DIAGNOSIS AND MONITORING OF SPORT-RELATED CONCUSSION

A STUDY IN AMATEUR BOXERS

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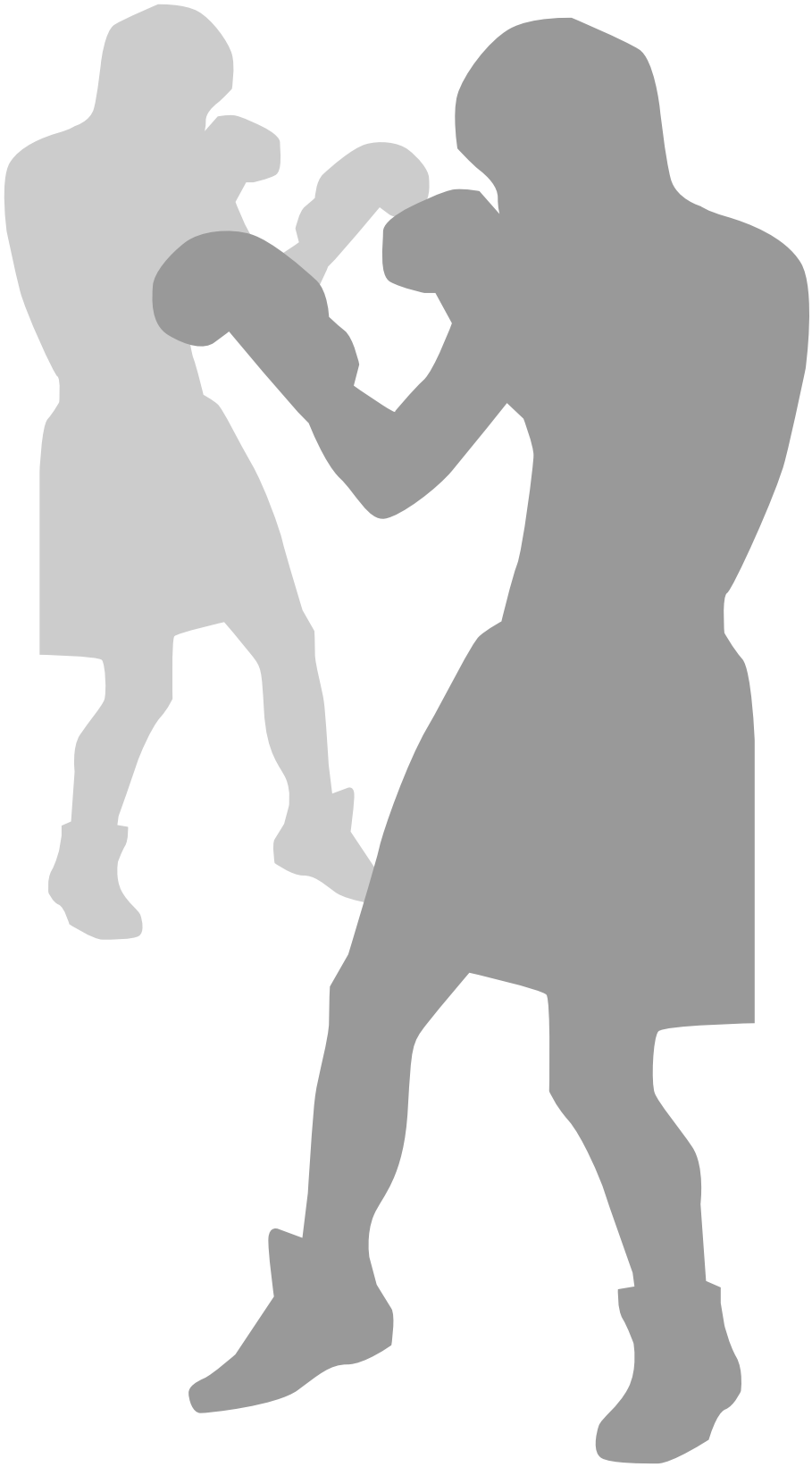
Cover: Sanna Neselius, 2001. Sanna was ranked as one of the best female boxers in the world, both at amateur and professional level.

For my two glittering stars, **Ingrid** **Lovisa** and You make my days *sparkle*



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ABSTRACT

Background: Concussions are one of the most common sport-related injuries and during recent years their consequence has been frequently debated. The aims of this thesis were to find possible methods, which may help clinicians to diagnose and monitor mild traumatic brain injury (TBI), analyse the *APOE*ε4 allele genotype that has been associated with poor outcome after TBI and evaluate the relationship between neuropsychological assessment and brain injury biomarkers in the cerebrospinal fluid (CSF).
Methods: In paper I-IV, 30 amateur boxers and 25 non-boxing matched controls were included. All study subjects underwent medical and neurological examination, neuropsychological evaluation and ApoE genotyping. Brain injury biomarkers were analysed in CSF and plasma/serum 1-6 days after a bout and after a rest period for at least 14 days. The controls were tested once. Paper V presents a knocked out boxer where CSF brain injury biomarkers were analysed at five time points until normalization.
Results: The CSF concentrations of neurofilament light (NFL), phosphorylated NFH (pNFH), glial fibrillary acidic protein (GFAP), Total-tau and S100B as well as tau in plasma were significantly increased 1-6 days after bout compared to controls. NFL, pNFH and GFAP remained elevated after the rest period. Possession of APOEε4 allele did not influence biomarker concentrations. The neurological assessment showed no significant differences between boxers and controls, however boxers with elevated CSF NFL by follow up performed significantly poorer on the Trailmaking A and Simple Reaction Time tests. The boxer in paper V showed marked elevation of CSF NFL, with a peak at 2 weeks post trauma, not reaching below the reference limit until week 36.

Conclusion: The subconcussive trauma in amateur boxing causes axonal and glial brain injury shown by elevated concentrations of brain injury biomarkers in CSF and plasma. CSF NFL was especially interesting since it correlated with the amount of head trauma and seemed to normalize after full recovery. The neuropsychological assessment seemed not to be as sensitive in the evaluation of a concussion. ApoE genotype was not found to influence CSF biomarker concentrations. Paper V showed that recovery from concussion, although in absence of symptoms, could take more than 4 months. The conclusion of this thesis is that NFL and other CSF biomarkers may be valuable in the management of injured athletes and in return-to-play decisions following concussion.

Keywords: concussion, head injury, boxing, traumatic brain injury (TBI), mild traumatic brain injury.

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DIAGNOS OCH UTVÄRDERING AV IDROTTSRELATERAD HJÄRNSKAKNING

EN STUDIE PÅ AMATÖRBOXARE

Bakgrund: Hjärnskakning är en av de vanligaste idrottsrelaterade skadorna och antalet som skadas ökar för varje år. De senaste åren har det intensivt diskuterats vilka effekter en hjärnskakning har på sikt och vilka riskerna är om idrottarna utsätts för nya hjärnskakningar innan nervcellerna har hunnit återhämta sig. Syftet med avhandlingen var: 1. Att undersöka om hjärnskakning kan diagnostiseras och utvärderas med hjälp av analyser av biomarkörer i blod och cerebrospinalvätska (CSF) 2. Undersöka om *APOE*ε4-allel genotyp, som associerats med försämrat utfall efter en traumatisk hjärnskada, påverkar förloppet efter en idrottsrelaterad hjärnskakning. 3. Analysera sambandet mellan en neuropsykologisk undersökning och analys av hjärnskademarkörer i blod och cerebrospinalvätska. **Metod:** I delarbete I-IV inkluderades 30 amatörboxare på elitnivå och 25 åldersmatchade kontroller. Samtliga studieobjekt genomgick en medicinsk samt neurologisk undersökning, neuropsykologisk utvärdering och ApoE genotypning. Hjärnskademarkörer i CSF och blod analyserades 1-6 dagar efter en match samt efter en viloperiod på minst 14 dagar. Kontrollerna testades endast en gång. I delarbete V rapporteras om en amatörboxare som förlorat sin match p.g.a. en knockout och där CSF analyserats via lumbalpunktion vid fem skilda tillfällen, under totalt 36 veckor efter traumat. **Resultat:** Neurofilament light (NFL), fosforylerad NFH (pNFH), glial fibrillary acidic protein (GFAP), total-tau, S100B i CSF samt plasma-tau ökade hos boxarna 1-6 dagar efter match jämfört med kontroller. NFL, pNFH och GFAP koncentrationerna i CSF var fortsatt förhöjda efter viloperioden. Boxerna som bar på *APOE*ε4-allelen hade inte mer påverkade biomarkör-koncentrationer än icke-bärare. Den neuropsykologiska undersökningen visade inga signifikanta skillnader mellan boxare och kontroller, men boxarna som hade förhöjda NFL koncentrationer i CSF vid uppföljningen (som tecken på större skada) presterade sämre på Trailmaking A och Simple Reaction Time testerna. I fallstudien visade NFL den tydligaste förändringen med kraftig förhöjda koncentrationer jämfört med normalvärdet 2 veckor efter skadan. Därefter sjönk nivåerna gradvis men hade inte normaliserats förrän vecka 36.

Slutsats: Fynden i denna avhandling tyder på att det repetitiva traumat i boxning orsakar en axonal och glial hjärnskada, även utan medvetslöshet eller symtom på hjärnskakning, som kan visas med analys av hjärnskademarkörer i CSF och plasma. CSF NFL var särskilt intressant, då den korrelerade med mängden av våld mot huvudet och verkade normaliseras när skadan hade läkt. De neuropsykologiska resultaten tyder på att neuropsykologisk undersökning inte är lika användbar i utvärderingen av hjärnskakning. *ApoE* genotyp kunde inte visas påverka vare sig förlopp eller läkning av en idrottsrelaterad hjärnskakning. Fallstudien i delarbete V visar att läkningen av en hjärnskakning, trots avsaknad av symtom, kan ta mer än 4 månader. Avhandlingens slutsats är att analyser av NFL och andra CSF hjärnskademarkörer kan vara värdefulla i den medicinska handläggningen av en hjärnskakning. Detta är särskilt betydelsefullt för idrottare, där det är viktigt att kunna bedöma när hjärnskadan har läkt så att idrottaren fortast möjligt kan återgå till idrotten, utan att riskera sin hälsa.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

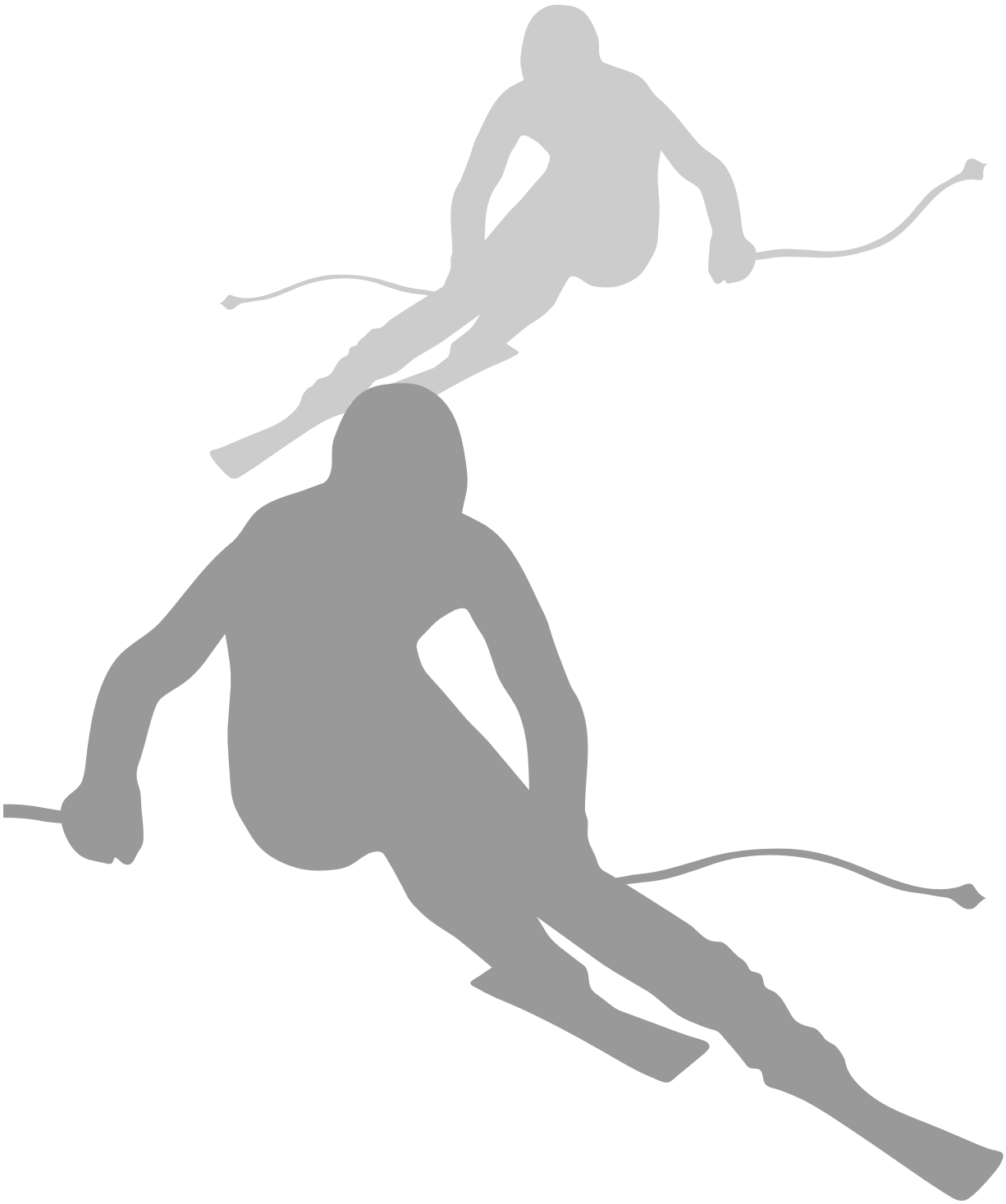
I. CSF biomarkers in Olympic boxing: diagnosis and effects of repetitive head trauma
Sanna Neselius, Helena Brisby, Annette Theodorsson, Kaj Blennow, Henrik Zetterberg H, Jan Marcusson.
PLoS One. 2012;7(4):e33606. Epub 2012 Apr 4.

