



# Long-term impact of mild traumatic brain injury on postural stability and executive function

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## Abstract

**Objective** A substantial number of individuals present with prolonged symptoms after a mild traumatic brain injury (mTBI) or concussion. This has warranted the development of assessment tools that can reliably detect prolonged symptoms after an mTBI. At present, a gold standard diagnostic tool for accurately identifying such prolonged symptoms is not available. The purpose of this study is to utilize specific measures of standing balance, cognitive function, and bimanual coordination to examine persisting long-term deficits in individuals with mTBI.

**Methods** A total of 18 (medically diagnosed with an mTBI within the last year) and 14 (healthy age-matched controls) individuals participated in the study. Assessment tools included NIH Toolbox Cognition Battery (NIHTB-CB), TEMPA, and Purdue pegboard (bimanual coordination) and standing balance on a force platform.

**Results** Individuals with mTBI demonstrated lower scores in all measures of cognition with statistically significant difference ( $p = 0.03$ ) in executive function. The clinical tests of bimanual coordination did not show any statistically significant differences between groups. Postural stability was significantly reduced ( $p = 0.039$ ) in the mTBI group.

**Conclusion** Our results show long-term performance deficits (cognition and postural stability) that persist in individuals with mTBI. In addition, to the best of our knowledge, this is the first study to identify cognitive deficits in individuals with mTBI by utilizing NIHTB-CB. Knowledge gained from this study might affect decisions of return-to-play or return-to-learn in individuals with a history of mTBI(s).

**Keywords** Brain injury · Balance · Cognition · Posture

## Introduction

Mild traumatic brain injury (mTBI) is an acute brain injury resulting from mechanical energy to the head from external physical forces [1]. Approximately 1.7 million TBIs occur in the USA annually, of which over 85% are mild [2]. It is estimated that 10–15% of patients who sustain an mTBI are not fully recovered at 1-year post-injury [3], though this estimate has been criticized and debated, with other groups observing half of their cohort exhibiting post-concussive symptoms (PCS) 12 months post-injury [4]. Persistent symptoms include physical (e.g., headaches, fatigue, balance issues), cognitive (e.g., poor concentration, memory impairments), and/or

emotional (e.g., mood disturbances, depression/anxiety) complaints and objective findings [5]. These symptoms can have a significant impact on an individual's ability to work, attend school, and perform activities of daily living. The National Institutes of Health has declared mTBI a major public health concern, and efforts to reduce post-mTBI symptoms are a top research priority [6].

Mild TBIs can have a significant impact on a student's academic performance and ability to engage in daily routine [7]. Encouragingly, research has shown high rates of compliance with current evidence-based recommendations from the National Collegiate Athletic Association (NCAA) for concussion management and return-to-play in sports. Compliance with NCAA recommendations for the management of the return-to-learn process, however, remains low [8], indicating the need for improvement in how universities facilitate student-athletes' return to the classroom. The NCAA notes that there is a lack of available evidence for return-to-learn protocols as compared to return-to-play, with most recommendations based solely on

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expert opinion [9]. Consequently, new screening tools are needed to accurately diagnose mTBI to help determine students' eligibility return to the classroom/play or time necessary to recover.

Another population with a high prevalence of mTBI is the military. With better and improved protective gear, a number of fatal injuries have reduced, but incidences of head and neck injuries—with resulting mTBI-like symptoms—have become more prominent [10]. The Department of Defense (DOD) reports that almost 85% of TBIs diagnosed in military personnel are considered mild [11], with a possible under diagnosis due to lack of standardized testing. Furthermore, many military personnel reportedly have persistent post-concussive symptoms. As a result, the DOD and VA are implementing new population-screening procedures for mTBI and increasing research to improve diagnostic accuracy of tests for mTBI especially in the post-acute period [12].

