DIRECT HITS TO THE HEAD DURING AMATEUR BOXING IS ASSOCIATED WITH A RISE IN SERUM BIOMARKERS FOR BRAIN INJURY

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Received November 11, 2010 – Accepted January 8, 2011

Boxing exposes participants to the physiological response to high intensity exercise and also to direct body and brain trauma. Amateur boxing is increasing and females have also been included in the Olympics. The aim of this study is to assess the stress response and possible brain injury incurred during a match by measuring serum biomarkers associated with stress and cellular brain injury before and after combat. Sixteen male amateur boxers were studied retrospectively. The study population was divided into two groups: (a) a group that received predominantly punches to the head (PTH) and (b) a group that received predominantly punches to the body (PTB). Blood samples were taken before and five minutes after each contest. They were analysed for S-100B, neuron-specific enolase (NSE), creatine kinase (CK) and cortisol. The PTH group received direct contacts to the head (not blocked, parried or avoided) and to the body (*n*=8, age: 17.6 ± 5.3, years; height: 1.68 ± 0.13, meters; mass: 65.4 ± 20.3, kg). The PTB group received punches to the body including blocked and parried punches, but received no direct punches to the head, (*n*=8, mean ± SD, age: 19.1 ± 3.2 years; height: 1.70 ± 0.75m; mass: 68.5 ± 15 kg). Significant increases (*P*<0.05) between pre- and post-combat serum concentrations in serum concentrations of S-100B (0.35 ± 0.61 *vs.* 0.54 ± 0.73, µg.L^{-1}) NSE (19.7 ± 14 *vs.*31.1 ± 26.6, ng.ml^{-1}) and cortisol (373 ± 202 *vs.* 756 ± 93, nmol.L^{-1}). Significant increases (*P*<0.05) of creatine kinase were recorded in both groups. This study demonstrates significant elevations in neurochemical biomarkers in boxers who received direct blows to the head. However, further work is required to quantify this volumetric brain damage and long term clinical sequelae.

Children as young as 11 years of age are eligible to compete in boxing bouts that involve full contact blows to the head (1). Furthermore, female amateur boxing has also recently been included as an Olympic sport and is a testament to equality in sport and the assumed confidence in the safety of amateur boxing (2). The perception of safety surrounding the sport can be attributed to the lack of consensus in the scientific literature on the potential dangers involved in boxing (3-5), compounded by the assumed protection afforded by the mandatory use of head-guards. This position is often assumed to be due to

Key words: boxing, brain injury, S100-B, Neuron-specific enolase (NSE)

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0394-6320 (2011)

the lower incidence of chronic brain injury amateur boxers incur when compared to their professional counterparts (5-6).

Although the disparity between amateur and professional athletes is often cited as evidence of the efficacy of head-guards in preventing neurological damage, other differences between professional and amateur codes may account for this (7). For example, chronic brain injury correlates positively with the number of head blows a boxer receives, the length of contests they participate in, the intensity and volume of sparring and the length of a boxer's career (7). These tend to be more prevalent in the professional sport (7).

Severe acute cerebral injuries, resulting in morbidity or fatality, are rare in amateur boxing compared with other sports (3). However, even with the use of head-guards, mild brain injuries (MBIs) account for 85-90% of all head injuries (3). Given their cumulative nature and the risk of long lasting symptoms are a major health concern (8). Multiple MBIs can lead to a transitory post-concussive syndrome which can develop into a chronic post traumatic stress disorder (9). Given that the probability and severity of post-concussive syndrome are greater for females (10), and children (11), the possibility of neurological damage being incurred in any sport that includes both participant groups, warrants closer investigation.

Interest in biomarkers of brain damage in clinical neurotrauma (12), and their association with neuropsychological sequelae is increasing (13). Protein S-100B (a glial marker) and neuron specific enolase (NSE, a neuronal marker), are the most frequently investigated biomarkers in clinical research, due to their commercial availability and detectability in serum. The S-100B protein cytokine, is physiologically produced and released predominantly by astrocytes on the central nervous system (CNS). It is present in the cytosol of glial and Schwann cells and can also be increased in high intensity exercise (14). NSE is predominantly found in the cytoplasm of neurons and in cells with neuroendocrine differentiation. Increased concentrations of NSE can be measured both in cerebrospinal fluid (CSF) and in peripheral blood after neuronal trauma (15). Serum cortisol is known to be elevated following head trauma (16) and prolonged hypercortisolaemia can lead to unipolar depression and dopaminergic, noradrenergic and thyroid dysfunction (17).

