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Serial monitoring of CO_2 reactivity following sport concussion using hypocapnia and hypercapnia

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Abstract

Primary objective: This study examined the effects of mild traumatic brain injury (mTBI) on cerebrovascular reactivity (CVR).

Research design: A repeated measures design was used to examine serial changes in CVR.

Methods and procedures: Twenty subjects who recently suffered a mTBI were subjected to a respiratory challenge consisting of repeated 20 s breath-holds (BH) and hyperventilations (HV). Testing occurred on days 2 (D2), 4 (D4) and 8 (D8) post-injury as well as a baseline (BASE) assessment (after return-to-play). Transcranial Doppler was used to assess mean cerebral blood velocity (vMCA) and expired gas analysis provided end-tidal carbon dioxide ($PETCO_2$) levels.

Results: There was no significant difference in resting vMCA across all testing days for mTBI. No significant differences in $PETCO_2$ were found throughout the testing protocol. A significant effect ($p < 0.001$) of testing day on vMCA was found during BH and HV challenges for mTBI. Post-hoc analysis revealed significant differences ($p < 0.05$) in vMCA between D2 and the other testing days.

Conclusions: These data suggest that, following mTBI: (1) CVR is not impaired at rest; (2) CVR is impaired in response to respiratory stress; and (3) the impairment may be resolved as early as 4 days post-injury.

Keywords: *Transcranial Doppler, mild traumatic brain injury, cerebral blood flow, breath holding, hyperventilation, cerebrovascular reactivity*

Introduction

Mild traumatic brain injury (mTBI) has become a serious concern in the medical, educational and athletic realms [1–3]. There has been increased media attention surrounding mTBI and the lasting effects it can have on an individual who suffers this type of injury. The terms post-concussion syndrome or post-concussive syndrome are commonly referred to when describing an individual experiencing prolonged effects of mTBI. Understanding the pathophysiological mechanisms contributing to these persistent symptoms has become a primary focus of recent research [4, 5]. In the most recent statement released by the Concussion in Sport Group it was noted that both the physiological and psychological aspects of the injury may play a role in recovery from mTBI [2]. Although there is considerable research pertaining to the neuropsychological

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aspects of mTBI, systematic investigations into the pathophysiology of these injuries are lacking [4].

The majority of mTBI's resolve spontaneously over a matter of days [2]. As an individual recovers from mTBI, his or her symptoms will gradually decrease. Current guidelines including a step-wise, symptom-limited program of exertion are dependent on the resolution of these symptoms [2]. However, as symptoms are self-reported, underlying physiological effects of mTBI may still be present when these subjective markers are reported to have resolved. In a study by Gall et al. [6], athletes who were asymptomatic at rest showed significantly different cardiovascular responses during low-to-moderate exercise. In another study, the same group of athletes illustrated significant differences in heart rate variability in response to the exercise protocol [7]. The fact that these differences were not apparent at rest supports the idea that underlying physiological disturbances are still present once subjective symptoms resolve [4]. In addition to the established subjective and neuropsychological symptoms, insight into an individual's response to physiological stress may prove to be useful as a marker for recovery from mTBI [6].

It has been reported that cerebral blood flow (CBF) is compromised following both mTBI and severe traumatic brain injury [8–13]. Previous studies have demonstrated that cerebral autoregulation [14, 15] and cerebrovascular reactivity [9, 16] have been found to be impaired following mTBI. In a previous study found in the December 2011 issue of *Medicine & Science in Sports and Exercise*, minor changes were illustrated in cerebrovascular reactivity between healthy control subjects and those suffering a recent mTBI ($x = 4.5$, $SD = 1.1$ days) [5]. Neuroautonomic cardiovascular dysregulation has also been suggested to occur post-mTBI [6, 7, 17]. In light of these changes, the current study was designed to examine the serial changes in cerebrovascular reactivity during the first 7 days following mTBI in competitive athletes. It is hypothesized that significant differences in cerebrovascular reactivity in response to respiratory stress would be evident throughout the testing protocol and, as subjects progressed through their recovery, these changes would return to normal.