II. Increased CSF Levels of Phosphorylated Neurofilament Heavy Protein following Bout in Amateur Boxers.
Sanna Neselius, Henrik Zetterberg, Kaj Blennow, Jan Marcusson, Helena Brisby.
PLoS One. 2013 Nov 15;8(11)

III. Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma.
Sanna Neselius, Henrik Zetterberg, Kaj Blennow, Jeffrey Randall, David Wilson, Jan Marcusson, Helena Brisby.
Brain Inj. 2013;27(4):425-33. Epub 2013 Mar 8.

IV. Neurological assessment and its relationship to CSF biomarkers in amateur boxer
Sanna Neselius, Helena Brisby, Jan Marcusson, Henrik Zetterberg, Kaj Blennow, Thomas Karlsson.
Submitted

V. Case report: Monitoring concussion in a knocked-out boxer by CSF biomarkers
Sanna Neselius, Helena Brisby, Fredrik Granholm, Henrik Zetterberg, Kaj Blennow.
Submitted



CONTENT

ABBREVIATIONS

AD	Alzheimer´s disease
ALS	Amyotrophic lateral sclerosis
ANAM	Automated Neuropsychological Assessment Metrics
Apo	Apolipoprotein
APP	Amyloid precursor protein
ATLS	Acute Trauma Life Support
BBB	Blood-brain-barrier
BDNF	Brain Derived Neurotrophic Factor
CNS	Central nervous system
COWAT	Controlled Oral Word Association Test
CSF	Cerebrospinal fluid
CT	Computed tomography
CTBI	Chronic traumatic brain injury
CTE	Chronic traumatic encephalopathy
DAI	Diffuse axonal injury
DTI	Diffusion tensor imaging
FIFA	Federation Internationale de Football Association
GCS	Glasgow coma scale
GFAP	Glial Fibrillary Acidic Protein
H-FABP	Heart type-Fatty Acid Binding Protein
H.H.F	Hellenic Hockey Federation
ICC	Interclass correlation score
ImPACT	Immediate Post-Concussion Assessment and Cognitive Testing

IOC	International Olympic Commission
IRB	International Rugby Board
KO	Knockout
LP	Lumbar puncture
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NFH	Neurofilament heavy
NFL	Neurofilament light
NFP	Neurofilament medium
NFT	Neurofibrillary tangles
NMO	Neuromyelitis optica
P-tau	Phosphorylated tau
pNFH	Phosphorylated neurofilament heavy
RLS 85	Reaction level scale 85
ROCF	Rey Osterrieth Complex Figure
RSC-H	Referee Stops Contest – Head
SCAT	Sport Concussion Assessment Tool
SWI	Susceptibility Weighted Imaging
T-tau	Total-tau
TBI	Traumatic brain injury
WAIS-R	Wechsler Adult Intelligence Scale – Revised

01 INTRODUCTION

1.1 EPIDEMIOLOGY

Traumatic brain injuries (TBI) have been reported as a serious concern in many sports and the incidence of sport-related TBI has more than doubled over the last 10 years in USA. In 2011, the incidence was 46 per 100,000 people in contrast to 1998 when it was 20/100,000 (123% increase) [1]. In this US study, 10% of the TBIs were intracranial haematomas or skull fractures, the rest were defined as unspecified/concussions. In Sweden, 62,300 (8.8 %) people attended the emergency departments due to a non-fatal head injury in year 2008 [2].

About 26% of all head injuries are sports related [3] and one fourth of these are caused by bicycling and football [4]. In a recently published study by Steenstrup et al [5], 245 FIS World Cup skiers were followed for 7 years. This revealed two fatal outcomes after head injury. The head injury risk was highest in freestyle skiing with injury incidence of 1.8% per 1000 runs. In boxing, acute TBI can be caused by knock out (KO) with loss of consciousness or by the cumulative effect of translational and rotational punches to the head [6]. The KO frequency in amateur boxing is less than 1% [7,8], in contrast to professional boxing, where about 24 % of all fights ends with knockout [9].

1.2 ANATOMY

The central nervous system (CNS) consists of the human brain and the brain stem (fig. 1). The human brain weighs about 1500 g and consists of nerve cells (sensory-, motor- and interneurons) and glial cells (for example astrocytes, ependymal cells, oligodendrocytes and Schwann cells). The nerve cells (neurons) are composed of the cell body, several dendrites and one axon. The function of the neurons is to process and transmit nerve signals. The function of glial cells is to protect and support the nerve cells, produce myelin and to supply nerve cells with nutrients and oxygen.

The CNS has two kinds of tissue: The outer grey matter and the inner white matter. The grey matter contains the cell bodies, dendrites and axon terminals of neurons. The white matter contains the axonal part of neurons and connects the different parts of grey matter to each other.

The skull and the 3 meninges Dura mater, Arachnoid mater and Pia mater protect the brain (fig. 1). The subarachnoid space lies between the Dura and Pia mater and contains cerebrospinal fluid which protects the brain against blows and functions in the cerebral auto regulation of cerebral blood flow [10].

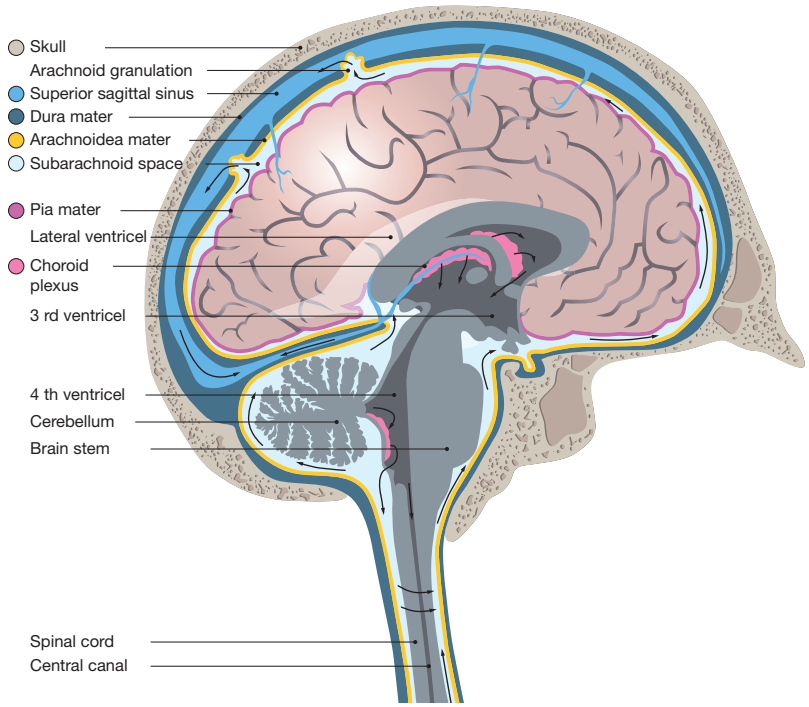


Figure 1. The human brain. *The choroid plexus of the brain produces the cerebrospinal fluid. The cerebrospinal fluid can exit to the blood stream via the arachnoid granulations. © Sanna Neselius*

1.3 ETIOLOGY

A traumatic brain injury can occur as a consequence of an external impact upon the head, by forces that cause sudden linear/rotational acceleration-deceleration within the scull or by a combination of both. Brain injuries can be of different severities, such as subdural haematoma (most severe), intracerebral haemorrhage [11] or concussion (least severe). Repetitive traumatic brain injury may eventually lead to chronic traumatic encephalopathy [12,13].

1.4 RISK FACTORS

Known risk factors for TBI are as follows [14]:

- Female gender
- Young age (12-18 years)
- History of previous concussions
- Pre-existing chronic disease (diabetes, cardiovascular disease, neurological disorders)

Although females have a higher risk for TBI, males have a higher incidence of severe TBI [14].

1.5 PATHOPHYSIOLOGY

Little is known about the pathophysiology and neurobiological changes after TBI, however it is known that it is caused by direct or indirect impacts causing translational or rotational acceleration of the head, leading to microscopic axonal injury and glial damage [15,16]. The brain injury is caused by tension on brain tissue that disturbs the cerebral physiology. A brain injury that is not fully recovered makes the brain more vulnerable for additional TBIs [17,18,19,20]. A young brain seems to be more vulnerable and needs a longer time for recovery [21]. The knowledge about the late effects of multiple TBIs is still limited, even though studies have suggested an association between repeated sport-related TBI and Chronic Traumatic Encephalopathy (CTE) [22,23,24].

1.6 PROGNOSIS

According to the 4th International Consensus Statement on Concussion in Sport 2012, organized by the IOC (International Olympic Commission), FIFA (Federation Internationale de Football Association), IRB (International Rugby Board) and H.H.F (Hellenic Hockey Federation), a concussion causes a neurological dysfunction with spontaneous recovery within 7-10 days [25], although it may take a longer time for the concussion to resolve in children [21]. Prolonged (> 7 days) recovery is a sign of more severe injury [26].

1.7 TREATMENT

The best way to treat a concussion for faster healing is unclear. Athletes are currently recommended to follow the "Return to play protocol", which starts with brain rest [27]. Further return to sport should follow a stepwise increase in activity (table 1). Athletes with an uncomplicated concussion and without any concussion symptoms can return to their sport within a week. Since no clinical tools currently exist for objectively diagnosing and quantifying the extent of brain damage after a mild TBI/concussion it is impossible to interpret when the injury has healed. The risk if returning too early, before the brain injury has recovered, is increased susceptibility to additional concussions in the short-term, and increased risk for developing a chronic traumatic encephalopathy in the long-term.

	Rehabilitation stage	Functional exercise at each stage of rehabilitation	Objective of each stage
1.	No activity	Symptom limited physical and cognitive rest	Recovery
2.	Light aerobic exercise	Walking, swimming or cycling keeping intensity < 70 % of max. permitted heart rate. No resistance training	Increase HR
3.	Sport-specific exercise	Skating drills in ice hockey, running drills in soccer. No head impact activities	Add movement
4.	Non-contact training drills	Progression to more complex training skills e.g. passing drills in football and ice hockey	Exercise, koordination and cognitive load
5.	Full contact practice	Following medical clearance participate in normal training activites	Restore confidence and assess functional skills by coaching staff
6.	Return to play/sport	Normal training and competing	

Table 1. Graduated return to play protocol
Stepwise post concussion program. The athlete should proceed to next level when asymptomatic at current level for 24 hours. If any concussion symptoms occur while the athlete is in the stepwise program, the athlete should return to previous level for at least 24 hours, before proceeding again [25].

1.8 INJURY PREVENTION

It is known that there is a huge problem with underreporting of sport concussions [19,28], which is why it is important to increase the knowledge about concussion among athletes, parents, trainers, medical staff and sport federations. Regular education and information is important as it increases the reporting frequency and reduces the risk of athletes returning to sport with persistent concussion symptoms [28,29]. Ensuring that athletes have time to recover may prevent complications in the form of severe TBI.

02 ACUTE TRAUMATIC BRAIN INJURIES

Acute TBIs can be severe, moderate or mild (concussions) and are presented in this section as:

- Epidural haematoma
- Subdural haematoma
- Subarachnoid haemorrhage
- Cerebral Contusion
- Second impact syndrome
- Diffuse axonal injury
- Concussion

2.1 EPIDURAL HAEMATOMA

An epidural haematoma usually results from a skull fracture caused by a direct blow that damages a meningeal artery, fig 2. Classically, there is no significant parenchymal injury in epidural haematoma. Epidural bleeding is most common in sports where the athletes do not wear a helmet.

2.2 SUBDURAL HAEMATOMA

A subdural haematoma is a bleeding between Dura Mater and Arachnoidea Mater and is the most common sports related intracranial bleeding, fig. 2. It accounts for 3-4% of all sport-related TBI [14] and is caused by a traumatic laceration to the brain or from linear/rotational acceleration–deceleration injury to the head. A subdural haematoma is often associated with some underlying parenchymal (brain) injury, including *diffuse axonal injury.*

Mortality risk is about 10% [31]. During a 30-year period from 1980 to 2009, 231 young athletes training 22 different sports, the majority of which were football players (138), died of subdural hematoma in the United States. Of these fatal traumatic brain injuries in football players, 12 % had a reported history of concussion with persistent symptoms within 4 weeks of death [32].

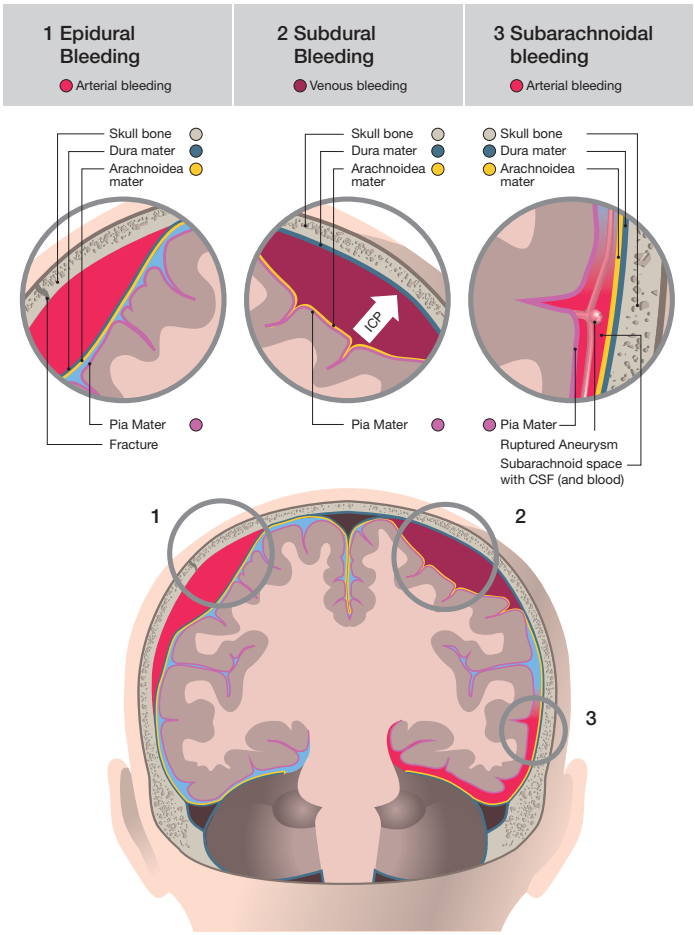


Figure 2. *Intracerebral bleedings. The subdural haematoma accounts for the majority of the sports related intracerebral bleedings in boxers with 40 % risk of poor outcome [30]. © Sanna Neselius*

2.3 SUBARACHNOID HAEMORRHAGE

Subarachnoid haemorrhage is a bleeding in the subarachnoid space, where the CSF circulates and the blood vessels run (fig.2). The subarachnoid space lies between the two meninges Arachnoid and Pia Mater. A haemorrhage is caused by a ruptured cerebral aneurysm that occurs either spontaneously or as a result of TBI [33]. It is difficult to find epidemiological studies about the incidence of sport-related subarachnoid haemorrhage in athletes but one study in young athletes suggested that sport-related subarachnoid haemorrhage accounted for 4% of all intracerebral haemorrhages [31].

