were completed with SPSS 21.0 for MAC. Two-way ANOVAs with Impact Force and Experimental condition as factors (control, no protective headgear, protective headgear) were run to examine outcomes from the behavioural tests and biomarkers. For all analyses p values of <.05 were considered statistically significant. Post-hoc comparisons were completed when appropriate. Error bars represent SEM.

Results

Behavioural outcomes

Time-to-right

The time each animal took to right itself (flip from a supine position on its back to a prone or standing position) following the injury was measured as the time-to-right and is thought to reflect a loss-of-consciousness (LOC). The data are shown in Figure 2 and demonstrate that animals experiencing a concussion, had significantly longer times-to-right than the control group, and this was irrespective of whether or not they had protective headgear, $F = 8.506$, $p < .001$). In addition, both headguard and no headguard groups that experienced a hit of 106 G took longer to right themselves than the 37 G groups (t = 3.488, $p < .01$).

Beam-walking

The beam-walking assay is intended to represent the deficits in balance and motor co-ordination often reported acutely following concussion. Testing in the beam-walking paradigm occurred 12–18 hours post-concussion, with the measures being hind-leg foot slips and the time needed to cross the beam. Although hind-leg foot slips are the primary outcome measure, some animals are able to cross the beam without slipping if they significantly slow their pace, whereas others need to slow their pace and still exhibit an increase in foot slips. We therefore use both measures to increase reliability of the findings. Animals with a concussion had significantly more hind-leg foot slips compared to control animals, with the exception of the 37 G group with protective headgear, $F = 13.74$, $p < .001$, (Figure 3A). Headgear animals in the 37 G group did not exhibit impairment either in the number of foot-slips (t = 0.215, $p > .05$) or time to cross the beam (t = 0.789, $p > .05$) (Figure 3 A and B). Although animals in the 106G group with protective headgear exhibited increased hind-leg foot slips, they did not show deficits in the time needed to cross the beam (t = 0.866, $p > .05$) (Figure 3B).

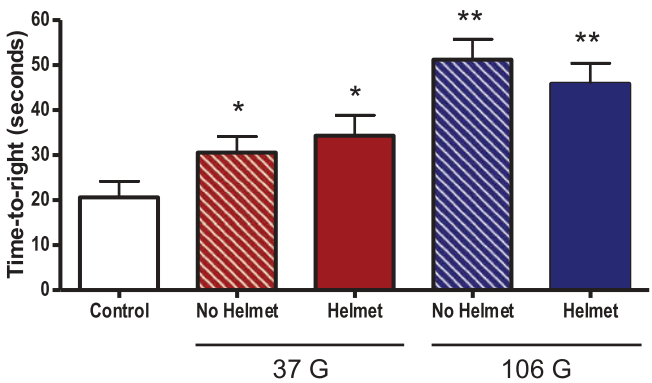

Figure 2. Regardless of protection or the severity of the impact, animals that experienced a mTBI took significantly longer to right after impact compared to controls. In addition, animals with a 106 G impact took longer compared to the 37 G group. (**: $p < .01$, *: $p < .05$).

Open field

Previous studies in our laboratory have shown that rodents are less active and reluctant to engage in exploratory behaviours following a concussion (16,18). Animals in this study were tested in the open field apparatus approximately 24 hours post-concussion. Similar to beam walking results, animals that experienced a concussion at 37 G with the protective headgear were indistinguishable from control animals. However, all other animals (37 G no headgear, 106 G no headgear, & 106 G with headgear), exhibited significant impairment in the distance travelled over the 10 minute session, $F = 6.701$, $p < .001$, (Figure 4A). Although distance is an important measure in the open-field the amount of time an animal spends in the center of the field and not in stereotaxic behaviour along the outside of the arena is also important factor. There was a significant group effect, $F = 3.480$, $p = .03$, exhibiting the similarities in locomotor/exploratory behaviour between control animals and 37 G animals with the headgear, along with differences between control animals and animals in the other 3 groups (Figure 4B).

Brain and serum biomarker analysis

Brain and body weight

There were no significant differences between any of the groups for body or brain weight ($p > .05$). The average body weight was 333.01 ± 19.88 and the average brain weight was 1.764 ± 0.087 (data not shown).