The primary aim of this study is to investigate if punches to the head (PTH), sustained during a single amateur boxing bout of five, two minute rounds, would result in subclinical cerebral injury as indicated by elevated serum levels of neurochemical markers and cortisol concentrations.

MATERIALS AND METHODS

Subjects

Approval for the study was obtained from the University Ethics Committee. The event complied with the Amateur Boxing Association (ABA) regulations. Subjects were all experienced male amateur boxers. Written, informed consent was provided by the boxers and their guardians when under the age of 18 years. Subjects were recruited from a national amateur boxing show, which was arranged three months prior to the contest. All subjects had fought at their appropriate weight on two previous occasions and no extraordinary dietary intervention was used to reach their appropriate weights. A full clinical examination, including hydration status, was conducted pre-contest, by the examining medical officer.

Study design

Punches received to the head or body were analysed retrospectively post boxing match for individual fighters using the Chowdhry scoring system (18). Following this analysis, male subjects (n=16) were assigned into the following:

(1): Punches to the head and body group (PTH) n=8, age range: 11-29, years; height range: 1.5-1.85 meters; mass range: 47.3-103 kg.

2): Punches to the body group (PTB) n=8, age range: 16-24, years; height range: 165-186 meters; mass range: 48-100 kg. (See Table I).

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 ante-cubital vein, one hour prior to the commencement of combat and after five minutes of cessation.

Values obtained following analysis of all serum analytes were adjusted to account for plasma volume changes pre- and post-exercise (19).

Body composition assessment

Total body mass (TBM) was measured using a

calibrated beam balance weighing scales (Seca, Cranlea Ltd, Birmingham, UK) and stature was measured using a calibrated stadiometer (Seca, Cranlea Ltd).

Measurement of punches

Three judges at ringside counted the number of punches thrown to the body and to the head and recorded the number on a hand held electronic boxing counter (Director, Europe, Ltd) and one independent recorder (experienced boxing referee), determined the number of direct head and body contacts (not blocked, parried or avoided) that landed for each individual during the contest.

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 19:00 and 23:00, between one and five 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 centrifusion at 20,900 g for 4 min in an Analox micro-haematocrit centrifuge (Hawksley & Sons Ltd., West Sussex, UK). Hb and PCV blood samples were taken in triplicate, and the mean recorded. Blood samples were also collected into vacutainers containing serum separation tubes (SST) and lithium-heparin (LiH). LiH tubes were centrifuged at 1250 g for 10 min at 4°C. The SST samples were allowed to clot at room temperature for exactly one hour before centrifusion. The plasma or serum supernatant was removed and placed into tubes (Eppendorf®) and stored at -80°C until analysis. Skeletal muscle damage, as quantified by measurement of total serum creatine kinase (CK) and cardiac muscle damage, during the boxing bouts, was excluded by the analysis of serum cardiac troponin T.

The serum NSE, serum cardiac troponin T, serum protein S100-B and serum cortisol were all measured by electrochemilluminescent immunoassay using a Modular Analytics E analyser, supplied by Roche Diagnostics; NSE part number: 1213313122; protein S100-B part number: 03175243190; Cortisol part number: 11875116122. CK was measured using a Specord 200 spectrohotometer with a Roche/Hitachi 917/MODULAR P analyzer. The assays showed excellent between-run precision (Co-efficients of Variation = 3.3-4.9%).

Statistical analysis

Data were analysed using the PASW 17.0 for Windows statistical package. Parametric data analysis is presented as mean ± standard deviation (SD) and analysed using Student's *t*-test. Non-parametric data is described as median and inter-quartile range, and analysed using the Wilcoxon rank-sum test. Statistical significance was accepted at the $P<0.05$ level.

RESULTS

There were no technical knockouts, knockouts or knockdowns. All bouts went the full distance. The demographic data is summarised in Table I. Following the boxing competition, significant increases ($P<0.05$) in serum concentrations of NSE, S-100B, CK and cortisol were encountered in the PTH group, but not in the PTB group (Figs. 1-3). There were no elevated levels of serum cardiac troponin. The shared variance between protein S100-B levels and punches received to the head was $r^2= 0.43$ suggesting that 43% of the variation found in post protein S100-B levels can be explained by the number of punches subjects received to the head (Fig. 4). Similarly, NSE was also strongly correlated with punches received to the head ($r^2= 0.25$) suggesting a 25% shared variance with head blows (Fig. 5).

