A double-blind, placebo-controlled intervention trial of 3 and 10 mg sublingual melatonin for post-concussion syndrome in youths (PLAYGAME): study protocol for a randomized controlled trial

Barlow et al.
A double-blind, placebo-controlled intervention trial of 3 and 10 mg sublingual melatonin for post-concussion syndrome in youths (PLAYGAME): study protocol for a randomized controlled trial

Karen M Barlow1,8*, Brian L Brooks1, Frank P MacMaster1, Adam Kirton1, Trevor Seeger1, Michael Esser1, Susan Crawford1, Alberto Nettel-Aguirre1, Roger Zemek2, Mikrogianakis Angelo1, Valerie Kirk1, Carolyn A Emery1,3, David Johnson1, Michael D Hill4, Jeff Buchhalter1, Brenda Turley1, Lawrence Richer5, Robert Platt6, Jamie Hutchison7 and Deborah Dewey1

Abstract

Background: By the age of sixteen, one in five children will sustain a mild traumatic brain injury also known as concussion. Our research found that one in seven school children with mild traumatic brain injury suffer post-concussion syndrome symptoms for three months or longer. Post-concussion syndrome is associated with significant disability in the child and his/her family and yet there are no evidence-based medical treatments available. Melatonin has several potential mechanisms of action that could be useful following mild traumatic brain injury, including neuroprotective effects. The aim of this study is to determine if treatment with melatonin improves post-concussion syndrome in youths following mild traumatic brain injury. Our hypothesis is that treatment of post-concussion syndrome following mild traumatic brain injury with 3 or 10 mg of sublingual melatonin for 28 days will result in a decrease in post-concussion syndrome symptoms compared with placebo.

Methods/Design: Ninety-nine youths with mild traumatic brain injury, aged between 13 and 18 years, who are symptomatic at 30 days post-injury will be recruited. This study will be conducted as a randomized, double blind, placebo-controlled superiority trial of melatonin. Three parallel treatment groups will be examined with a 1:1:1 allocation: sublingual melatonin 3 mg, sublingual melatonin 10 mg, and sublingual placebo. Participants will receive treatment for 28 days. The primary outcome is a change on the Post-Concussion Symptom Inventory (Parent and Youth). The secondary outcomes will include neurobehavioral function, health-related quality of life and sleep. Neurophysiological and structural markers of change, using magnetic resonance imaging techniques and transcranial magnetic stimulation, will also be investigated.

Discussion: Melatonin is a safe and well-tolerated agent that has many biological properties that may be useful following a traumatic brain injury. This study will determine whether it is a useful treatment for children with post-concussion syndrome. Recruitment commenced on 4 December 2014.

(Continued on next page)
Background

Traumatic brain injury (TBI) is one of the most common causes of neurological morbidity and it is more common in childhood and adolescence than at any other time of life [1-4]. Mild traumatic brain injury (mTBI) is the acute neurophysiological effect of blunt impact or other mechanical energy applied to the head, such as from sudden acceleration, deceleration or rotational forces [5,6]. It accounts for 90% of all TBIs [7]. Epidemiological studies suggest that one in five children will experience a mTBI by the age of 10, [1,8] and 799 out of 100,000 children under 14 years visit the emergency department (ED) with a mTBI in the United States [2] and Canada [9]. Falls (51%) and sports-related activities (25%) are the commonest causes [2,10,11].

One in seven school children sustaining an mTBI will suffer post-concussion syndrome (PCS) symptoms for three months or longer [10]. PCS is a combination of clinical symptoms including physical (such as headaches), cognitive (such as learning and/or memory dysfunction), and behavioral (such as mood) disturbances [7,10,12]. It is associated with significant disability in the child and his/her family [5,12-17]. It has been shown that 2% of mTBI children continue to have PCS symptoms one year later [10]. Using these figures, we estimate that annually over a 1000 children in Canada have PCS for over a year due to a ‘mild’ TBI and yet there are no evidence-based medical treatments available [18]. This suggests an urgent need to develop novel treatment options to improve outcomes for children suffering from PCS [19]. Furthermore, our neurobiological understanding of PCS is lacking [6,18], and routine clinical tests are not informative and so are not helpful in guiding treatment.

The complex pathophysiology of mTBI is well described [19-26], but the explanations for prolonged PCS are unclear [21,27]. The mechanisms leading to neuronal dysfunction, cell death and altered connectivity include: oxidative stress, metabolic dysfunction, neuroinflammation, axonal damage and alterations in cerebral blood flow [19,21,28]. Most animal studies demonstrate recovery from mTBI within 7 to 15 days [22,29,30], similar to the clinical experience of the majority of humans [6,10,12]. However, the pathophysiological explanations for prolonged PCS, seen in 11% of children with mTBI, have been elusive [10,27]. Recent animal and human research suggest that the explanations for the persistent PCS symptoms may be due to alterations in neuronal circuitry and neurotransmission [29-36].

Treatment of post-concussion syndrome

There are few evidence-based treatments for PCS and these studies usually do not include children, and so pediatricians have to rely on consensus guidelines for adults [37-44]. Avoidance of repeat injury is the mainstay in any treatment regimen for TBI as is rest until symptoms resolve [18,45]. As PCS symptoms resolve quickly in the majority of people, clinicians do not use pharmacological treatments (except for analgesics) in the first few weeks after injury [38,46-50]. There are few evidence-based treatments for PCS that persists for one month or more [37,40,43,44,51-53], providing the clinician with little guidance for the management of significantly debilitated patients [54]. Treatments are used without conclusive evidence [51] and are chosen to target the most problematic symptom [49,54-60]. A frequently recommended treatment for sleep dysfunction after mTBI is melatonin [61].