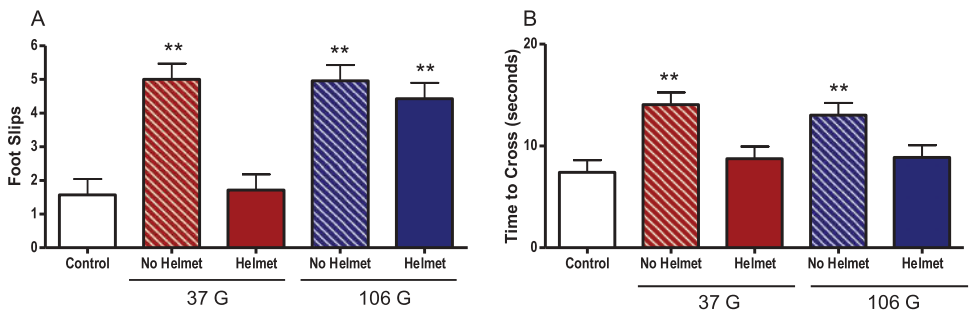

Figure 3. Motor dysfunction as measured by foot slips and time to cross in a beam walking paradigm. A) Rats with protective headgear at 37 G show no motor dysfunction in beam walking 14 hours post injury. Animals with no headgear at 37 G or at 106 G (regardless of protection) showed significant impairments. B) Rats with no protection for hits at 37 G or 106 G show longer times to cross the beam. Headgear groups are not significantly different than control animals. (**: $p < .01$).

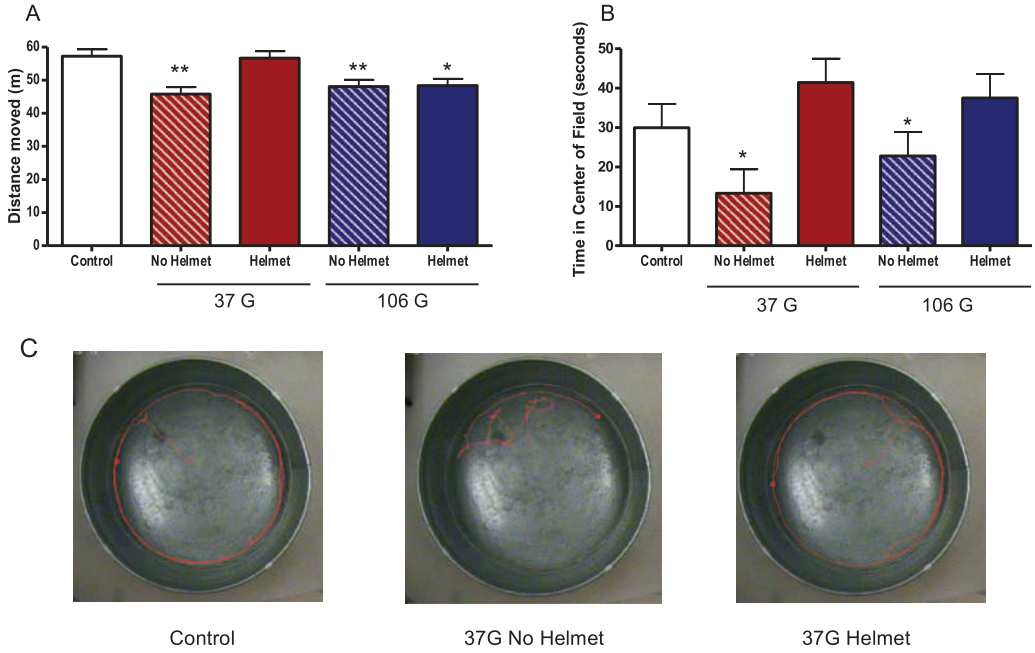

Figure 4. A) Average distance travelled by rats in each of the five experimental conditions in an open field for a 10 minute testing session. Animals in the 37 G impact group with a headgear showed no reduction in motor activity. The groups without headgear or at the higher impact have decreased distances travelled. B) Average amount of time the rats spent in the centre of the open field. C) Visual representation of the differences in travel patterns between control, headgear, and no headgear animals, (**: $p < .01$ or *: $p < .05$).