With the substantial number of individuals experiencing mTBIs and subsequent prolonged symptoms, it is important to understand the effects these injuries can have on the brain. The two brain regions most commonly injured from an mTBI are the frontal lobe and corpus callosum [13, 14]. Certain assessment tools can be utilized to evaluate involvement of specific brain areas post-mTBI. For example, a cognitive test can assess frontal lobe function, whereas bimanual coordination measures can provide insight related to integrity of the corpus callosum [15, 16]. A more complex task, such as standing balance, can be utilized to assess broader functioning of the brain and sensory motor integration [17]. Assessments that effectively allow for early identification of mTBIs can lead to early intervention, which has been shown to help reduce PCS [18, 19]. Thus, a diagnostic tool that allows for accurate identification of mTBI following trauma and that is sensitive to evaluate PCS is needed.

Currently available clinical assessment tools fail to identify all individuals with mTBI. While notably better at screening for acute mTBI, they have significant limitations in the subacute and chronic phases of injury. For example, the Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) instrument assesses neurocognitive function and symptoms post-concussion [20]. This instrument, however, cannot be effectively used without a baseline score [21]. Another simple tool to assess cognition is the National Institutes of Health Tool Box Cognitive Battery (NIHTB-CB). While the NIHTB-CB has been validated in individuals with either moderate or severe TBI, its effectiveness in diagnosing mTBI is unknown [22]. Other clinical measures assess multiple domains (e.g., cognition and balance) such as the Sports Concussion Assessment Tool 5 (SCAT5). The NFL uses this test for immediate assessment of suspected concussion, however, with variable accuracy [23, 24]. In addition, SCAT5 is not recommended for assessing mTBI in the subacute and chronic phases post-injury [25]. It is important to note that

while the corpus callosum is particularly vulnerable to TBI [26], none of the clinically available mTBI assessment tools test for deficits in bimanual coordination.

The purpose of this study was to develop and assess a battery of tests that would be fast and reliable to detect long-term deficits from mTBI. Due to the varied effects of mTBI on the brain and the most common areas injured, our assessment criteria are multidimensional in nature and includes measures of cognition, standing balance, and bimanual coordination. We hypothesized that compared with healthy controls, individuals with mTBI would (1) perform worse in all domains of cognition, (2) exhibit greater postural instability during standing, and (3) perform bimanual coordination tasks with more errors and increased delays.

## Methods

### Participants

A total of 32 individuals consented to participate, including 18 individuals with mTBI and 14 individuals with no history of mTBI (Table 1). All participants completed the cognition component of the testing. A subset of the participants (11 mTBI and all controls) also participated in tests involving balance and bimanual coordination. Inclusion criteria for the mTBI group required the presence of one or more of the following symptoms: confusion, disorientation, loss of consciousness for 30 min or less, post-traumatic amnesia for less than 24 h, and/or other transient neurological abnormalities such as focal signs or seizure. These manifestations could not be due to drugs, alcohol, medications, psychological trauma, coexisting medical conditions, or penetrating craniocerebral injury. Inclusion criteria for the control participants were the following: no previous history of head trauma, stroke, epilepsy, substance abuse, and decreased sensation or motor function in the arms or legs.

### Experimental procedure

Testing was completed in one session, lasting approximately 2.5 h including rest breaks between tests. Both groups performed the following assessments, with order of assessments randomized between subjects:

- a) *Cognition*: Cognitive abilities (COG) were tested using the iPad version of the NIH Tool Box Cognitive Battery (NIHTB-CB), which assesses attention, concentration, memory, executive function, recognition of spatial differences, and differentiation of patterns [27, 28]. The five tests used from the NIHTB-CB in our protocol were the Picture Sequence Memory Test Age 8+ (PSM), for assessing episodic memory; Flanker Inhibitory Control