Materials and methods

Subjects

Twenty athletic subjects (16 male, four female) who suffered mTBI while participating in ice hockey ($n = 17$), basketball ($n = 1$), snowboarding ($n = 1$) and fighting ($n = 1$) were included in this study. Average ($\pm$SD) age of the subjects was 19.7 ($SD = 3.3$) years, height was 179.3 ($SD = 10.4$) cm and weight was 77.8 ($SD = 13.0$) kg. Refer to Table I for the demographic information of each subject, including their sport concussion assessment score (SCAT) [2]. Subjects were scheduled to be tested on days 2, 4 and 7 post-injury. Due to unforeseen circumstances (i.e. scheduling conflicts, drop-out, late notification of mTBI), the average testing days fell on days 2 (D2), 4 (D4) and 8 (D8) post-injury. Seventeen of the 20 subjects were also baseline (BASE) tested either pre-injury ($n = 2$) or post-injury following their competitive season ($n = 15$) (see Table I). All athletes had returned to all activities of daily living at time of post-injury (BASE) testing. Thus, they were asymptomatic, returned to their previous level of play and did not display any lingering effects.

Concussion has been defined as a blow to the head causing an alteration in mental status and one or more of the following symptoms: headache, nausea, vomiting, dizziness/balance problems, fatigue, trouble sleeping, drowsiness, sensitivity to light or noise, blurred vision, difficulty remembering or difficulty concentrating [18].

For the purpose of this study all mTBIs were assessed using the Sport Concussion Assessment Tool (SCAT) guidelines [2, 19] which provides a list of 24 common symptoms, some of which are described above. Concussion was confirmed by a physician prior to laboratory testing. SCAT symptom scores were recorded prior to each testing session. There was no previous history of cardiovascular disease or other metabolic conditions nor were any subjects taking any medications. All procedures were approved by the Research Ethics Board of the University of Regina. Written informed consent was obtained and a thorough explanation of the procedures and objectives of the study were given to each subject prior to participating.

Equipment

Transcranial Doppler ultrasonography was used to monitor cerebral blood velocity as previously described [5]. A 2 MHz Doppler probe (Nicolet Companion III, VIASYS Healthcare, Burlington, ON, Canada) was placed over the right temporal window and adjusted until an optimal signal of the middle cerebral artery (MCA) was found. Ultrasound gel was applied to enhance the quality of the signal and search techniques previously described were used to ensure an adequate signal-to-noise ratio [20]. The probe was held in place by a thermally moulded bracket attached to an adjustable headband (VIASYS Healthcare) to prevent movement of the probe. The right MCA was selected for consistency of measurement and the same technician

Table I. Demographics of mTBI subjects.

							SCAT scores			
ID	Age	Gender	Height (cm)	Weight (kg)	Sport	Previous mTBI	BASE (day)	D2	D4	D8
1	20	M	183	82	Hockey	2	0 (21)	12	–	2
2	14	M	175	68	Hockey	3	0 (88)	36	16	4
3	22	M	182	81	Hockey	1	3 (129)	29	23	13
4	18	M	183	89	Hockey	1	–	23	23	–
5	15	M	176	65	Hockey	3	1 (121)	–	–	35
6	15	M	180	59	Hockey	0	1 (108)	63	42	37
7	17	M	172	57	Hockey	1	0 (99)	10	4	0
8	21	M	197	90	Hockey	0	3 (−233)	22	25	45
9	22	M	191	80	Fight	1	0 (258)	–	–	8
10	22	M	183	105	Snowboard	3	–	–	–	68
11	18	F	165	61	Hockey	0	0 (274)	10	26	18
12	23	F	165	77	Hockey	3	9 (88)	31	35	17
13	25	M	175	91	Hockey	2	–	–	16	0
14	15	M	188	75	Hockey	0	0 (93)	8	0	0
15	18	M	188	89	Hockey	1	0 (123)	9	2	0
16	21	F	163	63	Basketball	1	0 (165)	21	30	0
17	19	F	160	68	Hockey	0	0 (178)	28	4	0
18	23	M	185	83	Hockey	0	6 (25)	28	–	–
19	24	M	180	81	Hockey	1	0 (138)	45	–	11
20	21	M	194	91	Hockey	1	0 (−113)	–	3	–
Mean	19.7		179.3	77.8				25.0	17.8	15.2
SD	3.3		10.4	13.0				15.2	13.5	19.9

Means are expressed with standard deviation (SD). SCAT (Sport Concussion Assessment Tool); BASE = baseline SCAT score, with test day pre- or post-injury in parentheses; D2, D4, D8 = day 2, 4, 8 post-injury, respectively.