Melatonin as a potential treatment for PCS

Melatonin, naturally produced in the body by the pineal gland, has neuroprotective, analgesic, and anxiolytic properties and is a promising agent in TBI [62-67]. Melatonin’s role in the chronological regulation of major physiological processes (such as the sleep/wake cycle) [68-70] is well accepted. More recently, its therapeutic potential is being explored in other neurobehavioural conditions (for example chronic pain, headaches and anxiety) and TBI [67,71,72]. The many separate biological activities of melatonin are both receptor-mediated (at physiological levels) and non-receptor mediated (especially at supraphysiological levels) [63,72]. It is lipophilic, can cross cell membranes easily [73,74] and its neuroprotective mechanisms include i) reducing oxidative stress (for example decreasing oxidative and nitrosative abuse, lipid peroxidation, and increasing antioxidant enzymes) [75-81], ii) improving mitochondrial function [62,73,74,82,83], iii) inhibiting apoptosis (cell death) [84-86], iv) decreasing the neuroinflammation [64,87,88], and v) decreasing glutamate toxicity via GABA receptors [89-91].

The inherent biochemical and physiological characteristics of the brain, including high polyunsaturated fatty acids and energy requirements, make it particularly susceptible

Keywords: Concussion, Traumatic brain injury, Melatonin, Placebo, Pediatric, Randomized controlled trial

Trial registration: This trial was registered on 6 June 2013 at ClinicalTrials.gov. Registration number: NCT01874847.
to free radicals mediated insult. Melatonin has been shown to decrease oxidative stress induced by exercise in young athletes [92,93] and in patients with renal failure [94]. It protects against focal and global brain injury in adult and juvenile TBI [87,95-97], ischemic brain injury [98,99], cerebral edema [67,100], spinal cord injury [101,102] and radiation injury [103].

Further, melatonin improves many of the symptoms seen in PCS such as headaches, pain, and anxiety [104] probably via the gamma Aminobuteric acid (GABA)-ergic system and opiate receptors [66,105-107]. It is used to aid sleep in children with disabilities and visual impairment [108]. Melatonin has analgesic properties and has been shown to be useful in adult and pediatric migraine [109,110] and disorders of chronic pain (such as fibromyalgia and irritable bowel syndrome) [111,112]. It is also effective in treating anxiety. In a systematic review, premedication with melatonin significantly decreased preoperative anxiety [113].

The dose of melatonin in clinical pediatric practice ranges between 1 and 10 mg. Receptor-mediated effects occur at physiological doses (for example in children with chronic insomnia effects are achieved at 0.05 to 0.15 mg/kg) [114]. Lower doses do not achieve the same analgesic and anxiolytic effects [109,111,115-118]. In order to saturate melatonin receptors and achieve non-receptor mediated effects, supra-physiological doses are required [114,119,120]. A dose of 10 mg melatonin is likely to achieve this and yet stay within clinically-accepted parameters [70].

**Pilot data using melatonin in PCS**

We found that children with prolonged PCS and headaches had a significant response to melatonin treatment [61,121]. Post-traumatic headaches (PTH) are thought to be particularly resistant to treatment [55,122,123]. Few studies have specifically analyzed how patients respond to treatment [124,125], and none of these were in children. Our study aimed to: 1) describe the headache characteristics of PCS in children and 2) their response to pharmacological treatments [61]. A retrospective chart review of 48 children treated for PTH since 2007 was performed. The mean age was 14.1 years (SD 3.1) and 66% were female. The time since injury was 10.6 (SD 8.1) months. Melatonin was used as a first-line treatment where sleep dysfunction was a comorbidity. A total of 15 out of 18 children responded to melatonin treatment. Seven children were treated with 3 to 5 mg of sublingual melatonin; 11 children were treated with 6 to 10 mg. Significantly more children responded to treatment with melatonin (83%) when compared with the other treatments used (P <0.05) and no serious side effects were reported. As these children were on average 10.2 months post-injury, it is very unlikely that this response was due to time alone.

In summary, melatonin has potential as a safe therapeutic candidate for the treatment of PCS in children. It has efficacy in many of symptoms commonly encountered in PCS. In preliminary work, we found that children with prolonged PCS and headaches had a significant response to sublingual melatonin treatment [64]. Melatonin’s therapeutic potentials in mTBI include: 1) as a free radical scavenger and broad-spectrum antioxidant [75,76,82,88,126] and 2) symptomatically via the GABAergic system and opiate receptors [66,95,105,106,127]. The aim of this trial is to determine if treatment with melatonin improves PCS following mTBI in youths.

**Methods/Design**

We hypothesize that the treatment of children with PCS following mTBI with 3 or 10 mg of melatonin for 28 days will result in a decrease in PCS symptoms as compared with a placebo. The primary research question will be: Does the treatment of children with PCS symptoms following mTBI with 3 or 10 mg of sublingual melatonin for 28 days result in a decrease in PCS (physical, cognitive and behavioral) symptoms as compared with a placebo? The secondary research questions will be: 1) Is there a dose-response relationship? and 2) Is the treatment effect independent of the effect on sleep?

**Basic study design**

This study will be conducted as a randomized, double blind, placebo-controlled superiority trial. Three parallel treatment groups will be examined with a 1:1:1 allocation: 1) sublingual placebo, 2) sublingual melatonin 3 mg, and 3) sublingual melatonin 10 mg, see Figure 1. Individuals will be allocated to treatment groups using a randomization sequence that will be created in variable random block sizes (multiples of 3: 3, 6 and 9) to aid in the concealment of the next allocation, using random number-generating software. Participants, parents and investigators will be blinded to treatment groups. The primary endpoint is the change on the Post-Concussion Symptom Inventory Score for the parent (PCSI-P) and youth (PCSI-Y). Secondary outcome measures are listed in Table 1. The design allows for dose-dependent response assessment. This study was approved by Health Canada (clinical trial application number: 16391). Ethical permission was granted by the University of Calgary Health Research Ethics Board (number: 13-0372); protocol amendment version 02: 24 March 2014. Trial metadata are given in Addition file 1.
with an mTBI who remain symptomatic at 30 days post-injury.