**Table 1** Participant demographics

Demographics	mTBI	Control
Number of participants	18	14
Avg. age (years)	29.11 ± 10.82	23.79 ± 3.49
Avg. height (cm)	164.96 ± 7.22	167.19 ± 10.41
Avg. weight (kg)	64.84 ± 10.24	68.96 ± 22.40
Gender	F: 14/M: 5	F: 9/M: 5
Handedness	R: 15/L: 3	R: 12/L: 2
Avg. time post-concussion/mTBI (weeks)	14.72 ± 10	N/A
Number of participants /number of mTBIs	10/1, 6/2, 1/3, 1/6	N/A

mTBI, mild traumatic brain injury; Avg, average; NA, not applicable

and Attention Test Age 12+ (FICA), for assessing attention and executive function; Pattern Comparison Processing Speed Test Age 7+ (PCPS), for assessing processing speed; List Sorting Working Memory Test Age 7+ (LSWM), for assessing working memory; and a Dimensional Change Card Sort Test Age 12+ (DCCS), for assessing executive function. Cognition Fluid Composite (CFC) represented the composite score for all of the above tests. NIHTB-CB scores are age-adjusted scores which are comparative scores of participants in the NIH-TB nationally represented sample or database from the same age group.

- b) *Standing balance (BAL)*: Participants were asked to stand still on a standardized AMTI AccuSway force platform (AMTI Inc., Watertown, MA, USA) with arms crossed over their chest. Three trials of 60 s each were recorded for the following conditions: bipedal eyes open (BEO) and bipedal eyes closed (BEC). A dedicated rest period of 60 s was provided between these conditions to prevent fatigue. During eyes-open conditions, participants were asked to keep their eyes focused on a static point placed at eye level and at a distance of 1 m. Base of support (BOS) was recorded for each trial for all conditions. Balance Clinic V 2.03 software was used to calculate the center of pressure (CoP) data (sampled at 200 Hz) and to analyze spatial and temporal CoP variables.
- c) *Bimanual coordination*: Bimanual coordination (BMC) was assessed using two well-established clinical assessment tools: (I) TEMPA (Test d'Evaluation de la performance des Membres Superieurs des Personnes Agees) is composed of nine standardized tasks that simulate activities of daily living using both dominant and non-dominant hands and a grip strength task [29]. (II) The Purdue Pegboard Test involves manipulating small pins, washers, and collars on a pegboard as quickly as possible [30]; three trials of each task were completed. Dominant, non-dominant, and both hands were assessed for 30 s; the assembly task (involving both hands) was assessed for 60 s.

## Data analysis

SPSS 26 for Windows 10 (SPSS Inc., Chicago, USA) was used to analyze the data. Statistical significance was considered at  $p < 0.05$ . Shapiro-Wilk normality tests revealed that the data was not normally distributed for the following variables: demographics (e.g., age, height, weight, etc.), cognitive assessments, Purdue pegboard, grip force, and variables for standing balance. Thus, non-parametric tests (independent samples Mann–Whitney  $U$ ) were used to investigate the effect of groups (mTBI and Control) on these measures. TEMPA tasks were normally distributed, and thus a parametric test (MANOVA 9 tasks  $\times$  2 groups) was performed.