performed all examinations. Previous research has shown no difference in mean middle cerebral artery blood velocity (vMCA) from left to right sides at rest [21–23]. An electronic metronome was used during the respiratory protocol (see description below) to maintain proper cadence during hyperventilation (HV). Analysis of end-tidal carbon dioxide ($PETCO_2$) was conducted using a breath-by-breath expired gas analysing system (Sensormedics vMax 2200, VIASYS Healthcare), which was calibrated before and after each test with primary standard gases (16% O_2, 4% CO_2, balance N_2; 26% O_2, balance N_2) and a 3.0 L factory calibrated syringe for ventilation calibration.

Respiratory challenge: Hypercapnic and hypocapnic manoeuvres

Acclimation to the laboratory surroundings occurred while completing informed consent forms and medical histories. To account for any influence of circadian changes in CBF, subjects were tested at the same time on each testing day. Subjects were required to abstain from exercise as well as the use of alcohol and caffeine for 24 hours prior to testing. Once the transcranial Doppler probe was secured in place and the gas analysis system was set up, each subject remained seated upright in a chair and was instructed to move minimally throughout the first part of the procedure. Resting data were collected for 5 minutes, following which the subjects were instructed to 'take a normal breath and hold it for 20 seconds'. By using normal inspiration rather than a deep inspiration, a Valsalva effect was avoided, which can cause an initial decrease in vMCA and lead to under-estimation of cerebrovascular reactivity [24]. A countdown was given preparing each subject for this breath hold (BH) manoeuvre. The 20-second BH was followed by 40 seconds of recovery of normal breathing and was repeated 5-times. Following the BH sequence, 2 minutes of normal breathing was included before the subjects performed a 20-second hyperventilation (HV) manoeuvre at 36 breaths min^{-1} using a metronome to maintain cadence. After 40 seconds of recovery, HV was repeated again for a total of five repetitions (20:40 seconds ratio). This respiratory challenge protocol has been used successfully in a previous study [5].

Outcome measurements

Data were sampled from the Doppler probe at 1 Hz and were analysed off-line. $PETCO_2$ data were sampled following each breath. Mean resting values were computed by averaging the collected data over a 5-minute span. vMCA and $PETCO_2$ values were

recorded at 20 second intervals throughout both BH and HV.

Missing data

Minor technical difficulties related to equipment occurred from time to time resulting in randomly missing vMCA data. To help alleviate these methodological difficulties, multiple imputation (MI) was utilized and the randomly missing vMCA data points were replaced. MI predicts and replaces missing values based on all other information collected in the study and has been proven to be both a valid and reliable statistical alternative [25, 26]. MI employs several advantages in dealing with missing data, including relying on more plausible assumptions than do other commonly used approaches to missing data (listwise deletion or complete case analysis), thus allowing the use of the entire sample size for statistical analysis.

Statistical analysis

Mean and standard deviation was calculated for both absolute vMCA values and $PETCO_2$ and were analysed using SPSS (SPSS 17.0, Chicago, IL). A one-way within-group repeated measures ANOVA was used to compare vMCA responses to BH and HV across all four testing days. A Bonferroni corrected pairwise comparison among estimated marginal means was used to determine where differences existed. Due to equipment portability limitations and technical difficulties, $PETCO_2$ data were not collected in 10 subjects. As previous research [5] demonstrated that a group effect of testing day was not to be expected with $PETCO_2$, ANOVA was utilized in comparing $PETCO_2$ values across days.

Results

Using repeated measures ANOVA for the mTBI analyses, vMCA demonstrated a significant effect for the test days during the hypercapnic (BH) (Wilks' Lambda $= 0.135$, $F(51,122.87) = 2.306$, $p < 0.001$, $\eta^2 = 0.486$) and hypocapnic (HV) (Wilks' Lambda $= 0.126$, $F(51,122.87) = 2.421$, $p < 0.001$, $\eta^2 = 0.499$) challenges. The results for each section of the testing protocol are discussed in further detail below.

Resting values

Test day did not significantly affect resting values ($F(3) = 2.064$, $p = 0.115$) between BASE and any of the testing days, although it was observed that resting vMCA initially increased on D2 and remained decreased at D8. $PETCO_2$ differences at rest were also not significant across the four testing days ($F(3,22) = 0.620$, $p = 0.609$). Table II illustrates the changes in resting vMCA values through the four (BASE, D2, D4, D8) testing days.