2.4 CEREBRAL CONTUSION

Contusio cerebri is a bruise of the brain. It can be associated with multiple micro haemorrhages where small blood vessels leak within the brain tissue. The contusion is caused either by direct blow to the head or by acceleration/deceleration forces. Initial computed tomography (CT) scan can be normal, however the contusion often progresses within 24 – 48 hours and can thereby result in oedema with life-threatening rise in intracranial pressure. Cerebral contusion is often associated with other traumatic brain injuries and occurs in 20–30% of severe TBI.

2.5 SECOND IMPACT SYNDROME

Second impact syndrome is described as a rare, often fatal, traumatic brain injury with unclear pathophysiology that occurs when a repeat injury is sustained before symptoms of a previous head injury have resolved [34]. The incidence is unknown, since the literature only presents case-reports. Due to lack of evidence, its existence is also questioned by experts [35], who instead suggest this should be called a condition of cerebral swelling.

Weinstein et al present a ”second impact syndrome” case-report of a 17-year-old football player [36]. This athlete suffered from a TBI without unconsciousness but did not initially recognise the trauma as a concussion. He sought medical attention 3 days post trauma due to persistent headache, but his CT and medical examination were normal. The athlete was recommended to rest until symptom-clearance but returned to sport 5 days post trauma where he was hit during a drill exercise. He went down on his knees, reported dizziness and headache and was unable to feel his legs. Subsequently the athlete became unresponsive. He developed bilateral subdural haemorrhages, cerebral swelling, midline shift and elevated intra-cranial pressure. Three years post-injury, the patient had regained only limited verbal, motor and cognitive skills. The authors suggest that second impact syndrome results in cerebral blood flow dysautoregulation with massive hyperaemia and high risk of fatal hyperaemic herniation of the brain [36].

2.6 DIFFUSE AXONAL INJURY (DAI)

Diffuse axonal injury is a traumatically induced axonal injury and therefore occurs in the white matter. DAI is caused by blows leading to rapid rotational acceleration/deceleration of the head leading to axonal stretching, something that axons are poorly prepared to withstand [37].

The immediate loss of consciousness, for example due to a knock out in boxing, appears to be caused by rapid rotational acceleration with damage to axons in a specific region of the brain - the brainstem. This unconsciousness seems to be independent of the overall extent of axonal pathology [38].

The microscopic nature of DAI makes it difficult to diagnose the extent of axonal injury. There are usually no findings on routine imaging, such as CT, which is why DAI injury can be missed with classical investigations. It can be suspected with prolonged symptoms after a concussion [37]. Some studies have been able to detect DAI by magnetic resonance imaging as multiple round or ovoid lesions, representing multifocal punctuate foci, haemorrhagic or non-haemorrhagic [13,39]. The location of DAI is correlated with the severity of trauma and graded I-III [40]. According to Park et al, DAI grade I heals within 2 weeks, but recovery after DAI injury grade III takes up to 2 months, as shown by magnetic resonance imaging [40].

Grading of DAI according to Park et al [40]

- Grade I - Mild DAI. Scattered small haemorrhagic lesions on hemispheric white matter.
- Grade II - Moderate DAI. As grade I plus additional focal lesions on the corpus callosum.
- Grade III – Severe DAI. As grade I and II plus additional focal lesions on the brain stem.

2.7 CONCUSSION/MILD TBI

More than 90% of all traumatic brain injuries are concussions [14]. A concussion is caused by a direct or indirect head blow with/without loss of consciousness (fig. 3) [25]. Sport-related concussion, also called mild TBI, is a common injury in many impact sports such as football, ice hockey and boxing. Concussions have received increased attention in recent years in the media, and among medical professionals and sport organisations, since there is growing awareness about the acute and long-term consequences of concussion [1]. The effect of several concussions and subconcussive repetitive TBI has been under discussion, since this is a major issue in many sports. Human studies are limited, but in a mouse study, repetitive subconcussive traumatic brain injury has been demonstrated to be cumulative, leading to astrogliosis and tau phosphorylation and causing spatial learning and memory deficits for up to 6 months [41].

2.7.1 Definition

According to the International Conference on Concussion in Sport 2012, a concussion is defined as a ”complex pathophysiological process affecting the brain, induced by biomechanical forces with or without loss of consciousness, resulting in neurological symptoms” [25]. This is a revision of the 3rd Consensus Statement from 2009, where unconsciousness was considered obligational [27]. The classically used neuro-imaging investigations CT and magnetic reso-

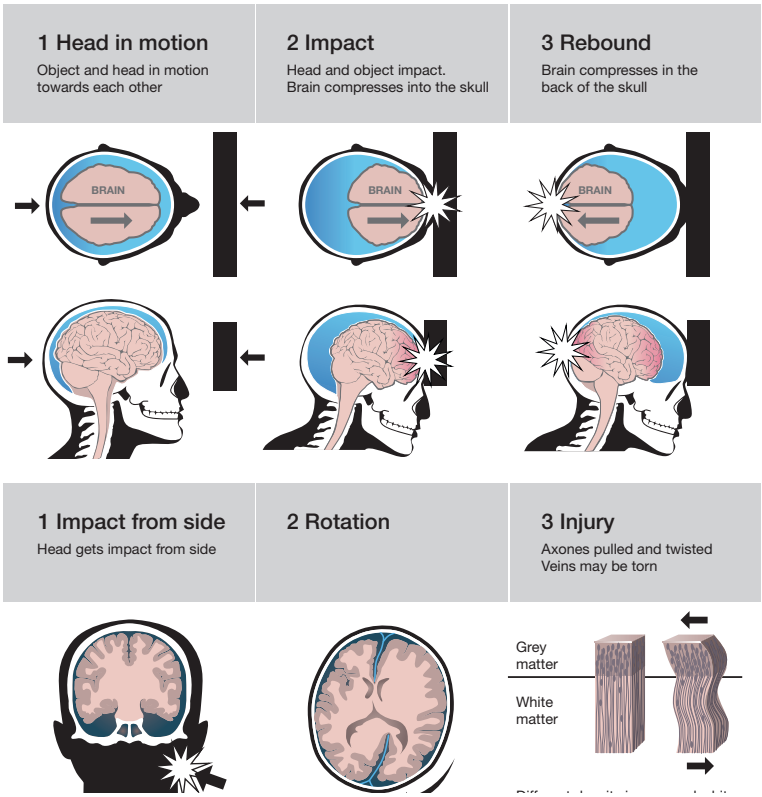


Figure 3. *Different mechanics in head impacts caused by translational vs. rotational acceleration. Rotational acceleration (when impact comes from side, as from a hook in boxing) causes more axonal shearing and tension on brain tissue and vessel than translational acceleration.* © *Sanna Neselius*

nance imaging (MRI) are normal. Spontaneous recovery occurs within 7-10 days [25], although it may take a longer time for the concussion to resolve in children [21]. Prolonged (> 7 days) recovery is a sign of more severe injury [26].
Commotio cerebri, contusio cerebri and mild TBI are used synonymously for concussion in the literature, although the latest "Consensus Statement on Concussion in Sport" prefers the term concussion [25].

2.7.2 Concussion symptoms

Symptoms of concussion can be subtle and injury reporting can also be influenced by factors such as stress, fatigue or unwillingness of the athlete to recognise the symptoms as being concussive in nature (table 2) [19].

ACUTE SYMPTOMS	LATE, AFTER A FEW DAYS
• Headache • Dizziness • "Mental clouding" • Nausea • Balance problems • Blurred vision • Memory problems • Neck pain	• Tiredness • Personality change • Irritability • Nervousness • Anxiety • Sleep disturbances • Sensitivity to light

Table 2. Concussion symptoms
The symptoms can vary but having one/several of the symptoms after TBI increases the suspicion for concussion [42].

2.7.3 Prognosis

According to the latest Consensus Statement, a concussion causes neurological dysfunction with spontaneous recovery within 7-10 days [25], although it may take a longer time for the concussion to resolve in children [21]. Prolonged (> 7 days) recovery is a sign of more severe injury [26].

2.8 GRADING OF TBI

The Glasgow Coma Scale (GCS) is an internationally accepted and used neurological scale to grade the level of consciousness in head injured patients and to grade the severity of brain injury, see fig. 4 [43]. In Sweden, the Reaction Level Scale 85 (RLS 85) in fig. 5, a modified version of the GCS, has been the preferred diagnostic tool at most emergency departments for more than 20 years due to indications of better reliability [44].

2.9 LONG-TERM EFFECTS OF TBI

Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative disease that is suggested to result from repetitive traumatic brain injury [45]. It was first presented by Martland 1928 in boxers when he introduced the term punch-drunk to a series of symptoms caused by the repetitive head trauma in boxing [46]. Even though CTE has been mostly studied in boxers, it has also been observed in football, ice hockey and soccer players [45].

Glasgow Coma Scale

Best Eye Response (E)	
No eye opening	1
Eye opening in response to pain stimulus	2
Eye opening to speech	3
Eyes opening spontaneously	4
Best Verbal Response (V)	
No verbal response	1
Incomprehensible sounds (moaning)	2
Inappropriate words (no conversation)	3
Confused, disoriented	4
Oriented	5
Best Motor Response (M)	
No motor response	1
Extension to painful stimulus	2
Abnormal flexion to painful stimulus	3
Flexion/withdrawal to painful stimulus	4
Localizing painful stimulus	5
Obeys commands	6
Total (E + V + M) of max. 15	

Figure 4. I*n the Glasgow Coma Scale [43] the eye, verbal and motor responses are tested. The sums of these three tests are calculated. The lowest possible GCS value is 3 (deep coma or death), while the highest is 15 (fully awake person). The eye response test consists of 4, the verbal of 5 and the motor response test of 6 grades. GCS 14-15 is calculated as a mild, 9-13 a moderate and 3-8 a severe TBI. GCS should be recorded for all athletes in case of subsequent deterioration. ©Sanna Neselius*

Reaction level scale (RLS85)

	Grade of alertness
1	Fully alert
2	Drowsy or confused, but responds to light stimulation
3	Very drowsy or confused, but responds to strong stimulation
4	Unconscious; localizes painful stimulus but does not ward it off
5	Unconscious; makes withdrawing movements following painful stimulus
6	Unconscious; stereotypic flexion movements following painful stimulus
7	Unconscious; stereotypic extension following painful stimulus
8	Unconcsious; no response to painful stimulus

Figure 5. T*he Reaction level scale is a scale for alertness used in Sweden instead of the Glasgow Coma Scale.*

2.9.1 Pathology

The relation between CTE and Alzheimer´s disease (AD) is debated, although it is shown that TBI is a risk factor for developing AD [47,48]. Neuropathologically, both conditions are characterized by Neurofibrillary Tangles (NFTs) but there are some differences; CTE patients generally have larger NFTs involving the superficial cortical layers II and III similar to Parkinson Disease and Amyotrophic Lateral Sclerosis (ALS), whereas AD patients have NFTs predominantly in layers V-VI. β-Amyloid plaques also characterize AD, but these seem to be absent in CTE [45,49].

CTE pathology according to the Boston Group

- Tau pathology with formation of neurofibrillary tangles (NFTs) in layer II and III of neocortex
- Axonal pathology
- No β-Amyloid or Amyloid Precursor Protein pathology
- Consists of 4 different stages correlating with symptom progress

2.9.2 Prevalence

The incidence and prevalence for concussed athletes to develop CTE is unknown since there are no published epidemiological, cross-sectional or prospective studies relating to CTE [50].

2.9.3 CTE symptoms

- Memory disturbances
- Behavioural and personality changes
- Parkinsonism
- Slower speech
- Gait abnormalities

2.9.4 Diagnosis

Today, CTE can only be diagnosed with certainty at autopsy, making it difficult to distinguish CTE from other neurodegenerative disorders with similar symptomatology such as AD, other dementias, ALS and Parkinsonism [49].

2.10 APOE GENOTYPE

The APOE gene has three common alleles (ε2, ε3 and ε4) and is located on chromosome 19. The presence of APOEε4 is a well-known risk factor for AD [51]. The role of the APOE gene in TBI is not fully understood, but it is asso-

ciated with unfavourable outcome after acute TBI [52] as well as chronic traumatic encephalopathy [53]. Since TBI is also a risk factor for AD [47,54,55], the presence of APOEε4 in combination with TBI is suggested to additionally increase the risk of developing AD [54,56].

2.11 MANAGEMENT OF SPORT CONCUSSION

2.11.1 On-field evaluation

The on-field physician makes the first evaluation according to the Acute Trauma Life Support (ATLS) principles or other emergency management guidelines [25]. The Consensus Statement 2012 also recommends assessment with the Sport Concussion Assessment Tool – 3rd edition (SCAT3) or Child SCAT3 for children under 13 years. The athlete is not allowed to return to play on the day of injury and if the physician decides that transfer to the nearest emergency department is not necessary, it is important not to leave the athlete alone, but to make serial monitoring for deterioation for 24 hours. If no physician is available, the athlete should be transferred to the emergency department for evaluation [25].

2.11.2 Evaluation at emergency department

The concussion diagnosis is established by symptoms and clinical evaluation including a detailed neurological examination with balance testing and cognitive function investigation [57,58]. After the first evaluation and according to Scandinavian concussion guidelines, admission for observation for 24 hours and/or discharging after a normal computer tomography (CT) scan of the brain should follow [59].