**Subjects**

*Eligibility criteria*

Parents and adolescents must provide written informed consent before any study procedures occur. Inclusion and exclusion criteria are shown in Table 2.

**Interventions**

Eligible patients will be randomized in equal proportions between three groups: placebo, 3 mg melatonin and 10 mg melatonin. Medication is taken sublingually at night, one hour before sleep time, for 28 days and will be continued even if there is symptom resolution. The placebo, 3 mg melatonin and 10 mg melatonin sublingual tablets are identically sized white tablets which are peppermint flavored.

**Adherence**

Administration of the study pill will occur at home under the supervision of the parent. When the study pill is dispensed, the research coordinator will review the importance of following study guidelines and instructions about taking study pills including timing, storage, and what to do in the event of a missed dose. Methodologies to maximize follow-up and compliance include convenient follow-up times, participant engagement strategies (such as newsletters and a website) and experienced research personnel. Adherence assessments will include a review of the medication log, a pill count every week, and a review of reasons for non-compliance. Unused will be counted and recorded on the appropriate case report form. With regards to concomitant care, there are no restrictions on the use of other medications. All participants will be advised to try to avoid analgesia overuse. Participants will be asked to complete a diary of any medications, medical appointments and alternative therapies.

**Primary outcome measure**

The Post-Concussion Symptom Inventory - Parent and Youth (PCSI-P and PCSI-Y). These standardized
questionnaires examining 26 symptoms, provide an overall rating of PCS. They have four specific domains: physical, cognitive, emotional and fatigue, and a high level of internal consistency reliability (alpha = 0.92) [140,141]. A change in PCSI scores allows us to account for baseline variability and gender (timeline: pretreatment, mid-treatment (day 14 to 15) and post-treatment (day 30 to 35) and 90 to 120 days post injury) [142]. A change in PCSI scores allows us to account for baseline variability and gender (timeline: pretreatment, mid-treatment (day 14 to 15) and post-treatment (day 30 to 35) and 90 to 120 days post injury) [142].

Secondary outcome measures (Table 1) will be collected at baseline and at the end of treatment (day 30 to 35). These will include parent and child rating of functional impairment using the 50-item Child Health Questionnaire (CHQ) [128-131], the Behaviour Assessment System for Children – 2 which is a standardized parent report measure of child behavior, and the Behaviour Rating Inventory of Executive Function (BRIEF) to assess daily executive abilities. Neurocognitive ability (including attention, executive functioning, memory, reaction time and information processing speed) will be measured using a computerized test, CNS-Vital Signs [132]. Actigraphy will be used to measure: sleep duration, bed time, sleep time, wake time; longest and shortest sleep time [135-137].

**Participant timeline and process**

Screening takes place by telephone around 30 days post-injury. Parental consent and youth assent will be obtained by the research assistant in the clinic.

**Sample size**

The main objective and hypothesis is based on the difference between the placebo and the 3 mg melatonin groups. The outcome of interest is the change in the PCSI score (day 30 minus day 59) and calls for a test of means between groups using one way analysis of variance (ANOVA). We used data obtained in our epidemiological study to calculate a reliable change score using the Jacobson Truax method [10,143]. A 10-point change on the PCSI-P score indicates a reliable change for subjects who are symptomatic at one month (SD 14.7). Further, we find that in practice a 10-point change is also clinically significant. Using a significance level of alpha = 0.05, expecting a power of 80%, assuming a within group SD of 14.7 and using as an effect size a difference between groups of 10 (the reliable change score), a sample size of 30 per group is required. Allowing for a 10% attrition rate, 33 participants per group are required. Recruitment will occur over three years with 36 to 48 patients being recruited each year.

**Statistical analysis**

Baseline demographic and clinical variables will be examined for group differences using ANOVA for continuous variables and chi-square tests for categorical variables. Subsequent analyses will involve controlling for possible confounding factors. All analyses will be done on an intention-to-treat basis (last observation carried forward). Group differences in the change in PCSI scores will be analyzed using ANOVA, with placebo, 3 mg melatonin and 10 mg melatonin as groups. Estimates and corresponding 95% confidence intervals for the a priori set pairwise comparisons of interest (placebo versus 3 mg, 3 mg versus 10 mg and/or placebo versus 10 mg) will be provided. Time to symptom resolution (as defined by a PCSI score equal or less than pre-injury) will be examined using the Cox proportional hazards model. Adverse events will be tabulated. Complete documentation will be kept on ‘non-completers’, including their reasons for non-completion. Differences between randomized groups for early termination will be descriptively reported. A secondary efficacy analysis will be done on ‘completers’ per protocol only, excluding protocol violations.

Analyses of secondary outcomes will examine group differences with a series of ANOVAs examining changes in scores for: 1) CHQ, 2) BASC-2 (parent), 3) sleep parameters, and 4) cognition (measured by CNS Vital Signs battery: attention, executive functioning, and reaction time, processing speed). Hierarchical multiple-regression modeling will be used to predict symptom improvement by entering sleep parameters (step 1), and treatment group (step 2) in order to determine how predictive treatment is above and beyond any effects of melatonin on sleep. Subgroup analysis will be based on the following

---

**Table 2 Inclusion and exclusion criteria**

| Inclusion criteria | Exclusion criteria |
|--------------------|-------------------|
| Age 13-18 years inclusive | Previous significant medical history |
| Mild traumatic brain injury [139] | Previous concussion within 12 months |
| Symptomatic (increase in PCS symptoms compared with pre-injury) at 30 days post injury | Lactose intolerance, as the placebo contains lactose |
| | Use of drugs that are likely to affect TMS, fMRI and/or sleep |
| | Inability to complete questionnaires or evaluation |
| | Claustrophobia or inability to tolerate MRI |
| | Contraindications to TMS (including history of seizures, unexplained loss of consciousness, metal in the head and/or implanted brain medical devices, cardiac pacemaker, and so on) |

Post Concussion Syndrome (PCS), Transcranial Magnetic Stimulation (TMS), Magnetic Resonance Imaging (MRI), functional MRI (fMRI).
dichotomies: personal or family history of migraine, loss of consciousness, and previous history of concussion. Bonferroni correction will be used for post hoc analysis.

