Neurochemical Aftermath of Amateur Boxing

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Background: Little solid information is available on the possible risks for neuronal injury in amateur boxing.

Objective: To determine whether amateur boxing and severity of hits are associated with elevated levels of biochemical markers for neuronal injury in cerebrospinal fluid.

Design: Longitudinal study.

Setting: Referral center specializing in evaluation of neurodegenerative disorders.

Participants: Fourteen amateur boxers (11 men and 3 women) and 10 healthy male nonathletic control subjects.

Interventions: The boxers underwent lumbar puncture 7 to 10 days and 3 months after a bout. The control subjects underwent LP once.

Main Outcome Measures: Neurofilament light protein, total tau, glial fibrillary acidic protein, phosphorylated tau, and β-amyloid protein 1-40 (Aβ1-40) and 1-42 (Aβ1-42) concentrations in cerebrospinal fluid were measured.

Results: Increased levels after a bout compared with after 3 months of rest from boxing were found for 2 markers for neuronal and axonal injury, neurofilament light protein (mean ± SD, 845 ± 1140 ng/L vs 208 ± 108 ng/L; P = .008) and total tau (mean ± SD, 449 ± 176 ng/L vs 306 ± 78 ng/L; P = .006), and for the astroglial injury marker glial fibrillary acidic protein (mean ± SD, 541 ± 199 ng/L vs 405 ± 138 ng/L; P = .003). The increase was significantly higher among boxers who had received many hits (>15) or high-impact hits to the head compared with boxers who reported few hits. In the boxers, concentrations of neurofilament light protein and glial fibrillary acidic protein, but not total tau, were significantly elevated after a bout compared with the nonathletic control subjects. With the exception of neurofilament light protein, there were no significant differences between boxers after 3 months of rest from boxing and the nonathletic control subjects.

Conclusions: Amateur boxing is associated with acute neuronal and astroglial injury. If verified in longitudinal studies with extensive follow-up regarding the clinical outcome, analyses of cerebrospinal fluid may provide a scientific basis for medical counseling of athletes after boxing or head injury.

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P ROFESSIONAL BOXING IS ASSOCIATED with risk for long-term neurologic injury.¹ The development of chronic neurologic symptoms in this setting was originally referred to as the punch-drunk syndrome or dementia pugilistica.² ³ The terminology has evolved with time, and the entity is now termed chronic traumatic brain injury and occurs in approximately 20% of professional boxers.⁴ The clinical manifestations vary depending on the accumulated number of blows to the head, career duration, performance as a boxer, and ability to withstand many hits.¹ ⁴ According to some studies, amateur boxers also show neuropsychologic and neuroimaging evidence of chronic traumatic brain injury,⁵ ⁶ although at a lower incidence than in professional boxers. There is, however, lack of consensus in the scientific literature, possibly because the expected effects are less severe in amateur boxing compared with professional boxing owing to less exposure to repetitive head trauma because of shorter bouts and the mandatory use of protective headgear.

Studies of chronic traumatic brain injury in boxers have been based on identification of the cumulative late effects of repeated hits to the head, such as brain atrophy and cognitive disturbances or neuropsychologic abnormalities. To our knowledge, no study has examined the short-term effects of amateur boxing on the brain in direct connection to a bout. We conducted a study to identify and monitor brain injury associated with amateur boxing by cerebrospinal fluid (CSF) analyses of biochemical markers for neuronal and astroglial injury in a cohort of amateur boxers after a bout and after extended rest from boxing. A control group of 10 healthy nonathletic subjects was included for com-

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 pariors of long-term biochemical evidence for neuronal impairment in amateur boxers.

**METHODS**

Fourteen Swedish amateur boxers (11 men and 3 women; age [mean ± SD], 22 ± 3.8 years) were enrolled in the study. Ten healthy male nonboxers with no known history of head trauma (age [mean ± SD], 30 ± 6.3 years) were included as control subjects. The study was approved by the Ethics Committee for Medical Research at Gothenburg University, Gothenburg, Sweden, and written informed consent was obtained from all participants. All CSF samples were stored at −80°C pending analysis. The participants were examined physically and neurologically before LP. Cerebrospinal fluid was collected in polypropylene tubes by lumbar puncture (LP) through the L3-4 or L4-5 interspace. In boxers, LP was performed both 7 to 10 days after boxing and after a 3-month period of rest from boxing. One boxer refused the second LP. Only 1 LP was performed in each of the healthy control subjects. Seven to 10 days was chosen as the optimal length of time for detection of a change in biomarker levels as a result of a bout on the basis of CSF biomarker kinetics in a study of stroke. All CSF samples were stored at −80°C pending analysis. The participants were examined physically and neurologically before LP. All were healthy and showed no signs of focal neurologic impairment in amateur boxers.