2.11.3 Return-to-play guidelines

At the 4th International Conference on Concussion in Sport 2012, athletes that no longer suffered from any concussion symptoms were recommended a stepwise return to sport following the "Return-to-play" protocol (table 1) [25].

03 DIAGNOSIS OF CONCUSSION

There are many investigations that can assist the physician in the diagnosis of TBI, although none of them has been shown to be sensitive enough for the diagnosis and monitoring of a concussion. According to the Scandinavian concussion guidelines, symptom & cognitive evaluation, medical assessment according to ATLS, neurological testing and classical neuroimaging investigations such as CT, are the standard tools for managing concussion at emergency departments and normally without pathology [59,60].

3.1 NEUROLOGICAL INVESTIGATION

Balance and coordination tests, such as Single Leg Stand Test (test of balance), Finger to Nose Test (test of coordination) and Gait tests (test of lower limb dynamic balance), are used to determine neurological function after brain injury [61,62,63]. However, a study published in 2010 on normative data showed a wide variability using the Single Leg Stand test. Only the Finger to Nose test and Tandem Gait test were found to be reliable and should be recommended when testing balance and neurological function after concussion [63].

3.2 NEUROPSYCHOLOGICAL ASSESSMENT

Neuropsychological evaluation has been considered as the most sensitive tool in the evaluation of concussion pathology, since numerous studies have shown that neuropsychological tests are sensitive in detecting the early cognitive impairment after concussion, up to 10 days post trauma [64,65,66]. Neuropsychological tests are also used after sport-related concussions as a tool in return to sport considerations [25]. It has been shown that the effects on particularly memory, processing speed and executive functions seem to correlate with size of injury and effects can persist for up to 3 months [67,68]. However, a limitation with neuropsychological evaluation is the need for baseline testing which restricts the usefulness as a diagnosis and monitoring tool of concussions in the emergency department. The question about the sensitivity of the tests in detecting small axonal injury remains, since these tests have not been able to show any pathology caused by the repetitive subconcussive trauma in amateur boxing [69,70,71].

3.2.1 Neuropsychological tests for TBI

There are several different tests for neuropsychological evaluation, both traditional "paper and pencil" and computerized evaluations, where the computerized tests have gained popularity in recent years as they are relatively cheap, fast and easy to administrate. To our knowledge, there is no scientific evidence that any one of the traditional, computerized or the hybrid neurocognitive evaluations are superior, although only a few studies are made using hybrid test batteries [72].

Computerized neurocognitive tests

Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT)
ImPACT is composed of several memory and mental speed tests with 89% sensitivity and 70% specificity for concussion [73] but with marginal reliability (interclass correlation score (ICC) of 0.49-0.89, average 0.62) [74,75].

Automated Neuropsychological Assessment Metrics (ANAM)
Similar to ImPACT, ANAM is composed of several memory and mental speed tests with marginal reliability (ICC 0.59-0.79, average 0.61) [75].

Traditional neurocognitive tests

Memory tests used to diagnose TBI

- **Rey Osterrieth Complex Figure (ROCF)** evaluates episodic memory and visuospatial skills [70] in TBI, Alzheimer´s disease and other neurocognitive disorders. It has been shown that AD patients have dysfunctions both with copying and recall, whereas TBI patients only suffer from dysfunctions in recall [76]. Episodic memory after severe TBI has been shown to be impaired [77] initially but no long-term consequences have been seen after mild to moderate traumatic brain injury [78]. The copy and recall are both affected by age and IQ [79].

- **Controlled Oral Word Association Test (COWAT)** is a part of the Multilingual Aphasia Examination and provides a measure of word generation and verbal fluency [80,81]. Declining word generation has been observed after mild TBI [82] and mild cognitive impairment [83], although the reliability is questionable since there are also studies reporting that COWAT cannot discriminate between sport-related concussion and control subjects [84].

- **Listening Span and Digit Span** addresses short-term auditory working memory and attention skills. Digit Span is part of the Wechsler Adult Intelligence Scale – Revised (WAIS-R) and is used to evaluate less effortful attention skills [85], whereas Listening Span is used in attention tasks and evaluation of complex, executive aspects of working memory related to short-term memory capacity. This function is essential for important cognitive abilities including reasoning, comprehension and problem solving [86,87]. It is impaired in preclinical stages of AD [88] but seems not to be affected by sport-related concussions [89]. In its entirety, the WAIS is designed to measure intelligence and is available in a revised form, WAIS-R [85] (available at the onset of the studies in this thesis) and the recently published WAIS-IV. WAIS-R or subtests are normally included in neurocognitive assessment to estimate general intelligence and education level, since they can interfere with neuropsychological evaluation results [79,90,91].

Tests of processing speed and executive functions

- **The Trailmaking test** consists of two parts, A and B, and assesses processing speed, attention and executive functioning [92]. There is evidence that especially the B part can detect brain damage and predict long-term outcomes after traumatic brain injury [93,94,95] with high specificity (90.6 %) although low sensitivity (19%). The sensitivity and specificity for test A are 40.6% and 84.4% respectively [91].

- **The Reaction time task** reflects impairments in information processing and failure to maintain executive control. Reaction time declines by increasing age [96], but regular physical activity can slow down or prevent functional decline associated with ageing [97]. Impairment of reaction time after single and multiple concussions has been demonstrated [98,99].

- **The Finger tapping task** is impaired after a mild TBI [100,101] and reduced performance in finger tapping by boxers compared to controls has been shown [102].

3.3 RADIOLOGICAL INVESTIGATIONS

Conventional computed tomography (CT) and magnetic resonance imaging (MRI) are not sensitive enough to diagnose DAI injuries or small microscopic changes after sport-related concussion. Advanced MRI techniques are diffusion tension imaging, magnetic resonance spectroscopy (MRS) and functional MRI such as Susceptibility Weighted Imaging.

3.3.1 Susceptibility Weighted Imaging (SWI)

SWI is a new technique using full-velocity-compensated high-resolution 3D gradient-echo sequence to evaluate diffuse axonal injury [103]. DAI is often associated with punctuate haemorrhages in the deep subcortical white matter, which are not routinely seen on computer tomography or magnetic resonance imaging sequences [103]. SWI has been shown to detect intracranial bleedings in concussed patients with GCS 13-15 despite normal CT [104].

3.3.2 Proton Magnetic Spectroscopy

Proton magnetic resonance spectroscopy has been able to evaluate metabolic alterations after a concussion by determining the brain energy-state marker N-acetylaspartate in concussed athletes. Even though the athletes reported symptom-clearance after 3-15 days, the metabolic brain alterations remained up to 30 days post injury, indicating persistent metabolic vulnerability of the brain despite the athlete declaring clinical recovery [105].

3.3.3 Diffusion Tensor Imaging (DTI)

Diffusion Tensor Imaging (DTI) detects axonal injury and has shown pathology in the right posterior limb of the internal capsule, the right corona radiata and the right temporal lobe of the brain in ice hockey players after concussion [106].

DIAGNOSIS OF CONCUSSION

- BIOMARKERS IN CSF AND BLOOD

04

The cerebrospinal fluid is a promising source of biomarkers in TBI, since the CSF compartment is a relatively closed system where biochemical changes within the brain are reflected. The CSF is produced by the choroid plexus at a rate of 20 ml/hour and the CSF compartment contains about 150 ml CSF [10]. The CSF is renewed in young adults about 4 to 5 times daily, by absorption and secretion into the blood [10]. Today, lumbar puncture is used routinely to collect CSF for the diagnosis of a variety of diseases, such as Alzheimer´s Dementia, other neurodegenerative disorders and infections (e.g borrelia burgdorferi).
The CSF compartment is protected by the blood-brain-barrier (BBB) – a permeable barrier that separates the blood from the central nervous system. The BBB allows transport of water, gases and some lipid soluble molecules and amino acids [107]. A disrupted BBB can cause leakage of biomarkers into the peripheral blood.
Several interesting biomarkers have been observed in the CSF after TBI and some of them have also been found in the peripheral blood (table 3). Peripheral blood is easily accessible and optimal for the clinical setting, but assays for TBI markers have been hampered by a lack of analytical sensitivity for accurate measurement in blood samples. Table 3 lists biomarkers that are particularly interesting for the diagnosis and monitoring of TBI.

4.1 MARKERS OF NEURONAL INJURY

4.1.1 Neurofilament

Neurofilaments are 8–10 nm heteropolymers with three major subunits: Neurofilament light chain (NFL), neurofilament medium chain (NFP) and neurofilament heavy chain (NFH). They are only found in neurons where their main function is to maintain neuronal shape, size and conduction of nerve impulses along the axons [120].

NFL is expressed predominantly in large-caliber myelinated axons [121], and increased concentrations reflect white matter disease and axonal degeneration. NFH is mainly a phosphorylated protein, pNFH [120]. CSF levels of NFL and pNFH are elevated in axonal disorders such as amyotrophic lateral sclerosis (ALS) [122,123], multiple sclerosis (MS) [110,113] and TBI. Increased NFL

MARKER	FUNCTION AND PATHOLOGY	PERIPHERAL BLOOD* ng/L	CSF* ng/L
NFL	Maintain neuronal shape, size and conduction of nerve impulses along the axons Reflects white matter disease, axonal injury CSF concentrations depend on age [98]	**Controls:** <26.6 [99] **AD** 23 – 42 [99] **ALS** 58 -157 [99] **Guillan-Barré** 24-260 [99]	**Controls:** < 125 ng/L [100], [13] **AD** (1139-1711 ng/L) [99], **ALS** (4151-7323 ng/L) [99], **MS** 1. <125 -1200 ng/L)[100], 2. no significant increase vs controls [101] **Boxing:** 845(125 -1140) ng/L [13] No loss of consciousness
pNFH	Major structural component of motor axons Reflects white matter disease, axonal injury	**ALS** (Boylan et al 2014), Serum: 660 (390-2240), Plasma: 590(220-3140) **Severe TBI children** Serum: (12-1442) [102]	**Controls:** 341(287-429 [103] **ALS** 4380(2760-7410) Boylan et al 2014, **MS** 442(336-548) [103]
T-tau	Axonal microtubule stabilizing protein Marker for axonal injury	**PLASMA:** Controls, 74 years 4.43(SD2.83) [104], AD 8.80(SD10.1) [104], MCI 4.68(4.25) [104] **SERUM: Controls vs concussion**, 32(16-65) years. No significant difference, 86(SD48) vs 188(SD210) [105] **Severe/moderate TBI**: Good outcome 51.6 (SD81.5), Bad outcome 436.2(SD473.6) ng/L [106]	**Controls:** 74 years, 507(SD254) ng/L [104], 30(6.3)years, 325(97.7) ng/L [13] **AD** 828(SD375) [104], **MCI** 550(SD421) [104] **Boxing**: No loss of consciousness - no differences vs controls [13]
pTau	Hyperphosphorylated tau. Abnormal, toxic form of tau. Forms neurofibrillary tangles in neocortex. Marker for axonal injury, causes axonal degeneration leading to dementia.	Not analysis found	**Controls:** 74 years, 73.4(SD20.5) ng/L [104], 30(6.3)years 46.4(14.5) ng/L [13] **AD** 123(SD49.2) [104], **MCI** 78.1(28.8) [104] **Boxing:** Without loss of consciousness-no differences vs controls [13]
GFAP	Presented in large amounts in the intermediate filaments of mature CNS astrocytes (glial cells) Highly specific marker of glial cell injury	**NMO Serum:** No diagnostic value **Severe TBI serum:** Significant elevation vs controls up to 3 days post trauma [107]	**Controls:** 30(6.3)years, 402(88.8) ng/L [13], 43(28-63) years, 358(SD122) ng/L **MS:** 566(SD322) ng/L [101] **Boxing:** Without loss of consciousness, 542(199) p=0.04 [13]
S100β	Reflects glial cell injury (astrocyte damage)	**Normal** < 100 ng/L [108,109] **Sport related concussion:** 99(SD32) ng/L	**Normal:** 250 (SD80) ng/L **AD:** 400(SD200) ng/L

Table 3. Brain injury biomarkers that may aid in the diagnosis and monitoring of concussion

concentrations are also seen after an amateur boxing bout and have been shown to correlate with the size of injury [16], but pNFH has not been analysed after mild TBI or sport concussions. In blood, NFL and pNFH have been detected in ALS in increased concentrations and have further been shown to correlate with CSF concentrations [109,124]. Increased concentrations of pNFH in serum has been observed in children with severe TBI [112].

4.1.2 Heart type– Fatty Acid Binding Proteins

Heart type-Fatty Acid Binding Protein (H-FABP) is one of nine different types of FABP. It is expressed in multiple tissues, mainly in cardiac myocytes, but also in skeletal muscle, kidney, lactating mammary gland, placenta and the brain. The function of FABP is in the transport and storage of lipids and also protection from harmful fatty acids. In a clinical setting, plasma H-FABP is analysed to diagnose myocardial infarction [125]. In the brain, H-FABP is located in the neuronal cell bodies in the grey matter and is released in conjunction with different types of neurodegenerative conditions, such as dementia [126]. Elevated serum H-FABP concentrations have been shown after mild TBI [127], however it is not known whether H-FABP is also elevated in the CSF.

4.1.3 Brain Derived Neurotrophic Factor (BDNF)

BDNF is a nerve growth factor protein expressed in neurons with neuroprotective effects on the brain. It affects long-time memory and the survival of existing neurons, and encourages the growth and differentiation of new neurons [128]. Increased concentrations of BDNF have been found in the CSF of patients with Parkinson's disease [129] and higher serum levels may protect against future occurrence of dementia and AD [130]. The role of BDNF after TBI remains unclear and serum analysis after amateur boxing has not shown any significant differences between boxers and controls [131].

4.1.4 Apoliproteins

Apolipoproteins are expressed in several tissues including the brain and their function is to transport lipids. There are six classes of Apolipoproteins (A, B, C, D, E and H) and several subclasses.