All analyses will be performed using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, United States). Outcome analysis will be performed after all participants have been recruited and reviewed by an independent biostatistician as part of the Data Safety Monitoring Board (DSMB). An interim safety analysis will be performed under the direction of the DSMB.

Safety and potential risks
Melatonin is available as an over-the-counter sleep aid in Canada. It is well tolerated by humans even at supra-physiological doses [104]. Systematic reviews of melatonin in sleep disorders have found melatonin to be safe in adults and children [144,145]. For all adverse events, there was no significant difference between melatonin and the placebo [144]. Any adverse events in PLAYGAME will be immediately reported to the principal investigator who will report any serious unexpected adverse event to the DSMB.

Trial management
The Trial Steering Committee (TSC) in accordance with Good Clinical Practice Guidelines will manage the trial (Chair: KMB). All lead investigators and authors of this paper will be steering committee members.

The Trial Management Committee (KMB, BLB, AK, FPM and BT) will be responsible for the day-to-day running of the trial.

The DSMB will be responsible for safeguarding the interests of trial participants, potential participants and investigators (JH, LR and RP).

Research team responsibilities
KMB (Director of Brain Injury and Rehabilitation Program) is a pediatric neurologist and expert in TBI. She will be responsible for the day-to-day management, adherence to protocol, patient clinical concerns, data interpretation, manuscript preparation and distribution/utilization of trial results. DD is an experienced research team leader and neurobehavioral scientist who, together with MDH, will ensure smooth trial operation, and DJ is an expert in knowledge translation. This research team brings together the expertise of a TBI neuropsychologist BLB (cognitive testing), pediatric emergency medicine MA (ED personnel management, data collection and recruitment), an expert in pediatric sleep disorders and VK (antigraph and sleep logs). Research methodological and biostatistical experience will be provided by SC and ANA (study design, data management and data analysis).

Expected results
It is expected that children treated with melatonin will have lower scores on the PCSI when compared with the placebo, and will have less behavioral (measured using the Behavioral Assessment System for Children) and functional impairment (measured using the Child Health Questionnaire). We expect to observe a dose-response relationship, with 10 mg melatonin treatment group having significantly lower PCSI scores, faster reaction times and increased processing speed compared with the 3 mg melatonin group. Sleep parameters are not expected to differ between the melatonin groups. It is expected that melatonin will be well tolerated without serious adverse side effects.

Discussion
Melatonin is available as an over-the-counter sleep aid in Canada. It is well tolerated even at supra-physiological doses. It has many biological properties that make it a promising treatment in traumatic brain injury. This study is a first step in elucidating whether sublingual melatonin is a useful treatment for children with post-concussion syndrome. This study will provide valuable information about the neurobiology of post-concussion syndrome in children, including the neurophysiological and structural properties of the brain during recovery from mTBI (not discussed here).

Trial status
The trial commenced on 4 December 2013 and is in recruitment. It will run until approximately November 2019.

Additional file

Additional file 1: PLAYGAME trial metadata.

Abbreviations
ACH: Alberta Children’s Hospital; ANOVA: Analysis of variance; BASC-2: Behavioral assessment system for children; CHEO: Children’s Hospital of Eastern Ontario; CHQ: Child health questionnaire; CHREB: University of Calgary Health Research Ethics Board; DSMB: Data safety monitoring board; ED: Emergency department; mTBI: Mild traumatic brain injury; PCS: Post-concussion syndrome; PCSI: Post-concussion symptom inventory; PCSI-P: Post-concussion symptom inventory-parent; PCSI-Y: Post-concussion symptom inventory-youth; PTH: Post-traumatic headache; TBI: Traumatic brain injury; TSC: Trial steering committee.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
KMB: principal investigator, conception and initiation, running of the trial, writing, critical revision, and final approval of manuscript. DD: conception and design of study. DJ: aided in design of study, emergency medicine, and knowledge translation. AK: conception and design of study, transcranial magnetic stimulation. FPM: conception and design of study, functional neuroimaging. BLB: conception and design of study, neuropsychological testing. AM: design of study, recruitment, emergency medicine. VK: conception and design of study, sleep medicine. MH: conception and design.
of study, trial operations. ME: conception and design of study, trial operations. BT: Implementation, data collection and trial operations. TS: Implementation, transcranial magnetic stimulation data collection and analyses. LR: Data monitoring, therapeutic. PR: Data safety monitoring. JR: Data safety monitoring. All authors read and approved the final manuscript.

Acknowledgements

This study is funded by Canadian Institutes of Health Research (grant number: 293375) and Faculty of Medicine, University of Calgary. These funding sources had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results. The design, management, analysis and reporting of the study are entirely independent of any manufacturers of melanotan.

Pedicatric Emergency Research Teams at Alberta Children's Hospital and Children's Hospital of Eastern Ontario, and Pediatric Emergency Research Consortium, Canada.

Author details

1Alberta Children's Hospital Research Institute, University of Calgary, Room 293, Heritage Medical Research Building 3330 Hospital Drive NW, Calgary, AB T2N 4N1, Canada. 2Pediatric Emergency Medicine, Children's Hospital of Eastern Ontario, 01 Smyth Road, Ottawa, Ontario K1H 8L1, Canada. 3Faculty of Kinesiology, 2500 University Drive NW, Calgary, AB T2N 1N4, Canada. 4Hotchkiss Brain Institute, University of Calgary, Health Research Innovation Centre, Room 1A10, 3330 Hospital Drive NW, Calgary T2Y 4N1 Alberta, Canada. 5Department of Pediatric Neurology, University of Alberta, Room 1D1, 8440 112 Street, Edmonton T6G 2B7 Alberta, Canada. 6Department of Statistics, Montreal Children's Hospital Research Institute, McGill University, 2300 Tupper Street, Montreal, Quebec H3H 1P3, Canada. 7Department of Pediatrics, Department of Critical Care Medicine, The Hospital for Sick Children, 555 University Ave. 2nd Floor, Atrium - Room 2830A, Toronto, Ontario M5G 1X8, Canada. 8Alberta Children's Hospital, 2888 Shaganappi Trail NW, Calgary, Alberta T3B 6A8, Canada.

