Should an increase in cerebral neurochemicals following head kicks in full contact karate influence return to play?

MR Graham,1 Pates J,1 B Davies,2 SM Cooper,3 K Bhattacharya,4* PJ Evans5 and JS Baker6

Abstract

**Background:** Cerebral neurochemicals are markers of traumatic brain injury (TBI).

**Objectives:** The aim of the study was to determine whether kicks to the head (KTH) in full contact karate significantly increased serum concentrations of protein S-100B, and neurone specific enolase (NSE). Kicks to the body (KTB) were also quantified to assess muscle tissue injury. Muscle damage was assessed by analysis of serum total creatine kinase (CK).

**Methods:** Twenty-four full contact karate practitioners were observed and filmed during actual competition and divided into two main groups post event: (1) Kicks to the head and body group (KTH): n = 12; mean ± SD; age, 30.4 ± 6.7 years; height, 1.74 ± 0.1 m; weight, 79.1 ± 2.1 kg; and (2): Kicks to the body group (KTB): n = 12; mean ± SD; age, 28.2 ± 6.5 years; height, 1.75 ± 0.1 m; weight, 79.2 ± 1.7 kg. The KTH group received direct kicks to the head, while group KTB received kicks and punches to the body. Blood samples were taken before and immediately post-combat for analysis of serum S-100B, NSE, CK and cardiac troponin.

**Results:** Significant increases in serum concentrations of S-100B (0.12 ± 0.17 vs. 0.37 ± 0.26, µg.L⁻¹) and NSE (11.8 ± 4.1 vs. 20.2 ± 9.1 ng.mL⁻¹) were encountered after combat in the KTH group and CK (123 ± 53 vs. 184 ± 103 U.L⁻¹) in the KTB group (all P <0.05).

**Conclusions:** Head kicks in full contact karate cause elevation of neurochemical markers associated with damaged brain tissue. The severity of injury is related to the early post-traumatic release of protein S-100B and NSE. The early kinetics and appearance post injury can reflect intracranial pathology, and suggest S-100B and NSE are extremely sensitive prognostic markers of TBI.

**Keywords**

concussion, NSE, S100-B, sport, TBI

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Introduction
Karate has never been an Olympic sport but is bidding to be included in the 2020 Olympics. Traumatic brain injury (TBI) as a result of boxing, Taekwondo and Judo, current Olympic combat sports, are potentially dangerous to health.

Approximately 1.5 million individuals with head injuries die every year, with several million receiving emergency treatment. Most of the burden (90%) is in low- and middle-income countries.

Sports’ concussive injuries account for approximately 2 million cases per year, in the United States alone, but an appreciation of the symptomatic and cognitive impairments that follow concussion has only been realised within the last decade. This type of injury should be addressed in the management of return-to-play (RTP) or the ability of the individual to perform.

Morbidity and neuropsychological sequelae are very common. Primary care management of the concussed sporting individual should begin immediately post event with analysis of specific neurobiochemical markers of brain damage, such as neuron specific enolase (NSE) and protein S-100B, because their prognostic predictor value of outcome.

Patients with specifically high levels of S-100B at initial assessment (>2.5 microg/L) may represent a high-risk group for disability after head trauma.

Routine computerised tomography (CT) or magnetic resonance imaging (MRI) scans of head injuries are not performed in sporting incidents, due to financial constraints and prejudicial views. The analysis of neurochemicals can reduce the need for CT or MRI scans, or admission by over 30%.

Moreover, the routine use of post-head injury surveillance imaging has been considered controversial in terms not only of cost, but also efficacy in identifying those who require operative intervention.

Despite these facts, the determination of neurochemical markers does not appear to be available routinely as either a diagnostic or prognostic markers of cerebral injury in primary or secondary care in the UK. Such information may be useful to set standards where elevations are observed post-head injury, providing a referral system to manage safe and effective return-to-play and prevention of any associated long-term sequelae.

The purpose of this study was to analyse whether kicks to the head (KTH) compared to kicks to the body (KTB), sustained during a karate contest, would result in elevated levels of neurochemical markers, NSE and S-100B, which may indicate cerebral damage. A further analysis was performed within the KTH group to assess if there was a significant difference between technical knockouts (TKOs) compared with kicks to the head, group 2 (KTH2) not resulting in a knock down or technical knockout (TKO).