Breath holding (hypercapnia)

Table II provides the results for the hypercapnic challenge. Throughout the entire hypercapnic challenge, significant effects of testing day were found when observing vMCA. Most notably, testing day had a statistically significant effect ($p = 0.011$–0.045) following all but one repetition. In general, the response to BH was increased on D2 and decreased throughout the next testing days (D4 and D8). Furthermore, there was a visual increase from rest on D2 that appeared to return to near-normal levels by D4. Upon post-hoc analysis, all significant differences in vMCA throughout the test trials indicated changes from D2 as compared to the other days. There were no significant differences in $PETCO_2$ across all four testing days.

Hyperventilation (hypocapnia)

Table II provides the results for the hypocapnic challenge. Analysis of vMCA indicated that there were two time points (20 seconds after the first and third repetitions) throughout the hypocapnic testing protocol where a significant effect of test day was present ($p = 0.026$–0.031). Additionally, post-hoc analysis revealed that no significant differences were present between any of the testing days throughout the testing protocol. There were no significant differences in $PETCO_2$ across all four testing days.

Discussion

To the authors' knowledge, this is the first study to monitor serial changes in cerebrovascular reactivity in the days immediately following mTBI. The primary finding was the altered cerebrovascular reactivity mechanism following mTBI that resolves over approximately the first 5 days of recovery.

There has been a multitude of research that has examined cerebral blood flow and blood flow velocity in response to head injuries [8, 10–13]. The majority of these studies are focused on traumatic brain injury where decreased Glasgow coma scale scores (<13) indicate a more severe brain injury. Resting vMCA values along with induced hyper- and hypocapnic states are the typical model used to evaluate these types of injuries. Numerous studies utilize cerebral autoregulation assessment as well via several different methods including carotid compression, cuff deflation and gas

Table II. vMCA results for resting data collection and respiratory challenge.

Repetition		Time (s)	Baseline	Day 2	Day 4	Day 8	*p*
Rest		–	61.5 ± 11.1	63.5 ± 9.8	58.6 ± 13.1	58.3 ± 14.3	0.115
Hypercapnia (breath-holding)	1	0	62.3 ± 10.7	66.2 ± 10.4	60.0 ± 15.3	58.8 ± 15.4	0.115
		20	71.7 ± 13.6	82.7 ± 12.9†	67.0 ± 18.0‡	63.0 ± 18.7‡	0.041
		40	59.5 ± 10.6	67.5 ± 12.5†	58.6 ± 14.6‡	56.0 ± 15.8‡	0.000
	2	0	60.1 ± 10.7	65.0 ± 9.4	57.8 ± 12.6	58.2 ± 13.6	0.001
		20	69.0 ± 13.8	78.0 ± 11.3†	64.2 ± 17.1‡	65.0 ± 17.4‡	0.045
		40	60.4 ± 11.3	66.3 ± 12.9	57.2 ± 15.3	53.7 ± 15.2‡	0.000
	3	0	59.2 ± 10.7	63.3 ± 11.5	57.6 ± 15.3	57.1 ± 12.4	0.001
		20	68.9 ± 14.7	75.8 ± 17.1	62.6 ± 17.1‡	63.8 ± 17.5‡	0.119
		40	60.1 ± 12.9	65.2 ± 11.9	58.5 ± 16.7	54.4 ± 17.5‡	0.003
	4	0	57.9 ± 11.3	65.0 ± 10.2	57.7 ± 12.2	55.6 ± 13.9‡	0.015
		20	69.8 ± 15.5	75.4 ± 14.2	64.9 ± 17.6	64.8 ± 18.2	0.011
		40	59.1 ± 12.2	62.3 ± 12.5	56.2 ± 15.7	55.1 ± 15.7	0.020
	5	0	57.9 ± 9.0	64.1 ± 10.9	57.2 ± 15.0	56.9 ± 12.7	0.068
		20	69.7 ± 13.3	75.5 ± 14.7	64.2 ± 19.9	69.1 ± 16.3	0.038
		40	56.5 ± 11.9	63.2 ± 10.1	56.9 ± 17.6	54.8 ± 15.1‡	0.010
		60	58.3 ± 9.7	63.6 ± 11.3†	56.8 ± 16.3	57.5 ± 12.9	0.030
Hypocapnia (hyperventilation)	1	0	61.5 ± 10.8	61.1 ± 11.5	58.6 ± 15.2	58.3 ± 13.2	0.120
		20	38.3 ± 9.3	41.1 ± 6.2	36.1 ± 10.1	35.1 ± 10.0	0.537
		40	54.2 ± 9.0	55.6 ± 9.9	51.2 ± 13.7	52.9 ± 11.7	0.026
	2	0	56.0 ± 11.0	58.2 ± 8.5	54.7 ± 13.0	56.0 ± 15.2	0.360
		20	38.0 ± 8.6	38.0 ± 3.9	35.5 ± 8.7	33.8 ± 8.5	0.740
		40	52.2 ± 10.6	53.0 ± 6.9	50.5 ± 14.6	49.3 ± 11.8	0.097
	3	0	54.8 ± 15.5	58.0 ± 8.4	50.0 ± 14.4	50.2 ± 14.2	0.463
		20	39.3 ± 10.1	35.3 ± 2.9	34.6 ± 9.2	33.4 ± 9.3	0.057
		40	48.8 ± 9.9	52.0 ± 6.2	47.4 ± 11.3	50.4 ± 11.8	0.031
	4	0	54.6 ± 16	51.6 ± 7.1	49.7 ± 14.6	49.7 ± 14.5	0.267
		20	37.6 ± 9.8	35.7 ± 4.4	34.9 ± 10.8	34.6 ± 9.7	0.393
		40	53.2 ± 13.8	54.8 ± 8.0	49.8 ± 13.1	50.9 ± 11.0	0.480
	5	0	52.2 ± 16.6	49.5 ± 4.7	48.6 ± 14.6	53.4 ± 16.9	0.180
		20	37.8 ± 11.9	36.5 ± 4.7	33.3 ± 9.1	35.4 ± 10.0	0.490
		40	53.0 ± 10.3	54.7 ± 8.7	52.0 ± 10.4	53.6 ± 12.5	0.203
		60	51.3 ± 16.4	52.9 ± 7.8	47.3 ± 12.4	51.2 ± 17.1	0.759