Received: 31 March 2014 Accepted: 17 June 2014

Published: 7 July 2014

References

1. McKinlay A, Grace RC, Horwood LJ, Ferguson DM, Riddell EM, MacFarlane MR. Prevalence of traumatic brain injury among children, adolescents and young adults: prospective evidence from a birth cohort. Brain Inj 2008, 22:175–181.

2. Langlois JA, Rutland-Brown W, Thomas KE. The incidence of traumatic brain injury among children and adolescents in the United States: differences by race. J Head Trauma Rehabil 2005, 20:229.

3. Thornhill S, Teasdale GM, Murray GD, McEwen J, Roy CW, Penny KI. Disability in young people and adults one year after head injury: prospective cohort study. BMJ 2000, 320:1631–1635.

4. Maas AIR, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. Lancet Neurol 2008, 7:229–241.

5. Geoghan J, Swain B, Friedman D, Forget R. Exploring children's self-efficacy related to physical activity performance after a mild traumatic brain injury. J Head Trauma Rehabil 2005, 20:436–449.

6. Barkhoudarian G, Hovda DA, Giza CC. The molecular pathophysiology of concussive brain injury. Curr Sports Med 2011, 3033: ii.

7. Heads-Up. Facts for Physicians about Mild Traumatic Brain Injury. http://www.cdc.gov/concussion/headup/pdf/Facts_for_Physicians_booklet_apdf.pdf

8. Corrigan JD, Selassie AW, Orman JAL. Epidemiology of traumatic brain injury. J Head Trauma Rehabil 2010, 25:72.

9. Ryu WH, Feinsein A, Colantonio A, Steiner DL, Dawson DR. Early identification and incidence of mild TBI in Ontario. Can J Neurol Sci 2009, 36:439–443.

10. Barlow KM, Crawford S, Stevenson A, Sandhu SS, Belanger F, Dawsey D. Epidemiology of postconcussion syndrome in pediatric mild traumatic brain injury. Pediatrics 2010, 126:6374–6381.

11. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. J Head Trauma Rehabil 2006, 21:377.

12. Yeates KO, Taylor HG, Rusin J, Bangert B, Dietrich A, Nuss K, Wright M, Nagni DS, Jones BL. Longitudinal trajectories of postconcussive symptoms in children with mild traumatic brain injuries and their relationship to acute clinical status. Pediatrics 2009, 123:735–743.

13. Moran LM, Taylor HG, Rusin J, Bangert B, Dietrich A, Nuss KE, Wright M, Yeates KO. Do postconcussive symptoms discriminate injury severity in pediatric mild traumatic brain injury? J Head Trauma Rehabil 2011, 26:348–354.

14. Emanuelson I, Anderson HE, Bjorklund R, Stahlhammar D. Quality of life and post-concussion symptoms in adults after mild traumatic brain injury: a population-based study in western Sweden. Acta Neurol Scand 2003, 108:332–338.

15. Ewing-Cobbs L, Levin HS, Fletcher JM, Miner ME, Eisenberg HM. The children's orientation and amnesia test: relationship to severity of acute head injury and to recovery of memory. Neurosurgery 1990, 27:138.

16. Ponsford J, Cameron P, Fitzgerald M, Grant M, Mikocka-Walus A. Long-term outcomes after uncomplicated mild traumatic brain injury: a comparison with trauma controls. J Neurotrauma 2011, 28:597–596.

17. Yeates KO, Taylor HG. Neurobehavioural outcomes of mild head injury in children and adolescents. Pediatr Rehabil 2005, 8:5–16.

18. McCrory P, Meeuwisse W, Johnston K, Dvorak J, Aubry M, Molfoy M, Cantu R. Consensus statement on concussion in sport: the 3rd international conference on concussion in sport held in Zurich, november 2008. Br J Sports Med 2009, 43(suppl 1):76–80.

19. Cernak I, Chang T, Ahmed FA, Cruz M, Vink R, Stoica B, Faden AI. Pathophysiological response to experimental diffuse brain trauma differs as a function of developmental age. Dev Neurosci 2010, 32:442–453.

20. Reed JA, LEonard AM, Fox DP, Tesler AR, Raghupathi R. Concussive brain trauma in the mouse results in acute cognitive deficits and sustained impairment of axonal function. J Neurotrauma 2011, 28:547–563.

21. Giza CC, Hovda DA. The neurotubulastic cascade of concussion. J Athl Train 2001, 36:228–235.

22. Giza CC, Maria N, Hovda DA. N-methyl-D-aspartate receptor subunit changes after traumatic injury to the developing brain. J Neurotrauma 2006, 23:950–961.

23. Gosselin N, Saluja RS, Chen JK, Bottari C, Johnston K, Pitts A. Brain functions after sports-related concussion: insights from event-related potentials and functional MRI. Phys Sportsmed 2010, 38:27–37.

24. Henninger N, Skard KM, Li Z, Kulkarni P, Dutzmann S, Urbanek C, Schwab S, Fisher M. Differential recovery of behavioral status and brain function assessed with functional magnetic resonance imaging after mild traumatic brain injury in the rat. Crit Care Med 2007, 35:2607–2614.

25. Henry LC, Tremblay S, Boulanger Y, Ellerembg D, Lassonde M. Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. J Neurotrauma 2010, 27:565–76.