DISCUSSION

In this study, the serum elevation of brain neurochemicals and cortisol, resulting from head trauma, concurred with past studies which have shown a significant increase and correlation between the neurochemical markers, NSE and S-100B, and systemic stress enzymes cortisol (13, 16).

MBIs are defined by an initial unconsciousness limited to 30 min, a Glasgow Coma Scale (GCS) score between 13 and 15, the absence of intra-cranial lesion on the computed tomography (CT) scan, and a post-traumatic amnesia period between one and 24 hours (20).

Increased serum concentrations of NSE have been found in 23% of patients admitted to hospital with minor head injury, and further CT analysis has shown contusion in 2% (21).

An association has been shown between clinical outcome and serum concentrations of protein S-100B and NSE in patients with severe traumatic

Table I. *Subject characteristics including pre- and post-bout outcomes.*

VARIABLE	PTH (PRE-BOUT) (n = 8)	PTH (POST-BOUT) (n = 8)	PTB (PRE-BOUT) (n = 8)	PTB (POST-BOUT) (n = 8)
Age (yrs)	17.6 ± 5.3	17.6 ± 5.3	19.1 ± 3.2	19.1 ± 3.2
Height (m)	1.68 ± 0.13	1.68 ± 0.13	1.7 ± 0.8	1.7 ± 0.8
Body Mass (kg)	65.4 ± 20.3	65.4 ± 20.3	68.5 ± 15	68.5 ± 15
Number of Head Punches		36 ± 18		
Number of Body Punches		51 ± 8		67 ± 23
CK ($U.L^{-1}$)	207 ± 107	244 ± 118*	150 ± 43	195 ± 63*
S100B ($ug.L^{-1}$)	0.35 ± 0.61	0.54 ± 0.73*	0.42 ± 0.19	0.43 ± 0.2
NSE ($ng.ml^{-1}$)	19.7 ± 14	31.1 ± 26.6*	16.4 ± 13	17.5 ± 14
Cortisol ($nmol.L^{-1}$)	373 ± 202	756 ± 93*	416 ± 140	417 ± 135

PTH = Punches to the Head Group; PTB = Punches to the Body Group;
*NSE = Neurone Specific Enolase; CK = Creatine Kinase Within the PTH-groups the S100B, NSE & Cortisol increased post trauma, *(P<0.05).*
*Within the PTH-group and the PTB-group, CK increased post trauma, *(P<0.05).*

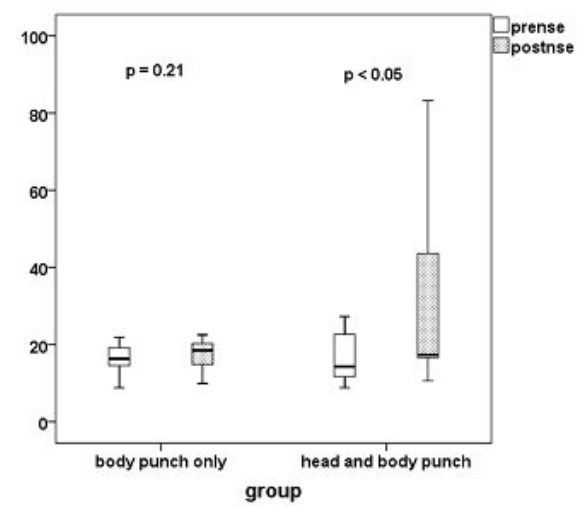

Fig. 1. *Number of head and body punches received and changes in Neurone Specific Enolase (NSE, $ng.ml^{-1}$) pre-bout and post-bout, following five, two min rounds of boxing.*
Y-Axis: Neurone Specific Enolase ($ng.ml^{-1}$)
X-Axis: Punches to Body or Head
Pre-bout Neurone Specific Enolase ($ng.ml^{-1}$) = pre-NSE
Post-bout Neurone Specific Enolase ($ng.ml^{-1}$) = post-NSE