Values are expressed as means ± SD. $n = 20$ for each group. Repeated measures ANOVA was used in comparing vMCA across the four testing days. p represents group effect of testing day. †indicates significant difference from Baseline ($p < 0.05$), ‡indicates significant difference from Day 2 ($p < 0.05$).

inhalation [14, 15, 27, 28]. A major drawback of using the traumatic brain injury model in humans is the inability to implement voluntary physiological stressors such as breath holding, hyperventilation and physical activity into a testing protocol such as the one used in this study. However, we were successful in this task by including transcranial Doppler ultrasonography together with expired gas analysis during serial testing. Recent literature supports the use of transcranial Doppler as an effective modality for cerebrovascular study [5, 29, 30].

The majority of published pathophysiological studies on mTBI have examined subjects on a single day or on a continual basis for a short period of time (i.e. 24 hours). The extended observation instituted in this study provides insight into the changes in vMCA that occur over the course of approximately a week following injury. This study supports previously presented but unpublished research that suggested serial monitoring of mTBI subjects is needed to fully understand the physiological mechanisms following injury [31, 32].

As mentioned above, one of the principal findings of this study was the significant difference in vMCA with no concomitant changes in $PETCO_2$ across testing days in the mTBI subjects. These findings indirectly suggest that the differences in vMCA were a result of changes in the cerebrovascular reactivity mechanism. Although the majority of the differences in this study were found in the BH (hypercapnia) trial, previous research has shown that response to HV (hypocapnia) may also be affected in mTBI subjects 4–5 days post-injury when compared to healthy, asymptomatic subjects [4, 5].

It was interesting to explore the effects of testing day throughout the protocol as this, to the authors' knowledge, has not been documented using objective measures of vMCA. A previous study using electroencephalography (EEG) made recordings on days 8 and 45 [33], but this does not provide

physiological information occurring in the days immediately post-injury as the majority of athletes are asymptomatic between 7–10 days post-injury [6]. Focusing on the respiratory challenges in this study, two important observations were noted. A significant effect of test day was observed throughout the entire hypercapnic challenge. On the other hand, as the subjects performed HV repetitions, there were only two time points throughout the protocol that test day was significantly affected. Furthermore, post-hoc analysis indicated that there were no significant differences between the four testing days (BASE, D2, D4, D8) in the HV portion of the respiratory challenge. These findings illustrate two important concepts.