Muscle damage was quantified by measuring total serum creatine kinase (CK). Cardiac muscle damage was excluded during the competition by the analysis of serum cardiac troponin.

Materials and methods
Subjects
Approval for the study was obtained from the University ethics committee. Prior to data collection all subjects read and completed an informed consent form, which outlined experimental procedures, data collection techniques and the purpose of the study. A pre-competition medical examination on the morning of the event was conducted on all contestants who entered a national full contact karate championship. Data collection procedures comprised of personal interviews, physical examinations and blood analysis. The event comprised four 3-minute rounds. Subjects were all experienced karate practitioners.

Study design
Subjects were divided into four groups, retrospectively following the karate contest, using the standard scoring system, provided by the organisation hosting the event. Tournament rules required a seated judge at each corner of the 10 × 10 m arena and a referee positioned at the centre of the combat arena, who could move anywhere within the arena. Two referee assistants and a match arbitrator were situated outside the competition arena. The tournament rules allowed punches to the body, below the neck, but not the head. However, kicks to the body and the head were permitted.

Measurement of punches and kick
Analysis of punches and KTB and KTH were quantified by the research team on the day of the tournament and further verified by subsequent video
analysis and performance histories obtained from the competitors. A TKO was deemed to have occurred when a competitor was either knocked unconscious, or when receiving a kick to the head, was incapacitated to such an extent, that the central referee ruled that the competitor could not continue.

Following this analysis, male subjects (n = 24) were assigned to: (1) Kicks to the head and body group (KTH): n = 12; mean ± SD; age, 30.4 ± 6.7 years; height, 1.74 ± 0.1 m; weight, 79.1 ± 2.1 kg; and (2) Kicks and punches to the body group (KTB): n = 12; mean ± SD; age, 28.2 ± 6.5 years; height, 1.75 ± 0.1 m; weight, 79.2 ± 1.7 kg.

KTH male subjects (n = 12) were further assigned into two groups: (3) Kicks to the head and body group 2 (KTH2): n = 6; mean ± SD; age, 27.3 ± 7.8 years; height, 1.73 ± 0.1 m; weight, 79.1 ± 2.1 kg; and (4) Technical knockout group (TKO): n = 6; mean ± SD; age, 33.5 ± 3.8 years; height, 1.74 ± 0.1 m; weight, 78.9 ± 1.9 kg.

Subjects acted as their own controls. Physiological tests were performed in the same order for all subjects. Subjects were examined and venous blood samples were taken from the brachial vein, prior to the commencement of combat and within 5 min of cessation.

Values obtained following blood analysis for serum analytes were adjusted to account for plasma volume changes as a consequence of exercise.11

**Body composition assessment**

Total body mass (TBM) was measured using a calibrated beam balance weighing scales (Seca, Cranlea Ltd., Birmingham, UK) and height was measured using a stadiometer (Seca, Cranlea Ltd., Birmingham, UK).

**Blood sampling**

Venous phlebotomy was conducted, using the standard venepuncture method into vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA). Blood was collected between the hours of 12:00 and 18:00, between 1 and 5 min, of contest completion. Venous blood was collected into an Ethylenediaminetetra-acetic acid (EDTA) vacutainer for assessment of full blood count. Haemoglobin (Hb) concentration was determined using the cyanmethaemoglobin method by placing venous blood in microcuvettes (Haemocue Blood Haemoglobin Photometer, Haemocue Ltd., Sheffield, UK). Packed cell volume (PCV) was measured using a Hawksley Micro-haematocrit Reader (Hawksley & Sons Ltd., West Sussex, UK) following centrifugation at 20,900 g for 4 min in an Analox microhaematocrit centrifuge (Hawksley & Sons Ltd., West Sussex, UK). Hb and PCV blood samples were taken in triplicate and the mean recorded. Bloods were also collected into vacutainers containing serum separation tubes (SST) and lithium-heparin (LiH). LiH tubes were centrifuged at 3500 rpm for 10 min at 4°C. The SST samples were allowed to clot at room temperature for exactly 1 h before centrifugation. The plasma or serum supernatant was removed and placed into tubes (Eppendorf®) and stored at −80°C until analysis. The serum NSE, serum troponin, and serum protein S-100B were all measured by electrochemiluminescent immunoassay using a Modular Analytics E analyser, supplied by Roche Diagnostics; NSE part number: 1213313122; protein S-100B part number: 03175243190.