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Neuropathological examination

Gross neuropathological examination indicated no structural or pathological abnormalities, and microscopic examination did not indicate any evidence of reactive astrocytosis or axonal damage in any of the animals (data not shown). However, a small number of tiny contusions were visible in the brains of three rats from the 106G impact group without protection (3/15, 20% of animals without headgear), and one rat from the 106G impact group with protection (1/15, 6.7 % of animals with headgear). All contusions seen were in the inferior frontal region. See Figure 5. These findings strengthen the validity of the model to mimic mTBI/concussion, as the behavioural deficits identified must have been associated with concussive injury rather than trauma to the brain. In addition, similar to the behavioural findings, at the high impact velocity, the headgear offered protection against the typical damage.

Tnf-α

Tumor necrosis factor alpha (*TNF-α*) is a pro-inflammatory cytokine that is rapidly released after CNS insult. The accumulation of literature, especially within the discipline of severe brain injury, now demonstrates that *TNF-α* has opposing effects on recovery from brain injury (19,20). An example of this duality is that *TNF-α* has been found to be increased after controlled cortical impact injury (21), but treatment with a receptor antagonist was not beneficial in clinical treatment (22). Although further studies are needed to understand the dichotomous relationship of *TNF-α*, this biomarker is generally examined after brain injury. At 3 hours post-injury in this study, levels of *TNF-α* were significantly reduced in serum collected from animals in the 106 G impact group, irrespective of protection, and the 37 G group that was not protected, $F = 3.540$, $p < .05$. The headgear prevented modification to *TNF-α* in the lower impact group ($t = 0.453$, $p > .05$) (Figure 6A). By 48 hours post-concussion there were no differences in *TNF-α* serum levels between the groups ($p > .05$) (Figure 6B).

Nef-l

Neurofilament light (*NEF-L*) is a component of the axonal cytoskeleton that is localized to axons, and involved in maintaining structural integrity (23). Studies show that severe brain injury, cerebrovascular incidents, and subarachnoid hemorrhages are often associated with increased *NEF-L* in cerebrospinal fluid (24). The headgear protected against NEF-L increases at both impact forces, when examined 3 hours post-concussion; *NEF-L* serum levels demonstrated significant increases in animals that did not have protective headgear at both 37 G ($t = 2.489$, $p < .05$) and 106 G ($t = 2.410$, $p = .05$) but not in animals with headgear (Figure 6C). Serum levels of *NEF-L* were elevated in all animals that experienced a concussion at 48 hours post-injury but this was not significant ($p > .05$) (Figure 6D).

GFAP

Glial fibrillary acid protein is an intermediate filament protein found in glial cells of the gray and white matter that is a highly specific biomarker of CNS damage and pathology. Levels of *GFAP* have been consistently related to brain injury severity and outcome, with recent studies indicating that increases in *GFAP* can be linked to axonal injury associated with mild brain injury and concussion (25). This study found that in the acute period (3 hours) there were no significant changes in serum levels of *GFAP* following concussion in any of the groups,