**Table 1. Biomarker Concentrations in Boxers After a Bout and After a Period of Rest From Boxing and in Healthy Nonathletic Control Subjects**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Boxers After a Bout</th>
<th>Boxers After Absence From Boxing</th>
<th>Control Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 14)</td>
<td>(n = 13)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>NFL</td>
<td>845 (1140)</td>
<td>208 (108)</td>
<td>≤125</td>
<td>.0001</td>
</tr>
<tr>
<td>P-tau</td>
<td>449 (176)</td>
<td>306 (78.1)</td>
<td>325 (97.7)</td>
<td>.008</td>
</tr>
<tr>
<td>GFAP</td>
<td>541 (199)</td>
<td>405 (138)</td>
<td>402 (88.8)</td>
<td>.006</td>
</tr>
<tr>
<td>T-tau</td>
<td>37.9 (10.2)</td>
<td>37.8 (9.30)</td>
<td>46.4 (14.5)</td>
<td>.003</td>
</tr>
<tr>
<td>Aβ[1-40]</td>
<td>19300 (2740)</td>
<td>19500 (3990)</td>
<td>19400 (5050)</td>
<td>.67</td>
</tr>
<tr>
<td>Aβ[1-42]</td>
<td>858 (128)</td>
<td>796 (133)</td>
<td>773 (114)</td>
<td>.15</td>
</tr>
</tbody>
</table>

**Abbreviations:** Aβ[1-40] and Aβ[1-42], β-amyloid proteins 1-40 and 1-42, respectively; GFAP, glial fibrillary acidic protein; NFL, neurofilament light protein; P-tau, phosphorylated tau; T-tau, total tau.

**Figure 1.** Individual cerebrospinal fluid biomarker values in boxers after a bout and after a period of rest from boxing. Cerebrospinal fluid levels of the neuronal and astroglial markers neurofilament light protein (A), total tau (B), and glial fibrillary acidic protein (C) after a bout are related to the number of hits to the head. Red squares indicate boxers who received many hits (>15) or high-impact hits to the head; blue circles, boxers who received few hits to the head. Each boxer underwent lumbar puncture twice, 7 to 10 days after a bout and after 3 months of rest from boxing.
All statistical calculations were performed using SYSTAT software (version 11.0; SPSS Inc, Chicago, Ill). For the paired observations, the Wilcoxon signed rank test was used. For the boxers vs control comparisons, the Mann-Whitney test was used. The Pearson product moment correlation coefficient was used for analyses of correlation between changes in the various biomarker levels after a bout and after a period of rest from boxing. The percentage increase of a certain biomarker after a bout was obtained by dividing the biomarker concentration after the bout by the biomarker concentration after rest from boxing times 100. The percentage increase in neurofilament light protein concentration after a bout compared with after rest from boxing correlates significantly with the change in total tau concentration (A) and glial fibrillary acidic protein concentration (B). Likewise, the change in total tau concentration correlates with the change in glial fibrillary acidic protein concentration (C). Red squares indicate boxers who received many hits (>15) or high-impact hits to the head; blue circles, boxers who received few hits to the head.

Table 2. Biomarker Concentrations in Boxers Who Received Many Hits or Were Groggy Compared With Boxers Who Received Few Hits and With Healthy Nonathletic Control Subjects*

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Many Hits (&gt;15) or Groggy* (n=7)</th>
<th>Few Hitsb (n=7)</th>
<th>Controlsc (n=10)</th>
<th>* vs b</th>
<th>b vs c</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFL</td>
<td>1490 (1360)</td>
<td>200 (65.8)</td>
<td>≤125</td>
<td>.002</td>
<td>.002</td>
</tr>
<tr>
<td>T-tau</td>
<td>551 (168)</td>
<td>346 (118)</td>
<td>325 (97.7)</td>
<td>.02</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>GFAP</td>
<td>650 (202)</td>
<td>431 (130)</td>
<td>402 (88.8)</td>
<td>.02</td>
<td>.59</td>
</tr>
<tr>
<td>P-tau</td>
<td>42.1 (12.8)</td>
<td>33.7 (4.4)</td>
<td>46.4 (14.5)</td>
<td>.10</td>
<td>.09</td>
</tr>
<tr>
<td>Aβ(1-40)</td>
<td>20 200 (3280)</td>
<td>18 300 (1820)</td>
<td>19 400 (5050)</td>
<td>.18</td>
<td>.92</td>
</tr>
<tr>
<td>Aβ(1-42)</td>
<td>884 (153)</td>
<td>832 (103)</td>
<td>773 (114)</td>
<td>.75</td>
<td>.28</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ(1-40) and Aβ(1-42), β-amyloid proteins 1-40 and 1-42, respectively; GFAP, glial fibrillary acidic protein; NFL, neurofilament light protein; P-tau, phosphorylated tau; T-tau, total tau.