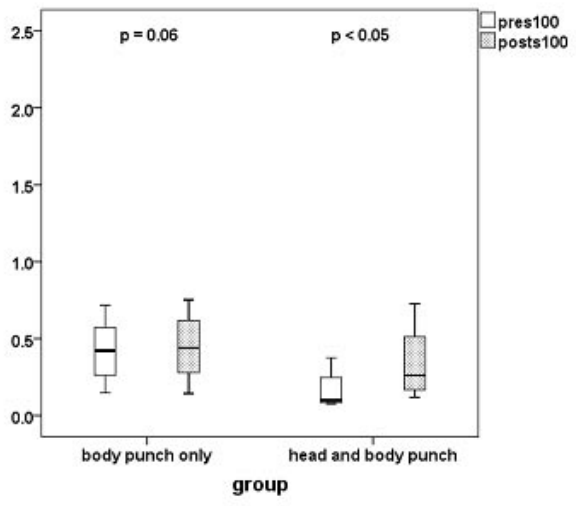

Fig. 2. *Number of head and body punches received and changes in Protein S100B ($\mu g.L^{-1}$) pre-bout and post-bout, following five, two minute rounds of boxing.*
Y-Axis: Protein S100B ($\mu g.L^{-1}$).
X-Axis: Punches to Body &/or Head
Pre-bout Protein S100 ($\mu g.L^{-1}$) = pre-S100B
Post-bout Protein S100 ($\mu g.L^{-1}$) = post-S100B

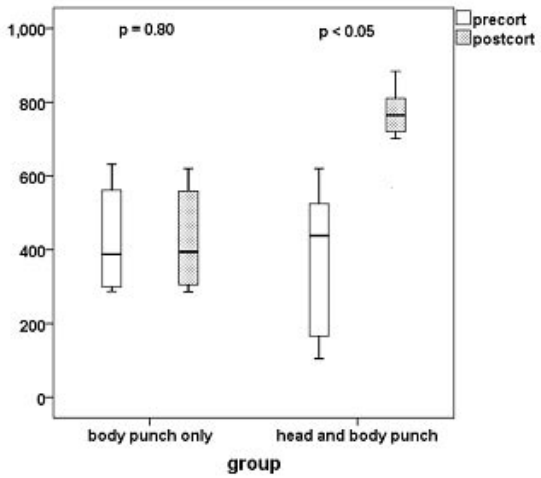

Fig. 3. *Number of head and body punches received and changes in Cortisol (ng.ml^{-1}) pre-bout and post-bout, following five, two minute rounds of boxing.*
Y-Axis: Cortisol (ng.ml^{-1}).
X-Axis: Punches to Body &/or Head
Pre-bout Cortisol (ng.ml^{-1}) = pre-Cort
Post-bout Cortisol (ng.ml^{-1}) = post-Cort

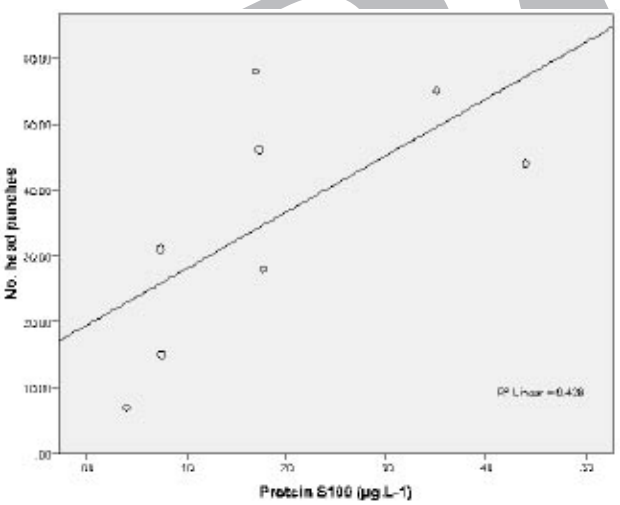

Fig. 4. *Scatterplot showing correlation between number of head punches received and changes in Protein S100 (µg.L^{-1}) following five, two minute rounds of boxing.*
Y-Axis: Number of Punches to the Head
X-Axis: Protein S100 (µg.L^{-1})

brain injury (22). The severity of traumatic brain injury is associated with the early post-traumatic release of protein S-100B and NSE, within five hours but not after 11 hours and S-100B appears to be more sensitive than NSE (22). Their early kinetics after