## Results

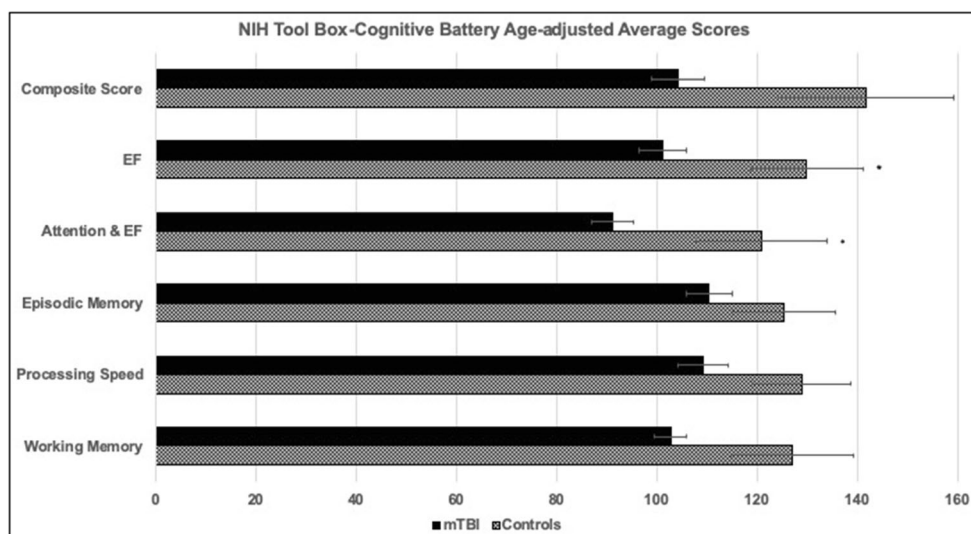
### Demographics

There were no significant differences between our mTBI and control groups with respect to age, weight, and height. The mechanism of injury for our mTBI participants was primarily sports-related, which included soccer, skiing, and biking.

### Cognitive abilities (COG)

Figure 1 shows scores across the NIHTB-CB domains of cognitive function for both persons with mTBI and healthy controls. In all of the age-adjusted measures of cognitive function, the mTBI group scored lower than the control group. DCCS test ( $p = 0.03$ ) and FICA test were statistically significant ( $p = 0.05$ ). The composite score (CFC) for the mTBI group was  $104 \pm 5.23$ , while the composite score of the control group was  $141 \pm 17.49$  ( $p > 0.05$ ).

**Fig. 1** NIH toolbox cognitive age-adjusted scores are shown here for all participants. mTBI participants scored lower on all measures of cognition compared to control counterparts (\* =  $p < 0.05$ ). EF, executive function



### Clinical tests for bimanual coordination

(i) *Purdue Pegboard Test*: For participants with mTBI, the average number of pegs completed with the dominant hand was  $14.61 \pm 0.88$  pegs, the non-dominant hand was  $14.27 \pm 0.70$ , and for both hands was  $11.76 \pm 0.72$  pegs. For the control group, the average number of pegs completed with the dominant hand was  $15.43 \pm 0.33$  pegs, the non-dominant hand was  $14.62 \pm 0.32$  pegs, and for both hands was  $12.38 \pm 0.39$  pegs. The average number of pieces inserted during the assembly trial for the mTBI group was  $33.30 \pm 4.03$  pegs, while the average for controls was  $37.48 \pm 2.86$  pegs. Although overall the control group was able to insert more

pegs than the mTBI group in a similar amount of allotted time, there was no statistically significant difference between groups ( $p > 0.05$ ).

(ii) *TEMPA*: All participants had upper extremity ranges of motion within normal limits and did not require assistance with any of the tasks. Results were analyzed separately for unimanual vs. bimanual activities. Average time to complete each of the nine TEMPA tasks for each group is shown in Table 2, with no statistically significant differences between groups. Non-parametric statistical analyses revealed no significant differences between groups in grip strength for both dominant (mTBI,  $35.24 \pm 2.44$  kg; controls,  $35.90 \pm 2.68$  kg) and non-dominant (mTBI,  $35.79 \pm 2.51$  kg; controls,  $34.28 \pm 2.65$  kg) hands.