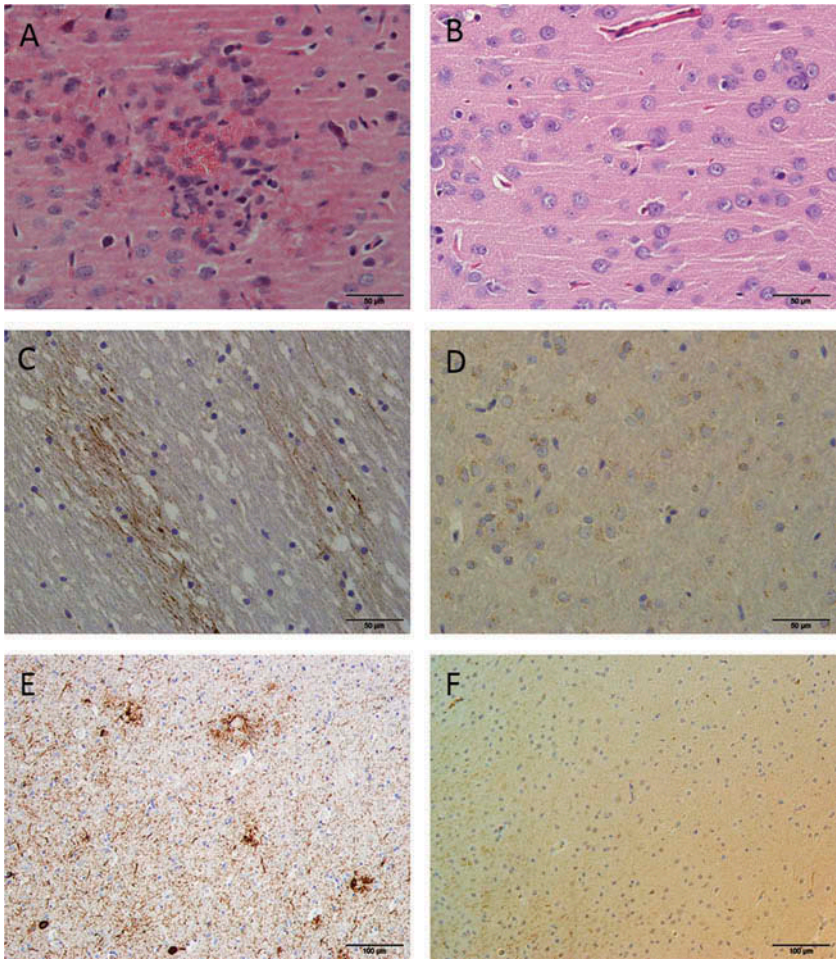

Figure 5. Histological analysis of brain tissue. In 3 of the 7 rats impacted at 10ms^{-1} without protection, tiny contusions were seen in the inferior frontal region of the brain (A). In 1 of the 7 animals impacted at this speed with protection, a contusion was also seen. Most of the rats in this group (B) showed no evidence of contusions. Immunostaining was carried out using antibodies against amyloid precursor protein and phosphorylated tau (pTau). Positive controls are APP and pTau are shown in (C) and (E). (D) and (F) show typical staining in the rat brains, seen across all groups. Scale bars = 50µm (A to D) and 100µm (E&F).

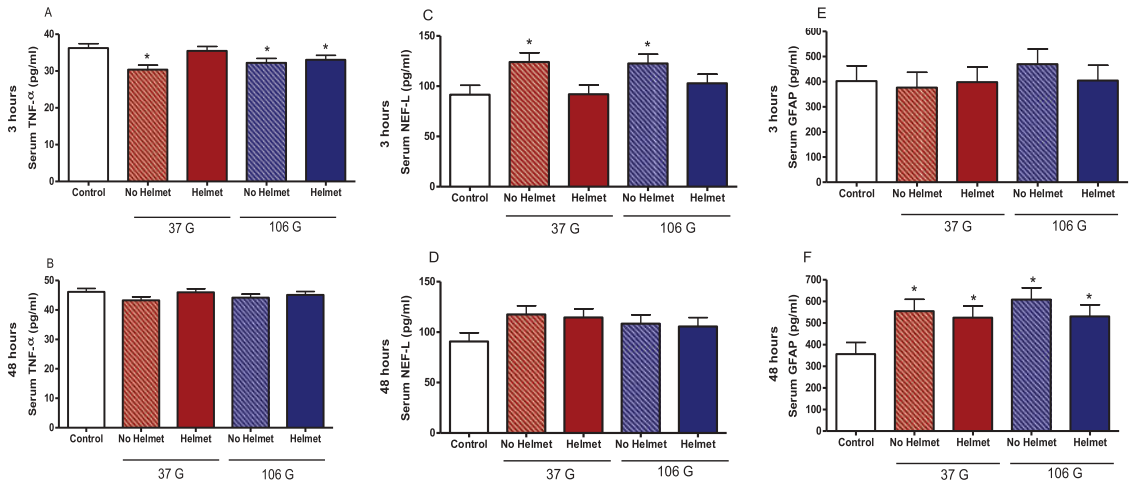

Figure 6. *TNF-α* serum samples at 3 hours (A) and 48 hours (B) after mTBI. There is a significant decrease at 3 hours for groups with no headgear at 37 G and for both groups at 106 G. There are no differences in *TNF-α* present at 48 hours post-concussion. (*: $p < .05$). *NEF-L* serum samples at 3 hours (C) and 48 hours (D) after mTBI. There is a significant increase in *NEF-L* levels at 3 hours for the 37 G and 106 G group with no headgear. At 48 hours, there is no significant difference between the groups. (*: $p < .05$). *GFAP* serum samples at 3 hours (D) and 48 hours (E) after mTBI. There are no significant differences at 3 hours for any of the groups. At 48 hours, all groups regardless of headgear or force of impact showed an increase in *GFAP*. (*: $p < .05$).