*Data are given as mean (SD) in nanograms per liter.

RESULTS

After a bout, there was a marked increase (4.1-fold) in the CSF levels of NFL compared with levels detected in the same individuals after a 3-month rest from boxing (Table 1 and Figure 1). The CSF levels of T-tau and GFAP also were significantly increased after a bout compared with after a 3-month rest from boxing (1.5-fold and 1.3-fold, respectively; Table 1 and Figure 1). The changes in NFL, T-tau, and GFAP concentrations in individual boxers were highly correlated (Figure 2).

The NFL and GFAP, but not T-tau, concentrations were significantly higher in boxers after a bout than in nonathletic control subjects (Table 1). No significant differences in biomarker concentrations were detected between boxers after the 3-month rest period and control subjects, except for NFL, which remained significantly elevated despite absence from boxing (mean±SD, 208±108 ng/L vs ≤125 ng/L; P=.001). The NFL, T-tau, and GFAP concentrations were higher in boxers who had received many hits (>15) or high-impact hits to the head compared with boxers who reported few hits (Table 2 and Figure 1). With the exception of NFL, boxers who received few hits had biomarker levels statistically indistinguishable from those in control subjects (Table 2). Levels of phosphorylated tau and Aβ(1-40), and Aβ(1-42), markers that reflect molecular changes in Alzheimer disease, were not significantly altered in boxers after a bout compared with after rest or levels detected in the nonathletic control subjects. No significant differences in CSF albumin concentrations were detected, indicating that amateur boxing does not significantly impair the blood-brain barrier function (data not shown).

COMMENT

The current study contributes new information about brain injury risks in amateur boxing. Data suggest that participation in an amateur boxing bout is directly associated with neuronal and astroglial damage, as re-
flected by the increase in NFL, T-tau, and GFAP concentrations in CSF. The findings that the increase in these CSF biomarkers is most pronounced in boxers who receive many hits or high-impact hits to the head and that the CSF levels show normalization after 3 months of rest from boxing indicate that the changes are directly related to brain trauma inflicted by hits to the head. The high correlation between biomarker changes in individual boxers supports this interpretation.

The most pronounced change was a marked increase in CSF NFL after a bout that also correlated with the severity of received hits, while a similar but less pronounced increase was found for CSF T-tau. Both NFL and tau are important constituents of neuronal axons. The CSF levels of these proteins increase in disorders with neuronal and axonal degeneration and damage, and the increase is known to correlate with the size of the brain lesion. When applied to the results of this study, the increases in NFL and T-tau probably reflect damage to neuronal axons from hits to the head during a bout. An increase after a bout was also found for CSF GFAP, which is an intermediate filament protein mainly expressed in astrocytes, for which it constitutes a selective marker. This finding suggests that there is also astroglial damage caused by amateur boxing. Similarly, in acute brain trauma, a marked increase in serum GFAP concentration was recently found. This increase also correlated with clinical outcome.

A large body of evidence supports the belief that professional boxers who have been exposed to repetitive head trauma are at increased risk for developing Alzheimer-like pathologic findings, with hyperphosphorylation of tau and formation of tangles and deposition of Aβ into plaques. We, therefore, tested whether amateur boxing results in any changes in CSF biomarkers reflecting these pathogenic processes, that is, elevated phosphorylated tau concentration and decreased Aβ(1-42) concentration and the ratio of Aβ(1-42)/Aβ(1-40). No significant changes were detected; hence, our data provide no evidence for any acute disturbances in these systems in amateur boxers.

In conclusion, our study results suggest that amateur boxing impairs axonal and astroglial integrity. The molecular changes detected are likely to be even more pronounced in professional boxers and in boxers who have received a knockout punch.

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Critical revision of the manuscript for important intellectual content: Zetterberg, Hietala, Jonsson, Karlsson, Popa, Rasulzada, Mehta, Rosengren, Blennow, and Wallin. Statistical analysis: Zetterberg and Hietala. Administrative, technical, or material support: Zetterberg, Styrud, Popa, Rasulzada, Wahlund, Mehta, Rosengren, Blennow, and Wallin. Study supervision: Andreasen, Rosengren, Blennow, and Wallin.

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