**Table 2** Average time (in seconds) to complete TEMPA tasks for both mTBI and control groups

Task	Hand(s) used	mTBI	Control
Pick up/move jar	ND	$1.81 \pm 0.22$	$1.65 \pm 0.11$
	D	$1.82 \pm 0.17$	$1.56 \pm 0.09$
Open jar/remove spoonful coffee	B	$10.58 \pm 0.78$	$10.50 \pm 0.47$
Pick pitcher/ pour water into glass	ND	$9.60 \pm 0.57$	$9.34 \pm 0.37$
	D	$9.34 \pm 0.64$	$8.90 \pm 0.40$
Unlock lock/open pill container	B	$13.99 \pm 1.56$	$13.10 \pm 0.84$
Write on envelope/stick stamp	B	$10.79 \pm 0.66$	$11.40 \pm 0.51$
Tie a scarf	B	$8.28 \pm 0.57$	$8.47 \pm 0.24$
Shuffle playing cards	B	$25.76 \pm 3.93$	$27.38 \pm 2.61$
Handle coins	ND	$7.92 \pm 0.54$	$8.29 \pm 0.26$
	D	$8.06 \pm 0.62$	$8.15 \pm 0.40$
Pick up/move small objects	ND	$7.38 \pm 0.53$	$7.35 \pm 0.23$
	D	$7.49 \pm 0.63$	$7.30 \pm 0.23$

mTBI, mild traumatic brain injury; ND, non-dominant; D, dominant; B, both

### Standing balance assessments

Various spatial and temporal measures of standing balance were assessed via a force platform (Table 3), including (a) the average displacement of the COP in the anteroposterior and mediolateral planes (COP Avg<sub>ap</sub> and COP Avg<sub>ml</sub>), (b) mean velocity of COP in the anteroposterior and mediolateral planes (MV<sub>ap</sub>, MV<sub>ml</sub>), (c) path length of the COP trajectory for total duration for each trial (path length), and (d) the effective COP area (area effective) which encompasses approximately 66% of the data of COP excursion on the force platform. Raw data of COP sway measures of a representative participant from each group is shown in Fig. 2.

- a) *Average displacement of COP:* In BEO condition, COP Avg<sub>ap</sub> for the mTBI group was  $-2.89 \pm 2.45$  cm, and for the controls, it was  $-2.83 \pm 2.44$  cm ( $p > 0.05$ ); COP Avg<sub>ml</sub> for the mTBI group was  $-0.77 \pm 1.13$  cm, and for controls, it was  $-0.06 \pm 0.09$  cm ( $p = 0.003$ ). In BEC condition, COP Avg<sub>ap</sub> and COP Avg<sub>ml</sub> for the mTBI group were  $-2.74 \pm 2.21$  cm and  $-0.75 \pm 1.06$  cm, and for the controls, they were  $-2.56 \pm 2.12$  cm and  $-0.18 \pm 1.34$  cm, respectively ( $p > 0.05$ ).
- b) *Mean velocity of COP:* In BEO condition, MV<sub>ap</sub> and MV<sub>ml</sub> for the mTBI group were  $0.61 \pm 0.12$  cm/s and  $0.38 \pm 0.15$  cm/s, and for the controls, they were  $0.65 \pm 0.12$  cm/s and  $-0.41 \pm 0.11$  cm/s, respectively ( $p > 0.05$ ). In BEC condition, MV<sub>ap</sub> for the mTBI group was  $0.85 \pm 0.19$  cm/s, and for the controls, it was  $1.01 \pm 0.41$  cm/s ( $p = 0.05$ ); MV<sub>ml</sub> for the mTBI group was  $0.39 \pm 0.14$  cm/s, and for controls it was  $0.55 \pm 0.48$  cm/s ( $p = 0.005$ ).
- c) *COP path length:* In BEO condition, path length for COP was  $57.99 \pm 7.11$  cm for mTBI and  $74.88 \pm 22.53$  cm for controls ( $p < 0.01$ ). In BEC condition, path length for COP was  $61.34 \pm 7.39$  cm for mTBI and  $79.50 \pm 20.50$  cm for controls ( $p < 0.01$ ).

- d) *COP area:* In BEO condition, effective COP area was  $0.90 \pm 0.58$  cm<sup>2</sup> for mTBI and  $0.58 \pm 0.32$  cm<sup>2</sup> for controls ( $p = 0.039$ ). In BEC condition, effective COP area was  $1.07 \pm 1.10$  cm<sup>2</sup> for mTBI and  $0.79 \pm 0.37$  cm<sup>2</sup> for controls ( $p > 0.05$ ).