Total creatine kinase (CK) was measured using a Specord 200 spectrohotometer with a Roche/Hitachi 917/MODULAR P analyser.

Cardiac troponin was measured by electrochemiluminescence immunoassay ‘ECLIA’ for use on ‘Elecsys and cobas e’ immunoassay analyser (Roche Diagnostics).

The lower detection limit of the S-100B assay was 0.015 μg/L (0 ± 3 SD) of S-100B protein. The intra-assay variability (CV) was 3.2% at 0.51 μg/L, 2.1% at 5.97 μg/L, and 2.3% at 11.4 μg/L of S-100B protein. The reference value for S-100B in blood was 0.069 ± 0.058 μg/L.

The lower detection limit of the NSE assay was 1 μg/L (0 ± 3 SD). The intra-assay variability (CV) was 2.99% at 10.3 μg/L, 4.7% at 83.7 μg/L and 2.0% at 184 μg/L NSE. The reference value for NSE in blood was 11.1 ± 4.7 μg/L.

The assays for CK and cardiac troponin showed excellent between-run precision (co-efficients of variation = 3.3–4.9%).

**Statistical analysis**

Data were analysed using the PASW 21.0 for Windows statistical package. Parametric data analysis is presented as mean ± standard deviation (SD) and analysed using Student’s t-test. Non-parametric
Table 1. Subject characteristics including pre- and post-bout outcomes for KTH and KTB.

<table>
<thead>
<tr>
<th>Variable</th>
<th>KTH (Pre-bout) (n = 12)</th>
<th>KTH (Post-bout) (n = 12)</th>
<th>KTB (Pre-bout) (n = 12)</th>
<th>KTB (Post-bout) (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.4 ± 6.7</td>
<td>30.4 ± 6.7</td>
<td>28.2 ± 6.5</td>
<td>28.2 ± 6.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74 ± 0.1</td>
<td>1.74 ± 0.1</td>
<td>1.75 ± 0.1</td>
<td>1.75 ± 0.1</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>79 ± 2.1</td>
<td>79 ± 2.1</td>
<td>79.2 ± 1.7</td>
<td>79.2 ± 1.7</td>
</tr>
<tr>
<td>CK (U.L⁻¹)</td>
<td>160 ± 88</td>
<td>186 ± 101</td>
<td>123 ± 53</td>
<td>184 ± 103†</td>
</tr>
<tr>
<td>S100B (µg.L⁻¹)</td>
<td>0.12 ± 0.17</td>
<td>0.37 ± 0.26‡</td>
<td>0.12 ± 0.16</td>
<td>0.11 ± 0.12</td>
</tr>
<tr>
<td>NSE (ng.mL⁻¹)</td>
<td>11.8 ± 4.1</td>
<td>20.2 ± 9.0†</td>
<td>13.8 ± 6.1</td>
<td>17.5 ± 7.4</td>
</tr>
</tbody>
</table>

*Within the KTB group, CK increased post trauma: P <0.05.
†Within the KTH groups, the S100B and NSE increased post trauma: P <0.05.
‡P <0.05 = significantly different to KTB.
CK: creatine kinase; KTB: kicks to the body group; KTH: kicks to the head group; NSE: neurone specific enolase.

Results

Following the karate contests, significant increases (P <0.05) in serum concentrations of S-100B, and NSE were encountered in the KTH group, but not in the KTB group. S-100B was significantly increased in KTH group, versus the KTB group, but not NSE.

Serum CK was significantly elevated (P <0.05) in KTB group (Table 1). There were no elevated levels of serum cardiac troponin.

There were significant increases (P <0.05) in serum concentrations of S-100B, and NSE in the TKO group and the KTH2 group (Figures 1 and 2). S-100B was also significantly increased post combat in both the TKO group and the KTH2 group versus the KTB group (Table 2).