Apolipoprotein A1 (ApoA1)

ApoA1 is a high-density lipoprotein in found in plasma and it may be a marker of neural degeneration. Increased CSF concentrations of ApoA1 have been seen in patients with AD, Parkinson's disease and multiple sclerosis [132],

although in another study plasma levels of ApoA1 were decreased in patients with Parkinson's disease [133]. TBI does not seem to have any effect on CSF ApoA1 concentrations [134].

Apolipoprotein E (ApoE)

ApoE is expressed in the central nervous system and secreted by glial cells and neurons, where it acts as a ligand for neuronal receptors and distributes cholesterol and phospholipids to injured neurons after brain injury [135]. ApoE plays a key role in the development of AD, where it is believed to promote plaque development. Reduced levels of ApoE are seen in AD [136] andafter severe TBI, CSF concentrations of ApoE are shown to decrease compared to controls the first 5 days post trauma [134].. One hypothesis for the decreased levels of ApoE is that it is consumed by neurons as a response to acute injury [134].

The effect on ApoA1 and/or ApoE concentrations in CSF/peripheral blood after concussion is unclear.

4.2 BIOMARKERS OF ASTROGLIAL INJURY

4.2.1 Glial Fibrillary Acidic Protein (GFAP)

Glial fibrillary acidic protein is present in large amounts in the intermediate filaments of the mature CNS astrocytes. The astrocytes, a type of glial cell, are star shaped and located both in the grey and white matter of the brain. The role of GFAP is not yet fully understood, but it is thought to be important in injury damage control and in modulating astrocyte motility and shape by providing structural stability to astrocytic processes [137].

GFAP is a highly specific marker for CNS injury [117]. Following tissue injury (trauma or disease), damaged astrocytes become reactive and respond by upregulating and releasing of GFAP [137]. The GFAP concentrations do not depend on gender, but CSF GFAP concentrations increase by 6.5 (SD5.9) ng/L annually in healthy patients [111]. Moderately elevated levels of CSF GFAP have been demonstrated in diseases such as progressive MS [138] and epilepsy [108]. The highest CSF GFAP concentrations of all are shown in the severe demyelinating, inflammatory disease neuromyelitis optica (NMO)[139], but for some reason, serum analysis of GFAP in NMO is of no diagnostic value [140]. After acute severe TBI elevated CSF GFAP concentrations have been shown in serum up to three days post trauma [117] and elevated CSF GFAP concentrations have also been shown after repetitive subconcussive head trauma received during an amateur boxing bout [16].

4.2.2 S100B

S100B is a calcium binding protein that is glial cell specific within the CNS and is expressed by mainly astrocytes, but also Schwann cells and oligodendrocytes [141]. Outside the brain it is produced to a lesser extent and released from adipocytes, chondrocytes and melanocytes [142].

S100B has five major intracellular functions [141]:

1. Regulation of phosphorylation mediated by protein kinase
2. Modulation of enzymatic activity
3. Maintenance of cell shape and motility
4. Part of signal transduction pathways
5. Promotion of calcium homeostasis

In the CNS, S100B is released after astrocytic damage and elevated concentrations are found in patients with Alzheimer's disease [143]. After TBI, S100B analysis has low specificity (40%), but high sensitivity (99%) for abnormal head CT evaluation [119]. After severe TBI with GCS < 8, CSF concentrations of S100B have been elevated for up to 5 days with a peak on day 1 [134]. Also, in serum S100B has been elevated within 3 hours after a sport related concussion, with normalization at follow up on day 2 post trauma [119].

Serum-S100B has also been studied in amateur boxers after bout with findings indicating both significantly increased [144] and normal concentrations [131]. In the study showing increased S100B serum levels, the samples were collected within 5 minutes after the bout. Boxers that received hits mainly to the head demonstrated higher S100B concentrations in comparison to boxers receiving hits only to the body [144].
Since 2013, analysis of S100B is recommended in Norway for the assessment of concussions at the emergency departments, as an initial diagnostic measure for mild head injury patients with low risk [118].

4.3 BIOMARKERS OF NEUROFIBRILLARY TANGLE AND PLAQUE PATHOLOGY

4.3.1 Tau

Tau is a phosphoprotein primary localized in the axonal compartment of neurons where it regulates microtubule assembly, dynamic behaviour and spatial organization, and the axonal transport of organelles, including mitochondria [145,146]. Phosphorylated tau (P-tau) is a toxic, abnormal condition of tau

that forms neurofibrillary tangles and causes axonal degeneration eventually leading to dementia [147]. P-tau is highly specific for AD with sensitivity and specificity of 80% [148] and is not recognised as a diagnostic biomarker for traumatic brain injuries.
Increased CSF and peripheral blood concentrations of T-tau and P-tau have been seen in neurodegenerative disorders such as Alzheimer's disease and mild cognitive disorder (MCI), although plasma/serum and CSF concentrations do not correlate [114]. Increased concentrations of T-tau have also been found in the CSF after epilepsy [108] and acute TBI [149], where the concentration of T-tau correlates with trauma severity [116]. No significant Total-tau elevation in serum/plasma has been shown after mild TBI/concussion [150,151].

4.3.2 Amyloid Precursor Proteins (APP)

Amyloid Precursor Protein (APP) is an integral membrane protein expressed in neurons. The physiological function for APP and its cleavage products are not fully understood, but the APP-family members among others have following functions [152]:

- Regulation of neurite outgrowth and axon guidance
- Involvement in the binding of metals
- Influence on synaptic function and long term potentiation
- Production of Aβ, a toxic cleavage segment of APP that plays a not fully understood role in the formation of Alzheimer's dementia

APP is initially cleaved by α- and β-secretases to form the Aβ-peptide. The Aβ-peptide is in turn cleaved to isoforms such as Aβ38, Aβ40, Aβ N42, Aβ 1-40 and Aβ1-42, where Aβ40 is the major isoform under normal conditions.

The Aβ-peptide is found specifically in senile plaques, the disease defining depositions found in the brains of of AD patients. [152]. TBI is considered a risk factor for Alzheimer's disease [153]. Low CSF concentrations of the aggregation-prone 42 amino acid isoform of Aβ (Aβ42), has been observed during the development of AD (due to deposition of Aβ42 in plaques) [154] and following TBI [155]. It has been shown that CSF levels of Aβ40 and Aβ1-42 decline the first 5 days after severe TBI (GCS < 8) [134], although it seems that the plasma concentrations remain unchanged [156]. Perhaps TBI induces APP-processing and Aβ formation, which eventually leads to Aβ aggregation.

05 AIMS OF THE STUDY

5.1 PAPER I-III

Sport-related concussions are common in many sports and it is currently difficult to determine when the injury has healed and when the athlete can safely be allowed to return to their sport. Therefore, our aims with papers I-III were:

- To evaluate the effects of subconcussive repetitive head trauma on the brain.
- To find possible brain injury biomarkers in the CSF and/peripheral blood that can assist clinicians in the diagnosis and monitoring of sports-related concussion.
- To analyse if being a carrier of the *APOEε4* allele genotype influenced biomarker concentrations after subconcussive repetitive head trauma.

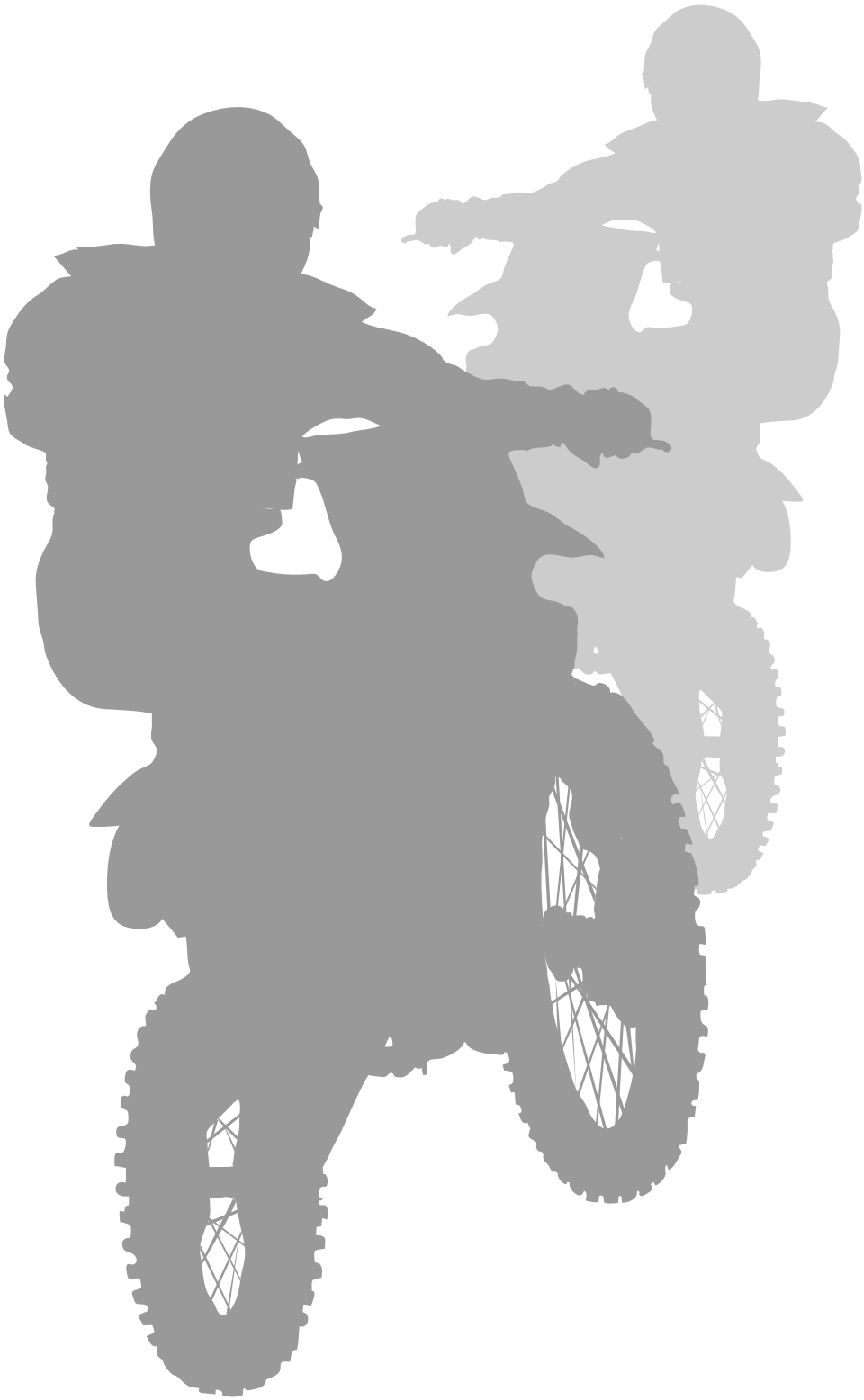
5.2 PAPER IV

The aims of paper IV were:

- To evaluate the sensitivity of neuropsychological assessment in the diagnosis and monitoring of mild TBI.
- To investigate the relationship between neuropsychological assessment and brain injury biomarkers.

5.3 PAPER V

The aim was to evaluate the brain injury biomarker concentrations in the cerebrospinal fluid over time as a measure of on-going axonal injury after a concussion with loss of consciousness.



06 METHODS

6.1 PAPER I-IV

6.1.1 Study Population

The study was designed as a prospective prognostic follow-up study. Thirty amateur boxers competing with a head guard, and at a high national and/or international level, were compared to 25 healthy, age-matched controls. All boxers had completed at least 45 bouts. This number was based on the regulation of the National Boxing Federation demanding an examination with MRI, CT or EEG every 50 bouts. The controls consisted of friends or relatives to the boxers, aiming to get controls with similar social background and education level. Exclusion criteria were athletes at a senior elite level in sports known to have a high incidence of sport-related traumatic brain injuries, for example, soccer, ice hockey and contact sports.

The regional ethical review board at Linkoping Health University, Sweden approved the study. Written informed consent was obtained from all participants.

6.1.2 Questionnaire Design

All participants filled in a questionnaire about medical history, medication, education, social background, and quantification of alcohol and drug intake. Previous sports career including sport-related concussions were recoreded, aiming to identify study participants with a higher risk for previous head injuries. The questionnaire was based on a previous study and included a 10-question survey regarding previous and current symptoms of head and neck injuries [157]. The number of symptoms that had worsened over the last 5–10 years was added up to produce score. The boxers reported information about their boxing career, fighting record, number of knockout (KO) losses, number of Referee Stopping Contest losses due to several hard punches to Head (RSC-H), present weight class, duration of career, age at career start, and age at first bout [157,158].

6.1.3 Grading of head trauma exposure

The boxers were asked to report the total amount of bouts they had participated in during the last week prior to testing (1–3 bouts) and estimated these bouts as easy (1), intermediate (2) or tough (3).

Three boxing experts blind to the CSF biomarker concentrations, who had good knowledge about the boxing career of the included boxers in the study, graded the boxers independently with regards to head trauma exposure during the boxer's total boxing career. When doing this, the experts took into account boxing style, skills of the boxer and the skills of the opponents. A grade from 1 to 5 was used, where 1 referred to a boxer with low head trauma exposure and 5 referred to a boxer with high head trauma exposure during their boxing career.

The total amount of bouts during the last week before test A, the boxers own grading of the bouts, and the mean of the expert grading over their total boxing career were added in a score. This score was named "Boxing Exposure". The aim was to calculate the total impact on the brain prior to testing.

6.1.4 Neurological examination

The medical and neurological assessments were made on all study participants prior to lumbar puncture. The investigations included anamnestic questions about concussion symptoms, a general somatic status (general condition, examination of mouth and throat, heart, blood pressure, abdominal palpation, peripheral circulation and skin status) and a neurological status (orientation, alertness, speech function, cranial nerves I-XII, motor skills, balance, coordination, gait, sensibility testing and testing of reflexes) [19].

6.1.5 Magnetic Resonance Imaging

MRI of the brain was performed in all participants without any structural injuries (haemorrhages, subdural haematomas) or other major findings observed.