### Discussion

Controversy over mTBI screening tools and standardization of such tools continues to be a growing issue. Current mTBI screening tools are based on symptomatic presentations, creating susceptibility for under diagnosis of mTBI as individuals who initially appear asymptomatic might still bear the burden of post-concussive symptoms. Thus, assessment tools to effectively diagnose individuals who have experienced an mTBI without overt symptoms are essential. In this study, individuals who sustained an mTBI in the past 8 months were compared with healthy age-matched controls on measures of standing balance, cognition, and bimanual coordination.

The results from this study support our first and second hypotheses, but not our third. Persons with mTBI exhibited deficits in cognition and larger postural sway in the mediolateral direction during eyes-open condition and slower sway in the eyes-closed condition. Clinical examination of bimanual coordination, however, was not able to successfully identify coordination deficits in individuals with mTBI. This study is novel in that it is the first to evaluate the NIH Toolbox Cognitive Battery in individuals with mTBI.

### Cognitive deficits after mTBI

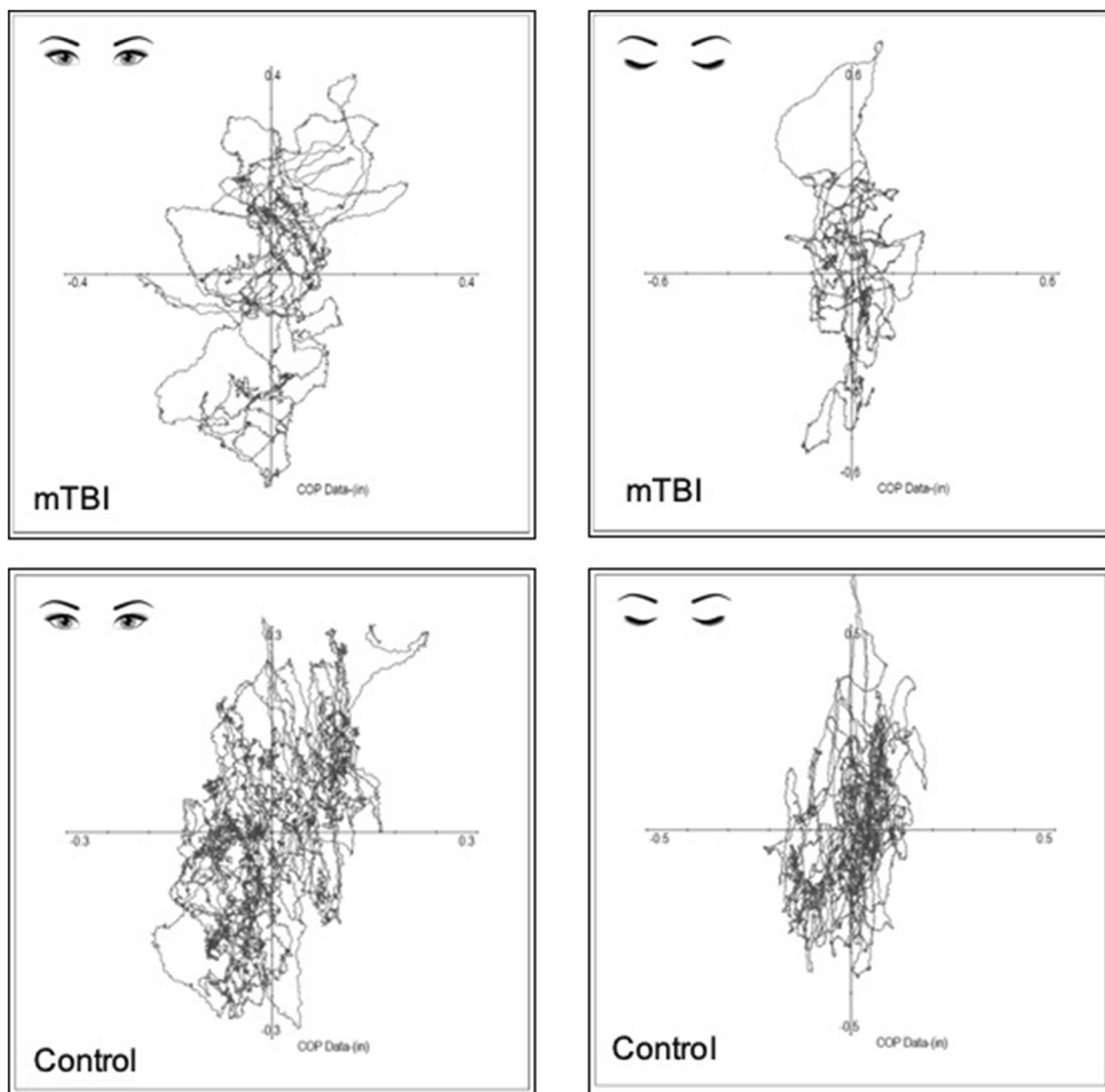
Our findings indicate that the NIHTB-CB may be applicable to individuals with mTBI as opposed to those with more