First, the asymptomatic brain seems to somewhat 'buffer' its response to BH and HV more effectively than a brain recovering from mTBI. Due to the nature of the repeated stressors involved in this study, one can argue that the BH portion of the respiratory challenge affected both the asymptomatic and symptomatic brain to a similar extent, which resulted in the lack of significant differences being observed with HV. Second, the results of this study indicate the possibility of BH being a more intense physiological stressor than HV. These findings support previous research where increases in cerebrovascular reactivity were greater with hypercapnia than hypocapnia [29, 34]. However, the mechanism(s) responsible remain unclear. In this study, subjects were still able to maintain an intake of oxygen with HV, whereas with BH oxygen supply was interrupted with a concomitant increase in CO_2. As the mTBI-injured brain may have an increased susceptibility to drastic changes in oxygen and carbon dioxide [8], responses to physiological stress such as BH and HV may be altered.

Perhaps the foremost question following the analysis of these results is why are these changes in cerebrovascular reactivity occurring in the days immediately following mTBI? Under normal circumstances, the brain can manage several types of physiological and neurological disturbances operating with many 'checks and balances'. However, it can be speculated that the mild amount of trauma that typically produces a mTBI may be enough to disrupt the physiological functioning of the brain to a point where these changes are noticeable; i.e. the regulatory mechanisms that control cerebral blood flow are disturbed [30]. As proposed by Giza and Hovda [8], the mildly injured brain is placed in a state of transient neurological (and neurometabolic) dysfunction. Following the initial insult, the brain's metabolism becomes affected which may contribute to some of the differences found in the present study.

Another aspect of mTBI to consider is its effect on neuronal activity. Diffuse axonal injury typically occurs with mTBI [8, 35] and may directly affect the linking of the neurological and cardiovascular systems. With the autonomic nervous system controlling many aspects of the cardiovascular system including vessel constriction and dilation, it is probable that any injury to the brain will not only induce a local effect but a systemic one as well. This concept of neuroautonomic cardiovascular dysregulation occurring following mTBI was initially proposed by Goldstein et al. [17]. A study by Gall et al. [6] observed cardiovascular abnormalities in response to exercise in junior hockey players suffering a mTBI. More recently, Hilz et al. [36] observed irregularities in autonomic function at an average of 20 months post-mTBI. The observation of autonomic impairment is a simple, yet effective tool and is emerging as a practical method of monitoring recovery from mTBI [4].

There were several limitations observed with this study. Foremost were problems situating from the recruitment of subjects. The primary subject groups (varsity hockey players) completed a baseline test which included the protocol described above prior to each season. However, not all of the concussed subjects in the study were recruited from these groups ($n = 2$). Thus, having to complete a post-season baseline test may have influenced the observations. Furthermore, the average time of the post-injury testing occurred at day 127 (SD = 71; range = 25–258 days) when all athletes were asymptomatic and had returned-to-play. With any post-season baseline testing, subjects were required to voluntarily disclose whether or not they had suffered an additional mTBI since the last testing session as well as any insult or circumstance that they felt may influence their results. None of the subjects informed of any such situation. However, the research into the long-term physiological changes that present themselves in asymptomatic subjects is lacking and it may be that the post-season baseline tests attributed to the differences seen. Further research is warranted to ascertain this.

Scheduling subjects on the appropriate testing days was not always possible, but did occur within 1 day. Although the authors worked closely with the primary subject group which provided enhanced access, initial contact with the remaining subjects was difficult at times. This was the primary reason for the missing data in this study. As stated in the methods, multiple imputation was used which has been shown to be statistically valid and reliable in dealing with missing data [26, 37], thus allowing for complete analysis of all subjects in this study. Understanding that the data was not collected but rather imputed may lead to assumptions that the results are not a true representation of the observations in the study. However, comparisons between

testing days were completed using independent *t*-tests and a Bonferroni correction and similar results were observed. Thus, multiple imputation was used to allow for a repeated-measures ANOVA.

The final limitation was that control group data using a repeated measures design to match the mTBI group was not collected. However, previous research illustrated that there were no statistical differences in vMCA between a control group and mTBI subjects when baseline tested [5].