6.1.6 CSF and blood sample collection

The lumbar puncture (LP) was performed between 10 a.m. and 3 p.m., with the study participants in a sitting position or lying on one side. For the first 18 subjects, a Quincke Type Point spinal needle (22 Gauge) was used, but since a few of the study objects suffered from post spinal headache, the needle was changed to a Sprotte (24 Gauge). Thereafter no more post spinal headache occurred. For each study subject, 5–10 ml CSF was collected in a polypropylene tube (Sarstedt, Nümbrecht, Germany), gently mixed to avoid gradient effects,

aliquoted and stored at -80°C pending analysis.
Blood was collected by venepuncture into whole blood and gel-separator tubes. The samples were centrifuged within 20-60 minutes, aliquoted and stored at -80˚C pending analysis.
CSF and blood samples were collected twice in the boxers: The first samples were collected 1 to 6 days after a bout (test A) and the second at least 14 days after competition or sparring (test B). The control subjects underwent one LP and venepuncture.

6.1.7 Biomarker analysis

Cerebrospinal fluid

NFL and GFAP were analysed using previously described ELISA methods [159,160]. The detection limit of the NFL ELISA was 125 ng/L. CSF NFH was analysed using a sandwich ELISA (Abnova, Walnut, CA, USA).
CSF total tau (T-tau), tau phosphorylated at threonine 181 (P-tau181), and Aβ1–42 levels were determined using xMAP technology and the INNOBIA AlzBio3 kit (Innogenetics, Zwijndrecht, Belgium) as previously described [161]. S-100B was determined by an electrochemoluminescence immunoassay using the Modular system and the S100 reagent kit (Roche Diagnostics).
H-FABP was measured using a commercially available ELISA method (Hycult Biotechnology, Uden, The Netherlands), following the instructions from the manufacturer.
CSF AβX-38, AβX-40 and AβX-42 levels were measured by the electrochemiluminescence technology using the MS6000 Human Abeta 3-Plex Ultra-Sensitive Kit, while β-secretase cleaved soluble APP (sAPP-β) and α-secretase cleaved soluble APP (sAPP-α) were measured using the MS6000 Human sAPPalpha/sAPPbeta Kit (Meso Scale Discovery, Gaithersburg, Maryland, USA), as described previously [162]. CSF levels of ApoE and ApoA1 were measured using the MILLIPLEX MAP Human Apolipoprotein Panel (Millipore Corporation, Billerica, MA, USA) in a Bio-Plex instrument (Bio-Rad Laboratories, Inc., Herts, UK). Quantification of Aβ1-42 in plasma was performed by single molecule digital ELISA, as described previously in detail [163].
Intra-assay coefficients of variation were <10% for all assays. For each marker all samples were analysed on one occasion to eliminate any inter-assay variability.

Blood

Plasma levels of total tau (T-tau) were determined using a novel digital immunoassay [164]. The limit of detection of the assay is 0.02 ng/L, which is over 1000-fold more sensitive than conventional immunoassays. The assay

utilizes the Tau5 monoclonal for capture (Covance), and HT7 and BT2 monoclonals for detection (Pierce/Thermo). These antibodies react with both normal and phosphorylated tau, and have their epitopes in the mid-region of tau, making the assay specific for all tau isoforms.
A similar assay was used to measure Aβ42 concentrations in plasma, as previously described in detail [163].
Serum levels of S-100B were determined by an electrochemoluminescence immunoassay using the Modular system and the S100 reagent kit (Roche Diagnostics).
GFAP levels in serum were determined using a previously described ELISA [165].
BDNF levels in serum were determined with the BDNF Emax® ImmunoAssay System according to instructions by the manufacturer (Promega, Madison, WI).
Experienced and certified laboratory technicians performed all analyses simultaneously. Intra-assay coefficients of variation were <10% for all analyses.

6.1.8 *ApoE* genotyping

APOE (gene map locus 19q13.2) genotyping was performed using TaqMan® Allelic Discrimination technology (Applied Biosystems, Foster City, CA). Genotypes were obtained for the two SNPs that are used to unambiguously define the ε2, ε3, and ε4 alleles (rs7412 and rs429358).

6.1.9 Neuropsychological evaluation

An experienced neuropsychologist designed the neuropsychological assessment with the aim to test memory, processing speed and executive functions, the main areas previously shown to be impaired after traumatic brain injury [67,68]. The cognitive testing was administered at 1-6 days after the last bout, prior to, but on the same day as the collection of CSF and blood samples (test A). It was performed at daytime at the University Hospital in Linkoping, in a quiet room without distraction. The same examiner administered all the tests following a standardized procedure. The duration of the neuropsychological assessment was approximately 60 minutes. A blinded experienced neuropsychologist analysed the neuropsychological assessment.

Rey Osterrieth Complex Figure Test, part 1

The examiners were given a task to draw an exact copy of a given figure (fig.6) without time limitation [70].

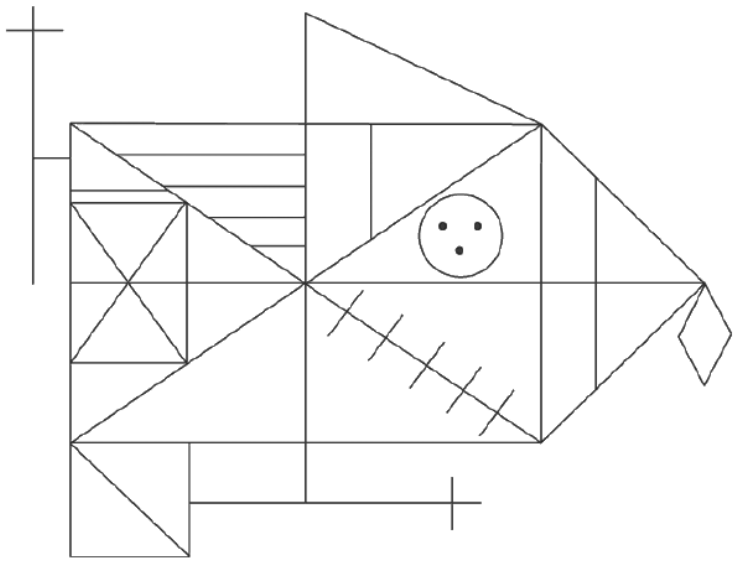


Figure 6. *Rey Osterrieth Complex Figure [70].*

Vocabulary

The vocabulary task involves the explanation of the meaning of words, ranging from common to less well-known items. Vocabulary intervention evaluates language and semantic memory and is part of the WAIS-R. Vocabulary is related to level of education and is critical for studies where educational background may interfere with neuropsychological results [85].

Controlled Oral Word Association Test (COWAT)

Participants were asked to generate as many words as possible that begin with a given letter, (i.e. F, A OR S, excluding proper names, numbers or words with different tenses or endings). Sixty seconds was allowed for each letter. The dependent variable was the total number of correct words produced, minus any repetitions [70].

Listening Span

The participants listened to a set of sentences, half of which were semantically correct and the other half incorrect. Participants were instructed to report whether each sentence was correct or not and to remember the last word in each sentence. This procedure was repeated for two to five sentences. After the sentences had been presented, the participants were asked to recall all the

target words in correct order. The task was repeated five times at each level of difficulty [166].

Rey Osterrieth Complex Figure, part 2.

The participants were again asked to draw the figure from memory as best they could, after been provided adequate distraction for about 30 minutes. To prevent rehearsal, the tasks between ROCF part 1 and 2 were not related to drawing or geometry. The scoring in the Rey-Osterrieth Figure Test was made according to established criteria developed by David Loring [167].

Computerized testing of episodic memory A

The examiners were presented a series of words, half of the words presented in writing on the computer screen and half of them presented with a recorded voice. The task was to try to remember all the words, even during following distractor tasks.

Digit Span

Participants were presented with a number series by the examiner, starting with two sets of three numbers, each stage adding one number. There were seven stages in total. The task was to immediately reproduce the words.

It is shown that performance on the Digit Span task is relatively insensitive to effects of mild TBI [168]. However, it ascertains that participants master

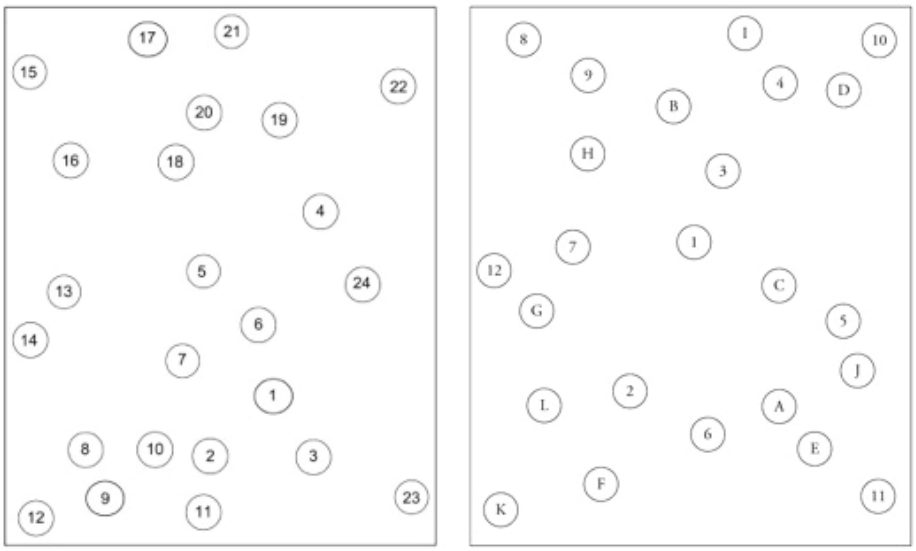


Figure 7*. Trailmaking A to the left and B to the right [169]. I part A the task is to draw lines and connect numbers in ascending order. In part B the examiner shall draw lines to connect circles alternating between numbers and letters in ascending order (1-A-2-B).*

necessary attention skills in order to allow meaningful interpretation of other neuropsychological data.

Trailmaking A and B

The task is to be as fast as possible without any mistakes and without lifting the pen from the paper (fig. 7).

Computerized testing: Simple and Complex Reaction Time

Reaction time is vulnerable to the effects of mild TBI [98]. Both Simple and Complex Reaction Time tests were constructed by a standardized model [170]. The examiners were presented with one of two geometrical figures (a circle or a triangle) on the computer screen.

When evaluating Simple Reaction Time, participants were instructed to respond as quickly as possible whenever the circle appeared on the screen.

Complex Reaction Time required participants to respond with right index finger to the circle and left index finger to the triangle.

Each stimulus was presented for 100 msec. A randomly varying interstimulus interval (ISI) was used, ranging between 300 and 5000 msec. The measurement was based on 40 repetitions of each condition. Individual mean values for the simple and complex reaction time were calculated and the difference between complex and simple reaction time for use in the further analysis of the results.

Computerized testing: Finger Tapping

The participants were asked to keep their dominant hand palm down; fingers extended, and rest the index finger on the space bar on a computer keyboard. The participants were instructed to press the key as many times and as fast as possible until a brief pause was introduced. The entire session consisted of five consecutive trials of 15 seconds each with a 15 second rest in between trials. We used the pace (= mean number of finger taps across trials) in our further analyses.

Computerized testing: Episodic memory – Part B

Following the part one of the Episodic Memory task, a self-paced, computerized, yes-no recognition test in two parts took place in the assessment of recognition memory performance. Written instructions explaining the nature of the recollection classification tasks (including particular examples) were presented on the computer screen during the recognition test.

In part one, words from Episodic Memory A were presented together with distractor words. The participants were asked to decide whether the word had occurred in the previous study lists and if they answered "yes", were also asked to decide if the recognition was accompanied by recollection (associations that took place during

the previous presentation, feelings or thoughts that linked the affirmative recognition decision to the previous presentation of the specific word).
In part two, the participant was asked to discriminate and identify earlier presented audial words among earlier visually presented words and fifteen new distracters. The subject was also asked to decide whether the recognition was accompanied by recollection.

6.2 PAPER V

Paper 5 presents a 21-year old amateur elite boxer using a head guard who was enrolled after suffering a knockout during a super heavy weight (+91 kg) fight. Written informed consent was obtained from the participant.

6.2.1 Baseline data

The boxer's fighting record included 33 wins out of 45 bouts (73%), without previous knockouts or losses due to RSC-H. Before the knockout bout, that was the focus of this study, the boxer had not competed for 6 weeks, but he had participated in a one-week long training camp, with tough sparring, ending one week before the knockout bout.

At the time of the knockout, the boxer received a rotational punch to the jaw in round 2 and lost consciousness for about 5 seconds. After gaining consciousness, the boxer reported that he felt fine. The on-field examination by the ringside physician was normal. At the local emergency department the boxer underwent a medical evaluation including CT scan of the brain without any pathological findings.

Medical anamnesis at enrolment revealed that the boxer was previously healthy and without history of previous concussions. The boxer reported a low alcohol intake and denied usage of drugs.

6.2.2 CSF collection and analyses

CSF was collected at 5 time points: 16 days after the knockout and then at 9, 18, 28 and 36 weeks. NFL, GFAP, T-tau, P-tau, Aβ42 were analysed. Damage to the blood-brain barrier was evaluated by analysing the CSF/serum albumin ratio (described in section 6.1.7). The concentrations were compared with laboratory reference values.

07 STATISTICS

Statistical analysis was carried out using the IBM Statistical Package for Social Sciences (SPSS). Version 17.0 was used to analyse the data presented in paper I. Version 16.0 was used for paper II and III and Version 21.0 was used for paper IV.
Descriptive data are reported as the mean, standard deviation (SD) and range (minimum-maximum). The level of significance was set at $p < 0.05$.

7.1 PAPER I-III

Differences between boxers and controls for the biomarker variables BDNF, FABP, GFAP, S100B, Aβ42, Total-tau and P-tau were tested using a Student's t-test. For the other biomarkers, the comparison between boxers and controls was calculated with the non-parametric Mann Whitney U test since some of the variables had skewed distributed data.
For the boxers, differences between time point A and B were compared using a paired sample T-test (for BDNF, FABP, GFAP, S100B, Aβ42, Total-tau and P-tau) and the Related Samples Wilcoxon's signed rank test.
Regression analysis was used as an exploratory tool to explain variation of the marker values as a function of different factors. Bayesian Model Selection was used to identify the best predictive model [171].
Correlation analyses were performed with a Spearman two-tailed test.

7.2 PAPER IV

Comparisons between groups were performed using the non-parametric Mann-Whitney U-test, as some of variables had skewed distributed data. Correlation analyses were performed with a Spearman two-tailed test.