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$F = 0.813$ $p = .54$, (Figure 6E). However, at 48 hours, all groups, irrespective of headgear, showed an increase in the levels of *GFAP*, $F = 3.092$, $p < .05$, (Figure 6F).

Discussion

This study used a rodent model of sports-related concussion that closely mimics the acceleration forces, impact speeds, and biomechanical properties of rugby-induced concussions, to evaluate the efficacy of soft shell headgear in preventing behavioural and pathophysiological deficits associated with brain injury. This pilot study was designed to demonstrate whether or not the novel protective material could reduce injury-associated pathophysiology, with the resulting information being used to inform subsequent studies that will examine repetitive impacts and long-term outcomes. For this study, the protective headgear was tested at two distinct impact forces, 37 and 106 G, which were designed to represent the impact forces typically identified in routine collisions (those occurring repetitively throughout the game) and forces above the injury threshold (6,32). Higher impacts are rare but almost always associated with concussion and loss of consciousness (33). Measures of three serum biomarkers (*TNF-α, NEF-L, GFAP*) were analyzed at 3 and 48 hours after mTBI to determine if the headgear could confer protection on a molecular level.

Results of this study demonstrated that a soft-shelled headguard constructed of viscoelastic foam was effective in preventing mTBI symptomology at 37 G, but was only marginally successful at 106 G. The behavioural evidence for the 37 G finding was a significant reduction in hind-leg foot-slips, a decrease in time to cross the beam in the beam-walking assay, and increased locomotor activity in the open field test compared to the group with no headgear and impact of 37 G. When the impact force reached the injury threshold at 106 G, the headgear provided some benefit as indicated by the time to cross the beam being indistinguishable from controls, but failed to protect the animals in the open field test. Conversely, animals that received the same head impacts at either force but did not have the protective headgear, presented with acute symptomology indicative of mTBI in both of these tests. The behavioural findings from this study suggest that the protective material would be effective at reducing acute post-mTBI behavioural symptomology that arise from majority of rugby hits, which are documented at or under 37 G (5). To our knowledge, this is the first demonstration of a potential headgear material effective at reducing mTBI symptoms following a sports related head injury using a rodent model. However, this is contradictory to the considerable evidence that has shown that current headgear does not protect against concussion (3,33,34). This novel viscoelastic material is believed to absorb greater energy than standard headgear, therefore transferring less energy and acceleration forces onto the brain. However, the differences in findings between our studies and others could also be due to the cumulative nature of brain injury; multiple sub-concussive hits (head collisions that do not produce a concussion) in practice or games could confer greater susceptibility to the experimental injury in question, opposed to our single hit to a naïve rodent (35). Irrespective of the underlying cause, the results are promising, indicating that this protective headgear may be beneficial to rugby players.

In addition to behavioural or functional measures, the TBI field also incorporates the use of molecular biomarkers in an effort to understand concussion pathophysiology. The primary goal of biomarker research is to identify a single, or panel of markers that would aid in early detection and diagnosis, in addition to predicting patient outcomes. However, to date, it has been very difficult to identify a reliable and predictive biomarker. This difficulty is primarily due to the fact that the underlying pathophysiology of concussion remains unclear, and there has been significant ambiguity and heterogeneity in clinical presentation of symptomology (20,36). In addition to variation in clinical presentation, there are also differences in biomarkers between experimental models (37–39). Therefore the interpretation of the findings in this study must be done with caution. This study chose to examine three serum biomarkers (*TNF-α, NEF-L*, and *GFAP*) that have been extensively examined for their roles in moderate and severe brain injury, as well as in neurodegeneration and neuroinflammation. As there is very little research that has actually shown that changes in these markers directly corresponds to patient outcomes following concussion, the discussion of these findings is theoretical and representative of the rodent model.