In summary, this study implemented serial monitoring of mTBI-injured subjects while subjecting them to a battery of physiological stressors including hyper- and hypocapnia. Taking into consideration that no significant differences were found in resting vMCA values (BASE, D2, D4, D8), the results indicate that, by serially monitoring minor head trauma associated with mTBI, pathophysiological changes in response to physiological stress are present. This is important to note as the most recent return-to-activity guidelines set forth by the CISG [2] advocate 'physical and cognitive rest until symptoms resolve and then a graded program of exertion prior to medical clearance and return-to-play' (p. 39). However, there is research supporting that, even with the resolution of symptoms, other regulatory irregularities still exist and need to be considered in future study designs [6].

While the accessibility to testing centres such as the laboratory that this study was conducted in may be restricted to a small amount of the population, the information provided through a protocol such as this may be a beneficial tool in the rehabilitation and return-to-activity decision-making process. Future research into cerebral blood flow response and cerebrovascular reactivity following mTBI is needed to not only support but also help further explain the findings of this study and should be encouraged. Furthermore, integrative physiological research that incorporates modalities including cerebral blood flow, cerebral oxygenation and continuous blood pressure monitoring will add significantly to understanding of the pathophysiology of sport-induced concussion or mTBI.

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References

1. Foreman M. Sidelined for safety: New laws keep student athletes with concussions benched. State Legislatures 2010;36:28–30.
2. McCrory P, Meeuwisse W, Johnston K, Dvorak J, Aubry M, Molloy M, Cantu R. Consensus statement on concussion in sport: 3rd International Conference on Concussion in Sport held in Zurich, November 2008. Clinical Journal of Sport Medicine 2009;19:185–200.
3. Tommasone BA, Valovich McLeod TC. Contact sport concussion incidence. Journal of Athletic Training 2006;41: 470–472.
4. Len TK, Neary JP. Cerebrovascular pathophysiology following mild traumatic brain injury. Clinical Physiology and Functional Imaging 2011;31:85–93.
5. Len TK, Neary JP, Asmundson GJG, Goodman D, Bjornson B, Bhambhani Y. Cerebrovascular reactivity impairment following sport-induced concussion. Medicine & Science in Sports & Exercise 2011;43:2241–2248.
6. Gall B, Parkhouse WS, Goodman D. Exercise following a sport induced concussion. British Journal of Sports Medicine 2004;38:773–777.
7. Gall B, Parkhouse W, Goodman D. Heart rate variability of recently concussed athletes at rest and exercise. Medicine & Science in Sports & Exercise 2004;36:1269–1274.
8. Giza CC, Hovda DA. The neurometabolic cascade of concussion. Journal of Athletic Training 2001;36:228–235.
9. Golding EM, Steenberg ML, Contant Jr CF, Krishnappa I, Robertson CS, Bryan Jr RM. Cerebrovascular reactivity to CO_2 and hypotension after mild cortical impact injury. American Journal of Physiology: Heart and Circulatory Physiology 1999;277:H1457–1466.
10. Grindel SH. Epidemiology and pathophysiology of minor traumatic brain injury. Current Sports Medicine Reports 2003;2:18–23.
11. McQuire JC, Sutcliffe JC, Coats TJ. Early changes in middle cerebral artery blood flow velocity after head injury. Journal of Neurosurgery 1998;89:526–532.
12. Werner C, Engelhard K. Pathophysiology of traumatic brain injury. British Journal of Anaesthesia 2007;99:4–9.
13. Bonne O, Gilboa A, Louzoun Y, Kempf-Sherf O, Katz M, Fishman Y, Ben-Nahum Z, Krausz Y, Bocher M, Lester H, et al. Cerebral blood flow in chronic symptomatic mild traumatic brain injury. Psychiatry Research 2003;124: 141–152.
14. Junger EC, Newell DW, Grant GA, Avellino AM, Ghatan S, Douville CM, Lam AM, Aaslid R, Winn HR. Cerebral autoregulation following minor head injury. Journal of Neurosurgery 1997;86:425–432.
15. Strebel S, Lam AM, Matta BF, Newell DW. Impaired cerebral autoregulation after mild brain injury. Surgical Neurology 1997;47:128–131.
16. Becelewski J, Pierzchala K. Cerebrovascular reactivity in patients with mild head injury. Polish Journal of Neurology and Neurosurgery 2003;37:339–350.
17. Goldstein B, Toweill D, Lai S, Sonnenthal K, Kimberly B. Uncoupling of the autonomic and cardiovascular systems in acute brain injury. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 1998;275:R1287–1292.