26. Upton MM, Galvella E, Lo C, Gold T, Ardekani BA, Shifteh K, Bello JA, Branch CA. Multifocal white matter ultrastructural abnormalities in mild traumatic brain injury with cognitive disability: a voxel-wise analysis of diffusion tensor imaging. J Neurotrauma 2008, 25:1335–1342.

27. Shrey DW, Griesbach GS, Giza CC. The pathophysiology of concussions in youth. Phys Med Rehabil Clin N Am 2011, 22:577–602, viii.

28. O’Connell KM, Littleton-Kearney MT. The role of free radicals in traumatic brain injury. Biol Res Nurs 2013, 15:253–263.

29. Sanders MJ, Sick TJ, Perez-Pinzon MA, Dietrich WD, Green EJ. Chronic failure in the maintenance of long-term potentiation following fluid percussion injury in the rat. Brain Res 2000, 861:69–76.

30. Sick TJ, Perez-Pinzon MA, Feng ZZ. Impaired expression of long-term potentiation in hippocampal slices 4 and 48 h following mild fluid-percussion brain injury in vivo. Brain Res 1998, 785:287–292.

31. Solomon GS, Olt SD, Lovell MR. Long-term neurocognitive dysfunction in sports: what is the evidence? Clin Sports Med 2011, 30:165–167, vi.

32. Vagnazzi R, SIGNORETTI C, CRISTOFOLI L, ALESSANDRINI F, FORNIS R, INGRO E, RIA A, MARZIALI S, ZOCATELLI G, TAZAVAZA B, DEL BOLGIA F, SORGE R, BROGLOP SD, McINTOSH TK, TAZZARINO G. Assessment of metabolic brain damage and recovery following mild traumatic brain injury in a multicentre, proton magnetic resonance spectroscopic study in concussed patients. Brain 2010, 133:3232–3242.

33. Len TK, Neary JP. Cerebrovascular pathophysiology following mild traumatic brain injury. Clin Physiol Funct Imaging 2011, 31:85–93.

34. Barlow KM, Coven D, Lerner R, Ozen C, Kasdak S, Yilmaz C, Yuecu E, Altnors N. The effect of repetitive concussions on cognitive functions in rats. Turk Neurosurg 2010, 20:442–448.
35. Nakajima Y, Horiiuchi Y, Kamata H, Yawaka M, Kuwabara M, Tsukobokawa T: Distinct time courses of secondary brain damage in the hippocampus following brain concussion and contusion in rats. Tohoku J Exp Med 2010, 221:29–235.

36. Green R, Koshimizu Y, Turner G: Research digest. Understanding the organic basis of persistent complaints in mTBI findings from functional and structural neuroimaging. Neuropsychol Rehabil 2013, 23:671–687.

37. Meehan WP III: Medical therapies for concussion. Clin Sports Med 2011, 30:115–124.

38. Mittenberg W, Burton DB: A survey of treatments for post-concussion syndrome. Brain Inj 1994, 8:429–437.

39. Al Sayegh A, Sandford D, Carson AJ: Psychological approaches to treatment of post-concussion syndrome: a systematic review. J Neurol Neurosurg Psychiatry 2010, 81:1128–1134.

40. Alsalaheen BA, Mucha A, Morris LO, Whitney SL, Furman JM, Camiolo-Reddy CE, Collins MW, Lovell MR, Sparto PJ: Preliminary study of subsymptom threshold exercise training for concussion in sports: post-concussive activity levels, symptoms, and neurocognitive performance. J Athl Train 2008, 43:265–274.

41. Schneider KJ, Iverson GL, Emery CA, McCrory P, Herring SA, Meeuwisse WH: Does routine follow up after head injury help? A randomised controlled trial. J Neurol Neurosurg Psychiatry 1997, 62:478–484.

42. Ponsford J, Willmott C, Rothwell A, Cameron P, Kelly AM, Nelms R, Curran C: Effect of melatonin on intracranial pressure and brain edema following traumatic brain injury: role of oxidative stresses. Arch Med Res 2013, 44:251–258.

43. Leidy JJ, Kozlowski K, Donnelly JP, Pendergast DR, Epstein LH, Willer B: Vestibular rehabilitation for dizziness and balance disorders after concussion. J Neurol Phys Ther 2010, 34:87–93.

44. Alsalaheen BA, Mucha A, Morris LO, Whitney SL, Furman JM, Camiolo-Reddy CE, Collins MW, Lovell MR, Sparto PJ: Routine follow up after head injury: a second randomised controlled trial. J Neurol Neurosurg Psychiatry 1998, 65:177–183.

45. McCoy P, Collie A, Anderson V, Davis G: Can we manage sport related concussion in children the same as in adults? Br J Sports Med 2004, 38:516–519.

46. Wade DT, Crawford S, Wenden FJ, King NS, Most NE: Does routine follow up after head injury help? A randomised controlled trial. J Neurol Neurosurg Psychiatry 1997, 62:478–484.

47. Zafonte R, Friedewald WT, Lee SM, Levin B, Diaz-Arrastia R, Antolin I, Rodriguez C: Glutamate induces oxidative stress not mediated by glutamate receptors or cysine transporters: protective effect of melatonin and other antioxidants. J Pineal Res 2001, 31:356–362.

48. Redman J, Armstrong S, Ng KT: Free-running activity rhythms in the rat: entrainment by melatonin. Science 1983, 219:1089.

49. Redman JR, Armstrong SM: Reentrainment of rat circadian activity rhythms: effects of melatonin. J Pineal Res 1988, 5:203–215.

50. Underwood H, Goldberg BD: Vertebrate circadian and photoperiodic systems: role of the pineal gland and melatonin. J Biol Rhythms 1987, 2:279–315.

51. Maldonado MD, Murillo-Cabezas F, Terron MP, Flores LI, Tan DX, Manchester LC, Reiter RJ: The potential of melatonin in reducing morbidity-mortality after cranioencephal trauma. J Pineal Res 2007, 42:1–11.

52. Carpentieri A, Díaz de Barboza G, Areco V, Peralta López M, Tolosadetalamon H: New perspectives in melatonin uses. Pharmazol Res 2012, 65:437–444.