Similar to the behavioural findings, analysis of the serum biomarkers chosen for this study demonstrated that the headgear material offered considerable protection against the 37 G head impacts and only marginal protection against the 106 G impacts. It is also important to point out that the timing of sample collection was significant. If samples had been collected at earlier or later time-points the interpretations would differ dramatically. For example, there were no changes in levels of *GFAP* at 3 hours post-injury, but significant differences in *TNF-α*. *TNF-α* is a pro-inflammatory cytokine that is rapidly released following insult and binds to both glial and neuronal cells in the brain. The vast majority of literature related to brain injury has demonstrated significant increases in *TNF-α*, but these studies utilized a moderate or severe brain injury and then examined either rodent brain or human cerebral spinal fluid (17,18,40). Based on these findings one could predict that mTBI would also increase *TNF-α*, but this study found that both the 37 and 106 G impacts reduced levels in serum at 3 and 48 hours after the injury, when animals did not have protective headgear. However, when comparing the control animals in this study to the experimental conditions, animals in the 37G impact group with headgear were indistinguishable from controls across all time points. It is possible that circulating *TNF-α* crossed into the brain through a damaged blood brain barrier (BBB). Previous work has shown that TBI can cause increased permeability in the barrier, which peaks at 4 – 6 hours after injury, which could explain why there is a decrease in *TNF-α* at 3 hours but not at 48 hours (41). Alternatively, the level of serum *TNF-α* could be unrelated to the brain's injury response and reflect a systemic reaction to the injury experience.

Neurofilaments are found exclusively in neurons and are involved in maintaining neuronal shape and function (21,42). Studies with boxers have demonstrated significant increases in *NEF-L* concentration in cerebral spinal fluid days after a fight whether the boxer reported a concussion or not (21). The present study found that the headgear material prevented a significant

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acute increase in *NEF-L* serum levels for both 37 and 106 G head impacts at the 3-hour time point. Serum analysis at the later time points demonstrated that *NEF-L* levels at 48 hours were increased in all animals that experienced an injury, regardless of whether or not they had the protective headgear. These results are still encouraging as they suggest that the pathophysiological processes differ for the headgear and non-headgear groups, which may indicate different, and improved long-term trajectories.

Unlike *TNF-α* or *NEF-L*, serum levels of *GFAP* have been examined in human populations following mild brain injury or concussion. The major finding has been that serum levels of *GFAP* increased in a portion of the population with mTBI (43,44). *GFAP* is used as an estimate of astrocyte activation and has been considered a characteristic marker of brain injury (45). In the present study, levels of *GFAP* rose as a function of time for animals that received head impacts at both 37 and 106 G. However, both headgear groups showed a less pronounced increase between 3 and 48 hours compared to groups with no protection; this may indicate that the injury pathophysiology is slowed or lessened in these animals. Further investigation, such as measuring at other time-points would be required to confirm this, but the novel protective headgear may be blunting the pathophysiological response of the concussion.

This study has found that the tested material eliminated behavioural deficits that represent mTBI pathology at impact forces of 37 G and lessened concussion symptomology at impact forces of 106 G. The biomarker analyses from serum samples provide further support for these findings. Although the initial results from this study are promising, additional experiments would strengthen support for the use of this material in headguards for rugby and similar recreational sports. Comparing our outcomes to another protective material, in addition to our non-injury group, could strengthen the benefit of this protective headgaurd. Furthermore, as the majority of players sustain multiple head impacts within a game (6,32), future studies should examine how many impacts a headguard can sustain before rendering it no longer protective. Although, the majority of current rugby players do not choose to wear the available headgear, this study indicates that head protection made from this viscoelastic open-cell foam could improve the outcomes after head injury. In addition, this pilot study examined only male rats as the majority of amateur and professional rugby players are male. However, given that accumulating evidence indicates sex-differences in pathophysiology and symptom presentation following mTBI (46–50), future studies need to examine the efficacy of headgauards in female populations. Based on these findings, headguards may assist with the protection and prevention of concussions by preventing acute behavioural symptoms for the player. In addition, if headguards can prevent molecular damage as suggested by the biomarkers in the 37 G impact group, wearing headgear may assist with the resulting long-term neurodegeneration and pathophysiology of concussion.

Declaration of interest

The authors report no declarations of interest

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