**Table 3** Comparison of standing balance force plate assessments between groups

Variable	Control	mTBI	Mann–Whitney <i>U</i>	<i>p</i> value
Condition: bipedal eyes open				
COP Avg <sub>ml</sub> (cm)	-0.06.09	-0.77 ± 1.13	455.5	0.003
Area effective (cm <sup>2</sup> )	0.58 ± 0.32	0.90 ± 0.58	547.0	0.039
Path length (cm)	74.88 ± 22.53	57.99 ± 7.11	376.0	< 0.0001
Condition: bipedal eyes closed				
MV <sub>ap</sub> (cm/s)	0.99 ± 0.41	0.84 ± 0.18	484.0	0.05
MV <sub>ml</sub> (cm/s)	0.53 ± 0.46	0.38 ± 0.13	406.0	0.005
Path length (cm)	79.50 ± 20.50	61.34 ± 7.39	337.0	< 0.0001

\*only statistically significant results reported

mTBI, mild traumatic brain injury; COP Avg<sub>ml</sub>, average displacement of center of pressure in the mediolateral direction; area effective, effective area of center of pressure; path length, total excursion of center of pressure from the centroid; MV<sub>ap</sub>, mean velocity in the anteroposterior direction; MV<sub>ml</sub>, mean velocity in the mediolateral direction



**Fig. 2** Postural sway data during eyes-open and eyes-closed conditions for two representation participants during single trials (top, mTBI; bottom, control). Note that postural sway increased in eyes-closed conditions for both the subjects. It is important to note the differences in maximum

units (in inches) of CoP sway measure which increased from  $-0.4/+0.4$  to  $-0.6/+0.6$  in the mTBI participant when eyes were closed. In the healthy control maximum units of CoP, sway measure increased from  $-0.3/+0.3$  to  $-0.5/+0.5$

severe TBI. Our sample of mTBI participants performed much better in all domains of cognitive function than the national average (a score of 100) except the FICA test that assesses attention and executive function. Thus, it is critical to note that in such an above-average sample, attention and executive function were significantly poorer in the mTBI group.

Our preliminary study is the first to validate the NIHTB-CB in individuals with mTBI, as this tool has only been previously validated in healthy individuals and those with complicated mild/moderate and severe TBI [22]. Furthermore, our findings suggest that executive function was especially diminished in individuals with mTBI, suggesting deficits in the frontal lobe. Similar deficits in executive function have been found in previous studies with TBI [31, 32]. Such deficits will

have negative implications on return-to-play/ return-to-learn protocols in athletes, students, and military personnel.

Other cognitive tools such as the ImPACT have been shown to be a reliable tool for assessing attention and processing speed in individuals post-mTBI [33]. However, a recent review reported that the diagnostic accuracy of ImPACT is inconclusive [34]. Our findings suggest that the NIHTB-CB can be used as an alternative to assess attention and concentration in this patient population.