53. Leon J, Acuña-Castroviejo D, Saizn RM, Mayo JC, Tan DX, Reiter RJ: Melatonin and mitochondrial function. Life Sci 2004, 75:765–790.

54. León J, Acuña-Castroviejo D, Escames G, Tan D-X, Reiter RJ: Melatonin mitigates mitochondrial malfunction. J Pineal Res 2005, 38:1–9.

55. Rodríguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, Reiter RJ: Interactions of melatonin and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. J Pineal Res 2003, 34:1–7.

56. Rosen J, Than NN, Koch D, Poegebler & Laatsch H, Hardeland R: Interactions of melatonin and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. J Pineal Res 2006, 41:374–381.

57. Tan DX, Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR: Significance of melatonin in antioxidative defense system: reactions and products. Biol Signals Recept 2000, 9:137–159.

58. Rodríguez C, Mayo JC, Saizn RM, Antolín I, Herrera F, Martín V, Reiter RJ: Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004, 36:1–9.

59. Kotler M, Rodríguez C, Sainz RA, Antonil I, Menéndez-Peláez A: Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. J Pineal Res 1998, 24:83–99.

60. Reiter RJ, Paredes SD, Korkmaz A, Jou M-J, Tan D-X: Melatonin combats mOllecular terrorism at the mitochondrial level. Interdiscip Toxicol 2008, 1:137–149.
Ozdemir D, Tugyan K, Uysal N, Sonmez U, Sonmez A, Acikgoz O, Ozdemir N, Uysal N, Gonenc S, Acikgoz O, Sonmez A, Topcu A, Ozdemir N, Beni SM, Kohen R, Reiter RJ, Tan DX, Shohami E: Barlow et al. Trials 2004, 15:271.

Görgülü A, Palaoglu S, Ismailoglu Ö, Tuncel M, Sürücü MT, Erbil M, Klnç K: Görgülü A, Palaoglu S, Ismailoglu Ö, Tuncel M, Sürücü MT, Erbil M, Klnç K: J Pineal Res 2013, 54:631–632.

Manda K, Anzai K, Kumari S, Bhatia AL: Manda K, Anzai K, Kumari S, Bhatia AL: J Pineal Res 2014, 63:142–143.

Barlow et al. Trials 2014, 15:271 Page 9 of 10 http://www.trialsjournal.com/content/15/1/271

84. Beni SM, Kohen R, Reiter RJ, Tan DX, Shohami E: Melatonin-induced neuroprotection after closed head injury is associated with increased brain antioxidants and attenuated late-phase activation of NF-kappaB and AP-1. FASEB J 2004, 18:149–151.

85. Keih J: Effect of snowboard-related concussion safety education for recognizing possible concussions. J Sports Med Phys Fitness 2011, 51:625–632.

86. Tsai MC, Chen WL, Tsai MS, Ching CH, Chuang JJ: Melatonin attenuates brain contusion-induced oxidative insult, inactivation of signal transducers and activators of transcription 1, and upregulation of suppressor of cytokine signaling-3 in rats. J Pineal Res 2011, 51:233–245.

87. Campolo M, Ahmad A, Crupi R, Impellizzeri D, Morabito R, Esposito E, Cuzzocrea S: Combination therapy with melatonin and dexamethasone in a mouse model of traumatic brain injury. J Endocrinol 2013, 217:291–301.

88. Das A, Belogodu A, Reiter RJ, Ray SK, Banik NL: Cytoprotective effects of melatonin on C6 astroglial cells exposed to glutamate excitotoxicity and oxidative stress. J Pineal Res 2008, 45:117–124.

89. Paula-Lima AC, Louzada PR, De Mello FG, Ferreira ST: Neuroprotection against Abeta and glutamate toxicity by melatonin: are GABA receptors involved? Neurotox Res 2003, 5:323–327.

90. Louzada PR, Paula Lima AC, Mendonca-Silva DL, Noel F, De Mello FG, Ferreira ST: Tauirine prevents the neurotoxicity of beta-amyloid and glutamate receptor agonists: activation of GABA receptors and possible implications for Alzheimer's disease and other neurological disorders. FASEB J 2004, 18:511–518.

91. Andrews-Zwilling Y, Bienen L, Xu Q, Li G, Bernardo A, Voon SY, Zwilling D, Yan TX, Chen L, Huang Y: Apolipoprotein E4 causes age- and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. J Neurosci 2010, 30:1370–13717.

92. Maldonado MD, Manfredi M, Ribas-Sema J, Garcia-Moreno H, Calvo JR: Melatonin administrated immediately before an intense exercise reverses oxidative stress, improves immunological defenses and lipid metabolism in football players. Physiol Behav 2012, 105:1099–1103.

93. Ochoa JJ, Díaz-Castro J, Kajariabille N, García C, Guisado IM, De Teresa C, Guisado R: Melatonin supplementation ameliorates oxidative stress and inflammatory signaling induced by strenuous exercise in adult male mice. J Pineal Res 2011, 51:373–380.

94. Velkov ZA, Velkov YZ, Galunska ST, Paskalev DN, Tabdjer AV: Melatonin: quantum-chemical and biochemical investigation of antioxidant activity. Eur J Med Chem 2009, 44:2834–2839.

95. Ozdemir D, Tugyan K, Uysal N, Sonmez U, Sonmez A, Ackigoz Q, Ozdemir N, Duman M, Ozkan H: Protective effect of melatonin against head trauma-induced hippocampal damage and spatial memory deficits in immature rats. Neurosci Lett 2005, 385:234–239.

96. Ozdemir D, Uysal N, Gonenc S, Ackigoz Q, Sonmez A, Topcu A, Ozdemir N, Duman M, Semin I, Ozkan H: Effect of melatonin on brain oxidative damage induced by traumatic brain injury in immature rats. J Pineal Res 2005, 45:631–637.