08 RESULTS

8.1 PAPER I-IV

8.1.1 Questionnaire design and neurological examination

The questionnaire about medical and social history and the 10-question survey was similar between boxers and controls (table 4). None of the boxers suffered from loss of consciousness during their last bout before test A. Only one of the boxers reported concussion-related symptoms after the bout (in this case headache) at the clinical examination, but the medical and neurological examinations were normal in all subjects with GCS 15. There was no correlation between age or the risk factors listed in table 5 and brain injury markers, when using a multiple regression model.

8.1.2 CSF biomarkers of neuronal injury

The markers of axonal injury, NFL, pNFH and T-tau were elevated in the boxers at test A compared to controls (Table 6). At test B, T-tau had normalised in the boxers but NFL and pNFH remained elevated compared to controls ($p<0.001$ and $p=0.018$).

CSF pNFH concentrations correlated with NFL ($r = 0.57$ after bout and 0.64 at follow up, $p<0.001$). 83 % of the boxers had NFL concentrations >125 ng/L after the fight (test A). Normal concentrations for this age group are considered < 125 ng/L (below the detection limit) [159]. One of the controls had a NFL concentration of 380 ng/L in the CSF; all of the others were <125 ng/L. At follow-up, 50 % of the boxers still had NFL concentrations >125 ng/L. Regression analysis for test A showed that NFL increased by 147 ng/L per day between days 1-6 after a bout (SD 67.0), $t=2.190$, $p=0.037$.

Boxing Exposure, the calculation of total amount of head trauma prior to test A, correlated with NFL concentrations ($r = 0.396$, $p = 0.030$). In the two boxers having the highest NFL concentrations at test A, 2340 ng/L and 2480 ng/L respectively, the collection of CSF was performed 5 days post fight. At follow up with a 14 day resting period, their NFL concentrations had decreased to <125 and 1600 ng/L, respectively. Both boxers had high Boxing Exposure grading with 1 and 2 tough bouts during the previous week and an expert score for their total boxing career of 4.0 and 5.0, respectively. The only boxer reporting

INFORMATION	BOXERS (30)	CONTROLS (25)
AGE **(years)**	Mean 22 (17-34)	Mean 22(17-30)
SEX	28 male	
2 female	20 male	
5 female		
EDUCATION		
Primary School	13%	20%
High School	67%	64%
University	20%	16%
OCCUPATION		
Student	33%	36%
Unemployed	20%	16%
Work	47%	48%
RISK SPORTS FOR TBI > 10 YEARS*		
	0%	24%
CONCUSSIONS	17% (max 2)	16% (max 1)
ALCOHOL		
No	40%	16%
> once per week	7%	8%
DRUGS		
Marijuana, Haschish	0%	12%

Table 4. Information about boxers and controls
*The boxers and controls were well matched. * Participants that had competed in sports where a head injury can occur e.g. soccer, ice hockey, martial arts*

AGE (years)			
Test A1	30 boxers, mean 22 (17-34)		
Test B2	26 boxers, mean 24 (17-34)		
AGE, WHEN START OF BOXING CAREER	Mean 14 (7-19) years		
AGE, FIRST BOUT	Mean 15 (10-19) years		
DURATION CAREER	Mean 7 (3-13) years		
DIPLOMA BOUTS3	Mean 18 (0-57) bouts		
REGULAR BOUTS			
Test A	Mean 74 (47-168) bouts		
Test B	Mean 92 (47->200) bouts		
WINS (%)			
Test A	Mean 70 (25-92)		
Test B	Mean 68 (25-92)		
KNOCKOUT			
One	8 (27%)		
Three	1 (3%)		
RSC-H4			
One	5 (17%)		
Two	1 (3%)		
WEIGHT (kg)	Mean 70 (54-91)		
BOXING STYLE			
Defensive boxer	7%		
Counterattack boxer	66%		
Attack boxer	27%		
EXPERT SCORING5			
Mean score ≤ 2.0	7%		
Mean score 2.1-3.9	74%		
Mean score ≥ 4.0	20%		
LAST BOUT (days)			
Test A	Mean 2.7 (1-6)		
Test B	Mean 148, median 26 (14-760)		
BOXING EXPOSURE			
Scoring last bout6	20% easy	47%	33% tough*
Number of bouts7	1 (40%)	2 (40%)	3 (20%)*
Concussion symptoms8			1 (3%)*

Table 5. Boxers' details

*[1]1-6 days after bout; [2]A rest period of a minimum of 14 days; [3]Boxing at age 10–14 years without hard punches; [4] Referee Stops Contest due to hard blows against head; [5]5Three experts graded the boxers 1 to 5, independently, (from low to high head trauma exposure considering total boxing career); [6] The boxers scored their last fight as easy, intermediate or tough; [7] Number of bouts in a row (maximum one per day) for the test A; [8] If a boxer experienced some sequelae after the last bout; *Boxers with increased risk for TBI*

CSF Marker	Boxer Test A[1] N=30 Mean(range)SD ng/L	Boxer Test B[2] N=26 Mean(range)SD ng/L	Controls N= 25 Mean(range)SD ng/L	P-value		
				Avs.C	Avs.B	Bvs.C
NFL[3]	532(125–2480)553	402(125–1780)220	135(125–380)51	**<0.001**	**0.072**	**<0.001**
pNFH[4]*	163(49–562)117	68(23–1503)298	33 (27–1265) 251	**0.000**	**0.018**	**0.018**
GFAP	496(70–1020)238	367(170–600)113	244(90–820)145	**<0.001**	**0.011**	**0.001**
S100B	0.76(0.34–1.68)0.29	0.63(0.33–0.99)0.16	0.60(0.30–1.16)0.23	**0.030**	**0.016**	0.67
T-tau	58(25–132)25	49(19–121)21	45(24–95)17	**0.025**	**0.024**	0.39
P-Tau	21(9–38)7	22(9–43)8	23(14–40)6	0.21	**0.09**	0.68
H-FABP	407(108–1089)208	334(40–769)195	458(67–1383)271	0.45	**0.07**	**0.07**
ApoA1*	1936(834–4673)1018	2435(1273–5589)986	2155(1033–5853)1247	0.710	**0.006**	0.221
ApoE*	4597(2659–9577)1647	4411(2751–7859)1321	3977(2131–7192)1432	0.128	0.534	0.109
APPα*	635(319–1122)189	666(227–1048)209	587 (359–988) 180	0.654	0.218	0.442
APPβ*	207(110–405)80	220(54–508)97	197(116–406) 78	0.565	0.334	0.462
Aβ1–42	306(191–411)52	294(178–423)54	297(231–362)39	0.43	0.37	0.83
Aβ38*	1541(715–2890)606	1538(566–2733)578	1578 (925–3066) 582	0.618	0.622	0.851
Aβ40*	7211(4017–11100)1919	7290(3315–10979)1836	7588(4887–10610)1658	0.993	0.501	0.638
Aβ42*	589(276–1380)268	606(227–1002)216	641(378–1119)214	0.846	0.657	0.468

Table 6. CSF brain injury biomarkers in boxers after a fight

[1]Test A: 1–6 days after last fight; [2]Test B: The boxers had rested from boxing for at least 14 days;
[3]For NFL the detection limit was 125 ng/L; [4]According to pNFH, the result from one of the controls was destroyed;
** Concentrations given as Median(Range)SD;*

sequelae (headache) after the fight reported 3 tough bouts over 3 days during the previous week and a mean expert score of 3.7 for their total boxing career. CSF NFL concentration was 600 ng/L one day after the last fight and increased to 1780 ng/L 15 days later. In total, 23 % of the boxers with elevated NFL concentrations at test A had even higher values at follow-up. Interestingly, one of these boxers had not been boxing for 360 days.

With regards to the other markers for neuronal injury; H-FABP, BDNF, ApoA1 and ApoE; no significant differences were found between boxers and controls (table 6).

8.1.3 CSF biomarkers of astroglial injury

GFAP and S100B are biomarkers for astroglial injury. All controls had GFAP concentrations between 90 and 380 ng/L except one subject who also had an elevated CSF NFL concentration. This individual had a GFAP concentration of 820 ng/L, which is considered abnormal for this age group [160]. 60% of the boxers had GFAP concentrations ≥ 410 ng/L (the value calculated by mean concentration of the controls, without the outlier, plus 2SD) at test A. 27% boxers still had GFAP concentrations ≥ 410 ng/L at follow up (table 6).

Concentrations of S-100B were significantly increased after a bout, but normalized at follow up, compared to controls (Table 6).

8.1.4 CSF biomarkers for neurofibrillary tangle an plaque pathology

No differences between boxers and controls were found for P-tau or any of the beta amyloids (table 6).

8.1.5 Biomarkers in peripheral blood

Tau in plasma was significantly increased in 20 % of the boxers after a bout (test A) compared to controls, but no significant difference was found between controls and boxers at follow up (test B)(Table 7).
One of the boxers had a plasma-tau concentration within the range of controls (0.80 ng/L) after the bout, increasing to 2.90 ng/L at follow up even though the boxer had not been boxing for over 3 months. This opposed the more common pattern of decreasing concentrations of plasma-tau between the two measuring occasions. Plasma-tau concentrations for the boxer that complained of headache after the last bouts were neither elevated at test A nor at test B (1.23 ng/L and 0.29 ng/L, respectively).

Correlation of plasma-Tau to risk factors for traumatic brain injury

There was no correlation of plasma-tau with any of the boxers' risk factors for TBI (table 5).

MARKER	Boxer Test A[1] N=30 Mean(range)SD ng/L	Boxer Test B[2] N=26 Mean(range)SD ng/L	Controls N= 25 Mean(range)SD ng/L	P-value		
				Avs.C	Avs.B	Bvs.C
T-tau	2.46 (0.13–26.73) 5.10	1.43 (0.02–11.60) 2.51	0.79 (0.02–4.76) 0.96	**0.038**	**0.030**	1.000
BDNF	28353(10331–42025) 7170	27836(13363–42164)7621	29146(19288–40417)5419	0.866	0.770	0.706
GFAP	All samples < 150*	All samples < 150*	All samples < 150*	-	-	-
S100B	0.037 (0.015–0.088) 0.018	0.043 (0.014–0.118) 0.024	0.041 (0.011–0.137) 0.025	0.685	0.400	0.674
Aβ42	12.1 (4.0–26.9) 4.8	11.2 (0.0–20.1) 4.2	11.6 (0.7–18.9) 4.4	0.906	0.200	0.672

Table 7. Concentrations of brain injury markers in serum/plasma
*Tau and Abeta42 are plasma samples and the rest are serum samples; [1]Test A: 1–6 days after last bout; [2]Test B: No boxing for at least 14 days; *Under the detection limit;*

Correlation of elevated plasma-Tau vs elevated CSF-biomarkers

There was no correlation between plasma-tau and any of the CSF biomarkers.

S100B, GFAP, BDNF and Aβ42

The concentrations of serum – GFAP were under the detection limits for all examinations. No differences within the groups were seen in the other analysed biomarker concentrations. The results are presented in table 7.

8.1.6 Role of *APOE* genotype

Possession of the APOEε4 allele did not influence biomarker concentrations.

8.1.7 Neuropsychological evaluation (paper IV)

The visuospatial ability was tested with the first part of Rey-Osterrieth Figure Test and revealed no differences between the groups.

No significant differences between the boxer and control group were seen in the assessment of episodic, language and semantic memory. Digit Span and Listening Span were used to assess working memory. No differences were seen in Digit Span but the boxers performed better than the matched controls in the Listening Span Task (p=0.049).

Evaluations of processing speed and executive functions were made using the tests Trail Making, Reaction Time and Finger Tapping. No differences between the groups were detected in any of these tests.

Relationship between neuropsychological evaluation and CSF NFL

When investigating the relationship between neuropsychological evaluation and CSF NFL, it was revealed that boxers with persisting NFL concentration elevation at test B (after rest) had significantly poorer performance on Trailmaking A (p=0.04) and Simple Reaction Time (p=0.04) compared to the other boxers.

8.2 PAPER V

The laboratory analyses showed marked elevation of CSF NFL 16 days post trauma, about 4-fold higher concentrations than in paper I and in a previous study on Swedish amateur boxers. Total-tau increased slightly between the first and second test occasions - 16 days and 9 weeks. The biomarker concentrations are presented in table 8.

During the whole follow-up period, the boxer did not report any concussion symptoms such as headache, memory deficits, dizziness or nausea. He was physically active but avoided all boxing training including head targeting for 5 months. After that normal training and sparring begun.
As shown in fig. 8, CSF NFL decreased gradually over the 18 weeks post trauma, while the boxer was resting, but was still significantly elevated at this time point. The boxer competed for the first time 6 months post trauma, 4 days before the 28-week test. Normalisation of NFL did not occur until the final CSF sample at 36 weeks post trauma.