### Bimanual coordination in mTBI

Imaging studies in individuals with moderate to severe brain injuries have identified disrupted interhemispheric

communication in prefrontal, sensory, and parietal regions leading to differential behavioral manifestations of bimanual motor functioning in young adults [35]. Moreover, in asymptomatic athletes post-concussion, increased recruitment of additional brain areas has been noticed after injury [36].

In our study, we chose to utilize two common clinical tools to evaluate upper extremity coordination: Purdue Pegboard test and TEMPA. Whereas validity and reliability of these tests have been shown in diseases such as multiple sclerosis and carpal tunnel syndrome, research is limited in brain injury, especially mTBI [29, 30, 37, 38]. Few studies have examined bimanual coordination deficits in persons with TBI, with inconclusive findings. For example, some individuals with TBI have been found to exhibit poorer performance on the Purdue Pegboard Test and take more time to complete the TEMPA activities compared with healthy controls [35, 37]. Another study by De. Beaumont et al., however, found no difference in bimanual coordination (i.e., velocity of rapid alternating movements) between athletes with mTBI who returned to sport at least 9 months prior and controls [39]. Similarly, our results did not show a difference in bimanual coordination between persons with mTBI and healthy controls. We infer that both TEMPA and Purdue Pegboard Tests for bimanual coordination may not be sensitive enough to identify subtle differences in mTBI. The inconclusiveness demonstrated by these studies highlights the need for additional research examining the role of bimanual coordination assessments by more sensitive measures in persons post-mTBI.

### Balance deficits after mTBI

Athletes without any history of injury show greater postural stability (i.e., less displacement of CoP) when compared with non-athletes [40]. Thus, even subtle balance deficits may be detrimental and potentially extend the return-to-play and return-to-learn windows in individuals with mTBI. In this study, when vision was intact, individuals with mTBI exhibited (a) increased displacement of CoP in the mediolateral direction, (b) increased CoP area, and (c) reduced path length compared with healthy controls.

Our results contradict the findings from Cavanaugh et al., as they did not find any changes in CoP displacements but rather saw differences in non-linear CoP measures such as approximate entropy [41]. It is important to note that their participants were assessed within 48 h of incurring a concussion, while our study included participants with more long-term injuries (average time following last concussion approximately 15 weeks). Thus, linear measures of CoP as reported in this study might be a better measure in patients with long-term deficits from mTBI. Moreover, our study adds to the findings of Baracks et al. [42] who found increased CoP area acutely after a concussive event, by demonstrating that CoP area remains increased even in chronic mTBI. Finally, our

study extends the finding of Quatman-Yates et al. [43] who showed decreased CoP path length in pediatric patients with mTBI, by adding new data and suggesting CoP path length also stays reduced in adults with mTBI.

### Limitations

While our study revealed differences in postural sway and cognition in mTBI, it has limitations. We had a small sample of participants in both mTBI and control groups which prevented any further statistical analysis. All mTBI participants were in the subacute stage which we considered as long term, while some may consider more chronic injuries as long-term deficits (> 6 months post-mTBI). Some of our participants had more than one mTBI that may have impacted our results as executive functions appear most sensitive to recurrent mTBI [44, 45]. Additionally, our testing sessions were moderately long and may have been fatiguing.

### Conclusion

A comprehensive assessment tool consisting of standing balance measured via a force platform and cognitive exams of concentration and attention via the NIHTB-CB is a valuable assessment to identify lingering effects in mTBI patients with long-term deficits. To better understand whether these screening tools would also be effective in identifying subtle symptoms in mTBI who are clinically asymptomatic, a follow-up study with a larger number of participants is needed. These preliminary findings provide support for designing an advanced multidimensional assessment tool in mTBI with long-term deficits. Potential assessments utilizing more sensitive measures of bimanual coordination should be further evaluated.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interests.

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