97. Tsai J, Whealin JM, Scott JC, Harpaz-Rotem I, Pietrzak RH: Examining the relation between combat-related concussion, a novel 5-factor model of posttraumatic stress symptoms, and health-related quality of life in Iraq and Afghanistan veterans. J Clin Psychiatry 2012, 73:1110–1118.

98. Wang WZ, Fang XH, Stephenson LL, Kihabani KT, Zamponi WA: Melatonin reduces ischemia/reperfusion-induced supersoxide generation in arterial wall and cell death in skeletal muscle. J Pineal Res 2005, 42:255–260.

99. Borlongan CV, Yarmamoto M, Takei N, Kumazaki M, Ungsupakorn C, Hida H, Sanberg PR, Nishino H: Giall cell survival is enhanced during melatonin-induced neuroprotection against cerebral ischemia. FASEB J 2000, 14:1307–1317.

100. Görgülü A, Palaoğlu A, Ismailoglu Ö, Tuncel M, Sünçü MT, Erbil M, Kök N: Effect of melatonin on cerebral edema in rats. Neurosurgery 2001, 49:1434.

101. Sanantary S, Sinibick EA, Das A, Kranian YH, Matzelle DD, Yalapragada AV, Reiter RJ, Ray SK, Banik NL: Melatonin attenuates calpain upregulation, axonal damage and neuronal death in spinal cord injury in rats. J Pineal Res 2008, 45:348–357.

102. Sanantary S, Das A, Thakore NP, Matzelle DD, Reiter RJ, Ray SK, Banik NL: Therapeutic potential of melatonin in traumatic central nervous system injury. J Pineal Res 2009, 47:134–142.

103. Manda K, Anzai K, Kurnan S, Bhata A: Melatonin attenuates radiation-induced learning deficit and brain oxidative stress in mice. Acta Neurobiol Exp (Wars) 2007, 67:63–70.
128. Ayr LK, Yeates KO, Taylor HG, Browne M: Dimensions of postconcussive symptoms in children with mild traumatic brain injuries. J Int Neuropsychol Soc 2009, 15:19–30.

129. Petersen C, Scherwath A, Fink J, Koch U: Health-related quality of life and psychosocial consequences after mild traumatic brain injury in children and adolescents. Brain Inj 2008, 22:215–221.

130. Ganesalingam K, Yeates KO, Ginn MS, Taylor HG, Dietrich A, Nuss K, Wright M: Family burden and parental distress following mild traumatic brain injury in children and its relationship to post-concussive symptoms. J Pediatr Psychol 2008, 33:621–629.

131. McCarthy ML, MacKenzie EJ, Dorbin DR, Atken ME, Jaffe KM, Paidas CN, Slomine BS, Dorsch AM, Berk RA, Christensen JR, Ding R: CHAT Study Group: The pediatric quality of life inventory: an evaluation of its reliability and validity for children with traumatic brain injury. Arch Phys Med Rehabil 2005, 86:1901–1909.

132. Brooks BL, Sherman EM: Computerized neuropsychological testing to rapidly evaluate cognition in pediatric patients with neurologic disorders. J Child Neurol 2012, 27(8):982–991.

133. Reynolds CR, Kamphaus RW: The clinician’s guide to the Behavior Assessment System for Children (BASC). New York: Guilford Press; 2002.

134. Doyle A, Ostrander R, Skare S, Crosby RD, August GJ: Convergent and Criterion-related Validity of the Behavior Assessment System for Children-Parent Rating Scale. J Clin Child Psychol 1997, 26:276–284.

135. Ayalaon L, Bonodkin K, Dishon L, Kanety H, Dagan Y: Circadian rhythm sleep disorders following mild traumatic brain injury. Neurology 2007, 68:1136–1140.

136. Garcia J, Rosen G, Mahowald M: Circadian rhythms and circadian rhythm disorders in children and adolescents. Seminars in Pediatric Neurology 2001, 8(1):229–240.

137. Hofstra WA, de Weerd AW: How to assess circadian rhythm in humans: a review of literature. Epilepsy Behav 2008, 13:438–444.

138. Donders J, DenRabber D, Vos L: Construct and criterion validity of the behaviour rating inventory of executive function (BRIEF) in children referred for neuropsychological assessment after pediatric traumatic brain injury. J Neuropsychol 2010, 4:197–209.

139. Griz CC, Rutcher JS, Ashwal S, Barth J, Getchius TS, Gioia GA, Gronseth GS, Guskiewicz K, Mandel S, Manley G, McKeag DB, Thurman DJ, Zafonte R: Summary of evidence-based guideline update: evaluation and management of concussion in sports: report of the guideline development subcommittee of the American academy of neurology. Neurology 2013, 80:2250–2257.

140. Glass RL, Natele MJ, Janusz GA, Gioia GA, Anderson S: Initial Development of a Parent Report of Post Concussion Symptoms in Children and Adolescents. Paper Presented at the Thirty-Third Annual Meeting of the International Neuropsychology Society: February 2-5 2005. St Louis, MO: Cambridge Journals; 2005:171.

141. Janusz JA, Sady MS, Gioia GA: Postconcussion Symptom Assessment. In Mild Traumatic Brain Injury in Children and Adolescents, Volume 1. New York: Guilford Press; 2012:241–263.

142. Gioia GA, Vaugh CG, Isquith PK: Manual for Pediatric Immediate Post-Concussion Assessment and Cognitive Testing. Pittsburgh: IMPACT Applications; 2011.

143. Jacobson NS, Truax P: Clinical significance: A statistical approach to defining meaningful change in psychotherapy research. J Consult Clin Psychol 1991, 63(1):12–19.

144. Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Baker G, Klassen TP, Vohra S: The efficacy and safety of exogenous melatonin for primary sleep disorders. A meta-analysis. J Gen Intern Med 2005, 20:1151–1158.

145. Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Vohra S, Klassen TP, Baker G: Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. BMJ 2006, 332:385–393.