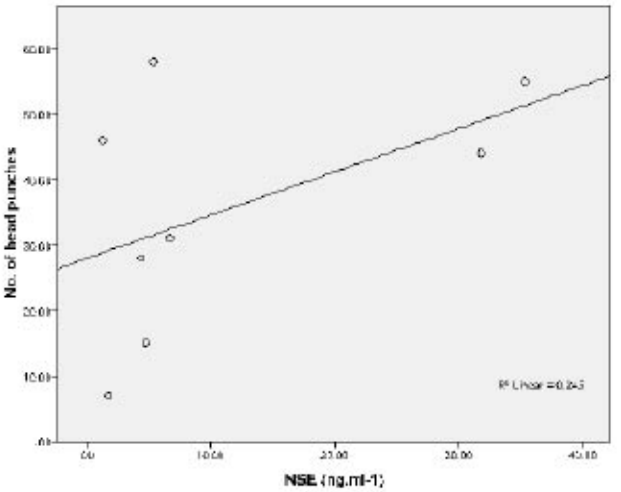

Fig. 5. *Scatterplot showing correlation between number of head punches received and changes in Neurone Specific Enolase (ng.ml^{-1}) following five, two minute rounds of boxing.*
Y-Axis: Number of Punches to the Head
X-Axis: Neurone Specific Enolase (NSE, ng.ml^{-1})

traumatic brain injury reflect intracranial pathology as demonstrated in cranial CT and they have become an extremely sensitive prognostic marker associated with neuropsychological outcome even in minor head injury (13).

There is evidence that markers for cerebro-spinal fluid levels of neuronal and axonal injury are still present three months post boxing matches (23). It is almost impossible to keep track of the majority of subjects to establish when serum levels return to baseline. This can be compounded by the fact that in between competitive bouts, training for the amateur boxer incorporates sparring in the gym and head-guards are not always worn, predisposing individuals to even more head trauma, outside competition. Persistent elevation of S-100B levels for two to six days, even in patients with favourable outcome, may reflect ongoing secondary damage after head injury (22).

The results of this study have identified changes which indicate that head blows in amateur boxing are not insignificant, and whilst head-guards may offer protection from superficial facial injury, the findings of this study indicate that they provide insufficient protection against cerebral contusion. Worryingly, boxing bouts are often stopped because

of facial cuts, which have little impact on neuro-cognitive performance. However, both professional and amateur bouts are allowed to continue after knockdowns, and following serious blows to the head. Such damaging effects could be further magnified by the fact that bouts can involve boxers of different experience and ability levels. While bouts comprise competitors of similar weight, there are no regulations within the sport that prevent discrepancies in experience or ability. This may predispose boxers to an even greater number of head blows, than may be the case in more equally matched bouts.

Further concern is that detailed clinical examination is of limited diagnostic value in detecting intracranial injuries. CT scanning is essential with loss of consciousness or post-traumatic amnesia and a GCS of 13-15, to avoid missing an intracranial injury (24). The risk of having an intra-cerebral haemorrhage from a mild brain injury (MBI) is 38% with seven % requiring neurosurgical intervention (25).

S-100B is not affected by haemolysis and measurements are crucial in the accident and emergency care environment (26). Admission values also have a significant correlation to injury severity and outcome, but show a highly time dependent temporal course (26). S-100B would appear to be more reliable as an early predictor of poor neurological outcome within 11 hours than NSE (27), and can reduce the need for CT scans or hospital admission by over 30% (28).

In the present study the stronger correlation between S100-B and punches to the head (43%) compared with NSE (25%) would also corroborate with these findings suggesting that measurement of this biochemical marker may contribute to initial assessment of MBIs.

Serum CK was significantly elevated in both groups following combat, demonstrating that the body blows were having a detrimental effect on skeletal muscle. Cardiac troponin was not elevated above normal values, five minutes sampling post body punches, but could not exclude cardiac muscle damage, either as a response to the high intensity physical exercise, nor as a result of commotio cordis (29).

In conclusion, pathological levels of brain neurochemicals, which are also produced as a result of heading the football, in soccer have been detected in female soccer players (30), potentially leading to traumatic brain injuries (31).

Considering the evidence outlined in this study, we observed a direct correlation between head punches, elevated serum neurochemicals and cortisol levels. However, further work is required in this area, which could include the introduction of force transducers in the head-guards, to quantify and establish this relationship further, with volumetric brain damage changes and long term clinical sequelae.

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