There was no further significant increase in the TKO group versus the KTH2 group. However Cohen’s d analysis of S-100B, in TKO vs. KTH2 demonstrated a large increase (31%) and a medium effect size (0.47), whereas there was no difference in effect size in NSE between the two groups (Table 2). All TKOs were provided with head injury advice and advised to attend hospital. One subject who incurred a knockout was taken to hospital by ambulance.

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Image captions:

**Figure 1.** Individual differences in protein S-100B (µg.L⁻¹) pre-contest and post-contest, following four 3-min rounds of karate (n = 12).
Y-Axis: Protein S-100B (µg.L⁻¹).
X-Axis: Individual subjects (n = 12).
Pre-contest kicks to the head (KTH) protein S-100B (µg.L⁻¹) = pre-contest KTH, S-100B. Post-contest KTH protein S-100B (µg.L⁻¹) = post-contest KTH, S-100B.

**Figure 2.** Individual differences in neurone specific enolase (NSE) (ng.mL⁻¹) pre-contest and post-contest, following four 3-min rounds of karate (n = 12).
Y-Axis: NSE (ng.mL⁻¹).
X-Axis: Individual subjects (n = 12).
Pre-contest kicks to the head (KTH) NSE (ng.mL⁻¹) = pre-contest KTH, NSE. Post-contest KTH NSE (ng.mL⁻¹) = post-contest KTH, NSE.
Alzheimer’s disease (AD). Plaques and tangles of DP are the same as those in dementia pugilistica (DP) or punch-drunk syndrome. The molecular markers present in the contact sport may lead to the development of prior to the commencement of the competition.

There were no elevated levels of serum cardiac troponin in the KTB group but not the KTH group, indicating an adverse effect from skeletal muscle trauma as a consequence of body kicks and punches (Table 1).

Increased concentrations of NSE and S-100B can both be measured in cerebrospinal fluid (CSF) and in peripheral blood after cerebral trauma and are accurate predictors of outcome, S-100B being more sensitive than NSE. Acute measurement of NSE and S-100B serum concentrations may provide a quantitative predictor of outcome after TBI in young children, which can be extrapolated to adults.

S-100B has multiple functions including the inhibition of protein phosphorylation through interacting with kinase substrates, regulating enzyme activity and interacting with cytoskeletal elements. It is also involved in calcium homeostasis and is believed to have a role in cytosolic calcium buffering. It has been shown to be raised in many organic brain disorders such as TBI, subarachnoid haemorrhage, stroke, epilepsy, multiple sclerosis, Parkinson’s disease and hydrocephalus. In addition, there is clinical and laboratory evidence that it is raised in certain neuropsychiatric disorders including post-traumatic stress disorder. S-100B has great potential to become a specific neurological screening tool that is predictive of outcome and responsive to treatment.

Secondary changes of TBI can include axonal injury, reduced cerebral blood flow, decreased cerebral cell glucose uptake, oedema, raised intracranial pressure (ICP), increased blood-brain barrier (BBB) permeability, elevated oxidative stress.
A suggested mechanism of TBI-induced cell damage is of excess free radical generation produced by mitochondria. During normal metabolism, the tricarboxylic acid cycle (TCA) generates reducing equivalents to produce adenosine triphosphate (ATP). Electrons are normally transferred along the electron transport chain, with only 1–2% of the oxygen generating oxygen radicals at Complex I in the respiratory chain. However, following TBI, changes in the availability of these reducing equivalents is diminished and the production of the superoxide anion (O$_2^-$) is increased. Following a TBI intracellular calcium (Ca$^{2+}$) increase activates specific enzymes, such as xanthine dehydrogenase, phospholipase A2 and nitric oxide synthase (NOS), which increase O$_2^-$ and nitric oxide anion (NO) production, which can result in oxidative damage.24,25