18. Kelly JP, Rosenberg JH. Diagnosis and management of concussion in sports. Neurology 1997;48:575–580.
19. McCrory P, Johnston K, Meeuwisse W, Aubry M, Cantu R, Dvorak J, Graf-Baumann T, Kelly J, Lovell M, Schamasch P. Summary and agreement statement of the 2nd International Conference on Concussion in Sport, Prague 2004. British Journal of Sports Medicine 2005;39:196–204.
20. Ringelstein EB, Kahlscheuer B, Niggemeyer E, Otis SM. Transcranial Doppler sonography: Anatomical landmarks and normal velocity values. Ultrasound in Medicine & Biology 1990;16:745–761.
21. Karadeniz U, Erdemli O, Ozatik MA, Yamak B, Demirci A, Kucuker SA, Saritas A, Tasdemir O. Assessment of cerebral blood flow with transcranial Doppler in right brachial artery perfusion patients. Annals of Thoracic Surgery 2005;79: 139–146.
22. Klingelhofer J, Hajak G, Sander D, Schulz-Varszegi M, Ruther E, Conrad B. Assessment of intracranial hemodynamics in sleep apnea syndrome. Stroke 1992;23: 1427–1433.
23. Netzer N, Werner P, Jochums I, Lehmann M, Strohl KP. Blood flow of the middle cerebral artery with sleep-disordered breathing: Correlation with obstructive hypopneas. Stroke 1998;29:87–93.
24. Markus HS, Harrison MJ. Estimation of cerebrovascular reactivity using transcranial Doppler, including the use of breath-holding as the vasodilatory stimulus. Stroke 1992;23: 668–673.
25. Little JA, Rubin DB. Statistical analysis with missing data. New York: Wiley; 1987.
26. Schafer JL, Graham JW. Missing data: Our view of state of the art. Psychological Methods 2002;7:147–177.
27. Czosnyka M, Smielewski P, Piechnik S, Steiner LA, Pickard JD. Cerebral autoregulation following head injury. Journal of Neurosurgery 2001;95:756–763.
28. Lang EW, Mehdorn HM, Dorsch NW, Czosnyka M. Continuous monitoring of cerebrovascular autoregulation: A validation study. Journal of Neurology, Neurosurgery, and Psychiatry 2002;72:583–586.
29. Ainslie PN, Duffin J. Integration of cerebrovascular CO_2 reactivity and chemoreflex control of breathing: Mechanisms of regulation, measurement, and interpretation. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 2009;296:1473–1495.
30. Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, et al. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. Journal of Neuroscience Methods 2011;196:221–237.
31. Tegeler C, Kim J, Collins G, Steelman D, Westwood K, Reynolds P, Martin D, Greenberg J, Stump D. Transcranial Doppler ultrasound for concussion in amateur athletes. Program and Abstracts of the 26th Annual Meeting of the American Society of Neuroimaging 2003;6:174.
32. Tegeler C, Kim J, Collins G, Steelman D, Westwood K, Reynolds P, Martin D, Greenberg J, Stump D. Dynamic vascular assessment of brain circulation for sports-related concussion. Cerebrovascular Diseases 2004;17(S4):1–38.
33. Barr WB, Prichep LS, Chabot R, Powell MR, McCrea M. Measuring brain electrical activity to track recovery from sport-related concussion. Brain Injury 2012;26:58–66.
34. Ogoh S, Hayashi N, Inagaki M, Ainslie PN, Miyamoto T. Interaction between the ventilatory and cerebrovascular responses to hypo- and hypercapnia at rest and during exercise. Journal of Physiology 2008;586:4327–4338.
35. Gaetz M. The neurophysiology of brain injury. Clinical Neurophysiology 2004;115:4–18.
36. Hilz MJ, Defina PA, Anders S, Koehn J, Lang CJ, Pauli E, Flanagan SR, Schwab S, Marthol H. Frequency analysis unveils cardiac autonomic dysfunction after mild traumatic brain injury. Journal of Neurotrauma 2011;28: 1727–1738.
37. Rubin DB. Multiple imputation for nonresponse in surveys. New York: Wiley; 1987.