Classification of TBIs begins at the scene of an incident and can be mild, moderate or severe and the Glasgow coma scale (GCS) is the most commonly used system. This measures a patient’s level of consciousness based on verbal, motor and eye-opening responses after injury and defines clinical severity. A GCS score of 3–8 (out of 15) is considered a severe TBI, 9–12 (out of 15) is moderate, and 13–15 (out of 15) is considered a mild TBI.26 Prior to identification of the neurochemical markers it was a very accurate predictor of outcome and no patient did significantly better than expected.27 In this cohort, one individual who received a direct kick to the head, suffered a knockout and endured a tonic-clonic seizure. GCS was recorded as 3/15 (E1, V1, M1) (Table 3). Full paramedical management was initiated. Because of the serious nature of the injury post combat, blood sampling by the research team was not possible. On regaining consciousness, the GCS recorded at 6 min was 13/15 (E4, V4, M5) (Table 3). Full paramedical management was initiated. Because of the occurrence of the seizure, the authors believed that he should have been admitted for 12 h for neurological observation or received a CT scan of his brain.

All knockouts in karate should be treated as serious head injuries and all subjects should be advised to attend hospital and, if warranted, conveyed to hospital. The GCS is still the most commonly used assessment in the field; however, following admission to hospital research indicates that it should be superseded by the ‘Full Outline of Unresponsiveness (FOUR) score’ which is a more accurate predictor of discharge outcome in TBI patients.28 Post-TBI concussive symptoms can be divided into three areas: (1) somatic, e.g. headaches; (2) emotional (behavioural), e.g. personality changes; and (3) cognitive, e.g. decreased mental acuity.29 Such sequelae may be permanent and compounded by the fact that athletes seldom report concussive symptoms, which makes the diagnosis and management challenging. The pathophysiology of concussion requires traditional management, and categorisation and return-to-play guidelines should be for individual assessments and management. Once diagnosed with a concussion, a sportsperson must not be allowed to return-to-play before the concussion symptoms have completely resolved.30 Neuropsychological testing may also be used in such management to prevent any decreased neurocognitive functioning.31

### Table 3. Glasgow Coma Scale: Individual subject readings.26

<table>
<thead>
<tr>
<th>Time post trauma</th>
<th>Score</th>
<th>Best eye response (max 4)</th>
<th>Best verbal response (max 5)</th>
<th>Best motor response (max 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1. No eye opening</td>
<td>1. No verbal response</td>
<td>1. No motor response</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Eye opening to pain</td>
<td>2. Incomprehensible sounds</td>
<td>2. Extension to pain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Eye opening to verbal command</td>
<td>3. Inappropriate words</td>
<td>3. Flexion to pain</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The improved nutrition, training regimes and fitness in competitive sporting individuals over the last century has resulted in physiques that can generate increased pace and therefore concussive forces, following TBIs. The incidence of TBIs in sport has dramatically increased resulting in socioeconomic deficits owing to any ensuing disability. Even a single mild TBI can lead to impaired function.32

There is a duty of care by professionals within different sporting disciplines to manage acute and any potential long-term consequences of brain injury.

Adding the measurement of S-100B serum concentration to the clinical decision rules for a CT scan or hospitalisation in patients with mild TBI could allow a 30% reduction in scans and in hospitalisation for clinical observation.33

Research suggests that patients with severe and moderate TBI and post-concussion syndrome should be managed in multidisciplinary neuroscience centres, regardless of the need for neurosurgical intervention to improve outcome.34

In conclusion, a recent review provided evidence that cognitive function only improved in the month after mild TBI (MTBI) and that verbal learning can be impaired up to 6 months after MTBI. Evidence also exists that MTBI is associated with a significant increase in the incidence of psychiatric disorders, and a three-fold increase in the risk for suicide.35 The extrapolation of the effects of management of even MTBI influencing return to play policies in sport requires serious consideration, further investment and research.36

The recent increased attention in the media and medical profession regarding the sequelae of MTBI, suggests the requirement of an expert neurological report including serial biochemical markers (a ‘neurobiological passport’) prior to return-to-play in cases with clinical symptoms of impaired cognitive function and elevated post-concussive neurochemicals following MTBI, as a minimum.

Following MTBI, serum neurochemicals can remain elevated for several weeks, but every case is different and the exact time to return to baseline is unknown. The authors also advocate the addition of another even greater predictive neurochemical biomarker (total tau, a protein signalling axonal damage in the brain) be added to this passport.37 This may assist future psychological and pharmacological rehabilitation at primary and secondary care level.

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