CSF	REFERENCE	WEEK				
Biomarker	**ng/L (years)**	**2**	**9**	**18**	**28**	**36**
NFL	< 380 (<30)	3250	2190	680	600	370
T-tau	< 300 (18-45)	303	326	224	223	151
P-tau	< 60 (< 60)	37	51	38	37	35
GFAP	< 750 (20-60)	280	230	290	360	120
Aβ42	> 550 (>18)	>550	>550	>550	>550	>550
Albumin ratio	< 7.2 (15-45)	4.2	3.0	3.6	3.7	3.5

Table 8. CSF brain injury biomarkers in a boxer following concussion

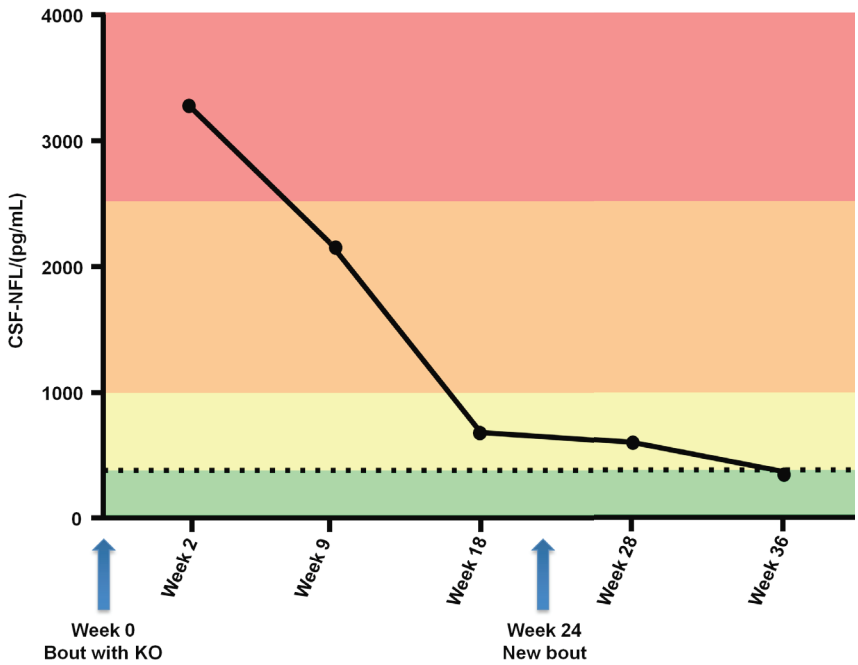


Figure 8. CSF NFL concentrations in a boxer following a knockout.
Time-course of cerebrospinal fluid neurofilament light protein changes in an amateur boxer after a knockout. Lumbar punctures were taken 2 weeks after trauma and then at regular intervals until normalisation. The dotted line represents the reference limit (<370 ng/L) for the boxer's age group.

09 DISCUSSION

Concussions are one of the most common sport-related injuries. These injuries have received increased attention in recent years, since there is now an awareness that an important type of change following concussion is axonal damage, often called diffuse axonal injury (DAI) [13] and that there is risk for long-term effects. In order to learn more about the pathogenesis and neurobiological changes after a concussion, this thesis has evaluated both the effects of the repetitive subconcussive head trauma as well as the effects of a knockout (concussion) in amateur boxing when boxing with a head guard.

9.1 CSF BRAIN INJURY BIOMARKERS

Analysis of biomarkers in the CSF and peripheral blood (paper I-III) revealed that, even though none of the boxers had been knocked out and only one complained of concussion symptoms (headache), the repetitive subconcussive head trauma in boxing leads to acute axonal and glial damage, that can persist more than 2 weeks. The case report of a knocked out boxer (paper V) further shows that after a concussion, in total absence of concussion symptoms, full recovery takes more than 4 months.

9.1.1 Biomarkers for axonal injury

Since diffuse axonal injury is defined as the mildest form of traumatic brain injury [37], it is not surprising that the axonal injury marker NFL was the most sensitive of the CSF biomarkers in detecting traumatic brain injury. There are several explanations for why the other axonal injury biomarkers were not sensitive enough to detect TBI.. For NFH, one important factor is that it is mainly analysed in its phosphorylated form, pNFH. Therefore only a part of the total circulating NFH in the CSF is analysed. Tau, in turn, is localized in the distal parts of the axons [146], whereas neurofilaments are expressed in large amounts along the large, myelinated axons [121] and thereby most affected by the translational/rotational acceleration of head impacts.

NFL and T-tau concentrations gradually increased daily during the 6 days post trauma, similar to findings from previous studies [172].

9.1.2 Biomarkers for glial injury

GFAP was significantly increased in boxers as compared to controls, but it remains unclear if concentrations correlate with size of injury and when they reach maximum levels post injury. At follow up, only GFAP was still significantly elevated in the boxers. The boxer having the highest GFAP concentration at test A was the only one complaining of concussion symptoms after a bout. This boxer had boxed three tough fights in a row (one daily). CSF was collected one day after the last bout. At follow up 15 days later, the CSF concentration of GFAP had decreased (960-500 ng/L), but was still elevated compared to the control group. In the knocked out boxer presented in paper V, the CSF GFAP concentration, analysed 16 days post trauma, was 280 ng/L. This boxer had only boxed one fight and was knocked out in the second round. More studies are needed to investigate whether GFAP correlates with the size of glial injury.

S100B was also significantly increased in boxers as compared to controls. The increased concentrations of S100B in the boxers at test A were somewhat surprising, since the CSF was collected on average 2 days post bout and it has been shown to peak at day 1 post head trauma [134]. Except in the CNS, S100B can be found in adipose tissue, muscles and skin [173]. Increased serum concentrations have been found both after TBI and after physical activity such as marathon running [174], although the half-time of serum-S100B released from muscles is short with normalization within 20 hours [174]. Since no studies to our knowledge have shown transport of S100B from serum to the CSF, S100B in the CSF most likely reflects the true cerebral S100B concentration [175]. The role of released S100B after TBI is not clearly understood but it might have both neurotrophic and neuroprotective functions, or simply reflect injury-related release.

9.1.3 Interpretation of CSF NFL concentrations

According to our findings as well those from previous studies, the normal CSF NFL concentration for this age group is < 125 ng/L, which was the detection limit for the analysis method used in our studies [16,110]. In paper I it was shown that after a "normal" boxing bout, the NFL concentration can increase up to 20 times, but after a knockout (paper V), the increase is much higher – at least 30-fold. This leaking of CSF NFL is interpreted as a sign of injured or ruptured axons.

9.1.4 Correlation with head trauma exposure

NFL was the only biomarker in this thesis shown to correlate with the boxing exposure score, which could be considered as an indirect measure of the expected size of injury. CSF NFL has also been shown to correlate with the size of head injury in a previous study on Swedish amateur boxers [16].

9.1.5 CSF biomarker changes at test A and B (paper I, III)

At test A (1-6 days after the last bout) the markers of axonal injury NFL, pNFH and T-tau, and the markers for glial injury GFAP and S100B, were significantly increased in boxers when compared to controls. At follow up (median 26 days post bout) the concentrations had decreased, but NFL was still elevated in 50% of the boxers, pNFH in 12% and GFAP in 27 % of the boxers. This can be interpreted as sign of acute injury that has not yet fully recovered or it might also be a sign of long-term effects of repetitive subconcussive head injury. Interestingly, one of the boxers with increased NFL concentrations at follow up had not trained or competed in boxing for 360 days since test A. All three boxers with elevated concentrations of pNFH at follow up had higher concentrations than at test A.

9.2 BIOMARKERS IN PERIPHERAL BLOOD

In peripheral blood only the axonal injury marker T-tau was elevated in plasma in a subgroup of boxers compared to controls after a bout (paper III). However, plasma T-tau concentrations did not correlate with boxing exposure, risk factors or any of the CSF biomarkers.

Since tau is exclusively found in axons, the increased plasma-tau likely reflects axonal damage caused by the repetitive subconcussive trauma incurred while boxing. The analysis results were somewhat unexpected. Firstly, all but one of the boxers were non-symptomatic concerning concussion symptoms. Secondly, the blood samples at test A were collected 1-6 days after a fight, whereas an animal study on rats was not able to show plasma-tau elevation after 24 hours post TBI; Thirdly, these results contradict the findings of two previous studies based on patients with mild TBI (GSC 13-15) [115,176]. One probably explanation for the differences between our study and previous studies is thatwe used a more sensitive analysis method (detection limit of 0.02 ng/L) than previously used (Innogenetics ELISA). The same analysis method for

plasma-tau used in this thesis has also been used in a recently published study on concussed ice hockey players. In that study, plasma-tau was elevated for up to 144 hours post trauma and it also seemed to correlate with outcome [177]. The potential and clinical relevance of tau, the transport of tau from CSF to peripheral blood, and the kinetics of tau after TBI in peripheral blood need to be further explored.

9.3 CSF VERSUS BLOOD BIOMARKERS

To measure biomarkers in serum after a mild TBI is challenging, since a smaller amount of released markers from the CNS can be analysed in peripheral blood compared to CSF. The role of the blood-brain-barrier and kinetics are not fully understood.

A limitation of the broader clinical use of CSF analysis in return-to-play considerations after a sport-related concussion is that lumbar puncture is more invasive and demands more skills than blood sample collection. In contrast to CSF, blood samples can be readily collected; hence reliable quantification of NFL and other brain injury biomarkers in serum/plasma would be a major stride towards using biomarkers in the diagnosis and monitoring of sport-related concussions.

9.4 NEUROPSYCHOLOGICAL ASSESSMENTS

Neuropsychological evaluation has been advocated as the most sensitive tool in diagnosing and monitoring a sports-related concussion [64]. Therefore it was valuable, for the first time ever, to relate neuropsychological assessment with CSF concentrations of the brain-specific brain injury markers. It was found that without baseline testing, neuropsychological assessment was not able to identify those boxers that had obtained small axonal injuries. One argument could be that the detected axonal injury is so small that it does not have any relevance, but interestingly, the boxers with elevated NFL concentrations performed significantly worse in Trailmaking A ($p=0.041$) and Simple Reaction Time ($p=0.042$), than the boxers with normal NFL concentrations. Both tests evaluate processing speed and executive functions, abilities that have been shown to be impaired after TBI [94,95,98].

9.5 WHEN HAS THE CONCUSSION HEALED?

At the latest International Consensus Statement on Concussion in Sports 2012, organized by IOC, FIFA, IRB and H.H.F, it was stated that all concussions should be treated individually, based on the grade of concussion. Athletes were recommended to follow the Return-to-play programme with a stepwise increase in physical activity during rehabilitation [25]. The problem with the Return-to-play protocol (table 1) is that it is based on recovery from symptoms rather than knowledge about healing time. It is likely that a concussion undergoes a healing process, just like other organs in the body, but in contrast to a bone fracture for example, it has been possible to follow the healing process with objective investigations. In the light of our findings, we suggest that absence of symptoms is not equivalent with full recovery. This assumption is based on following facts:

- There is an enormous problem with underreporting of concussions and masking of symptoms [178].
- Athletes with a history of previous concussions are more likely to have future concussive injuries than those with no history [19].
- Several concussions are associated with slower recovery than a single concussion [19].
- At least 12 % of fatal sport-related subdural hematomas had a reported history of concussion with persistent symptoms within 4 weeks of death [32].
- It is suggested that a concussion precedes second impact/cerebral swelling [36].

According to the "Return-to-play guidelines", the knocked out boxer in paper V would have been allowed to return to sport within a week. Nevertheless, unlike in many other sports, competing and training in amateur boxing is prohibited for 28 days after a knockout. Also this interval may well be too short, since our findings indicate that the brain needs a much longer time to recover after a mild TBI/concussion than previously known. It took more than four months before full recovery after a concussion due to a knockout, when measured by CSF biomarker analysis. The recovery time was much longer than the expected 7 days, even though the unconsciousness duration was only a few seconds and the boxer did not have any concussion symptoms.

Concussion in boxing can be caused either by translational acceleration or, as in our case study, after rotational acceleration caused by the hook. It is suggested that the brain is more vulnerable to forces caused by angular rotation, creating axonal stretching and greater tension on brain tissue and on the bridging vessels which partly can explain the long recovery time [179].

Since sport-related concussions are a major concern in many sports and there are risks of long-term consequences, we believe that it is important to work proactively and apply present knowledge and combine available examinations and tests to present practical advice to the concussed individual and to everybody involved in the care and training of athletes.

9.6 PREVENTION

Although the many health benefits of sports participation widely over-weighs the risk of sports-related concussion, preventive interventions should be implemented to decrease the concussion rate and risk for complications. Education, better sport-specific regulations to increase medical safety, and improvement of safety equipment, together with development of efficient assessment tools can help to reduce the concussion rates and complications to lowest possible level.

To be more specific:

- Continuous education of trainers, athletes, relatives, medical staff and sport federations is necessary to increase the reporting frequency and to reduce the risk of athletes returning to sport with persistent concussion symptoms.
- Exposure to competitions every weekend and/or tough sparring several times per week in boxing and other full contact sports can be questionable and should perhaps be regulated.
- It would be advisable to slow down the "return-to-sport" time after a concussion, especially in young individuals with more vulnerable brains.
- Suspension from further play over at least 4 weeks as in boxing should be compulsory in all sports with increased risk of TBI such as martial arts, soccer, rugby, ice hockey and alpine skiing.
- Even though more studies are needed, knowing the concentration of CSF NFL may be of assistance to the clinician in the diagnostic and prognostic counselling of concussed athletes, thereby aiding in return-to-play considerations.

10 STRENGTHS AND LIMITATIONS

10.1 STRENGTHS

- The boxers and controls were very well matched according to age, education, social background and previous concussions although the alcohol/drug intake was higher in the control group.
- Many of the controls had a long sports career in high impact sports with high risk of TBI, such as soccer and ice hockey, just like in the normal population.
- All participants were screened for risk factors for TBI and other factors that might influence the results (previous concussions, education, social background, drugs).
- The boxers were thoroughly evaluated according to boxing exposure.
- Several different assessment tools were used in the evaluation; medical and neurological evaluation, analysis of biomarkers, neuropsychological evaluation and MRI.
- When comparing with other similar studies, the groups consisting of 30 boxers and 25 controls were relatively large.
- All specific biomarker analysis was made simultaneously to eliminate any interassay variability
- The evaluation of the neuropsychological assessment was blinded to the neuropsychologist.

10.2 LIMITATIONS

- One limitation of this study is the variation of time points for CSF sampling for test A and B. The test A was performed 1-6 days after bout and test B hade even a greater variation (14-760), with a median of 26 days after bout. Ideally we would have liked to collect the CSF at the same time points for all participants. This was not possible since we had to adapt to the boxers' schedules and take the risk for side effects in the form of post spinal headache into account.

- Although the sample size in our study was larger than in previous studies on boxers, the sample sizes of the groups were still small and more studies are needed.

- The lack of a developed analysis kit, or kits that were not sensitive enough, limited the evaluation of the brain injury biomarkers in peripheral blood. Therefore all GFAP concentrations were under the detection limit. When the studies included in this thesis were performed, there was no available method to quantify NFL in plasma/serum, something that now seems possible [109].

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CONCLUSIONS

The conclusions of this study were:

- Repetitive subconcussive head trauma due to boxing caused axonal and glial injury, detectable with analysis of NFL, pNFH, Total-tau, GFAP and S100B in the cerebrospinal fluid and Total-tau in plasma, even though the boxers did not have concussion symptoms or lost consciousness.

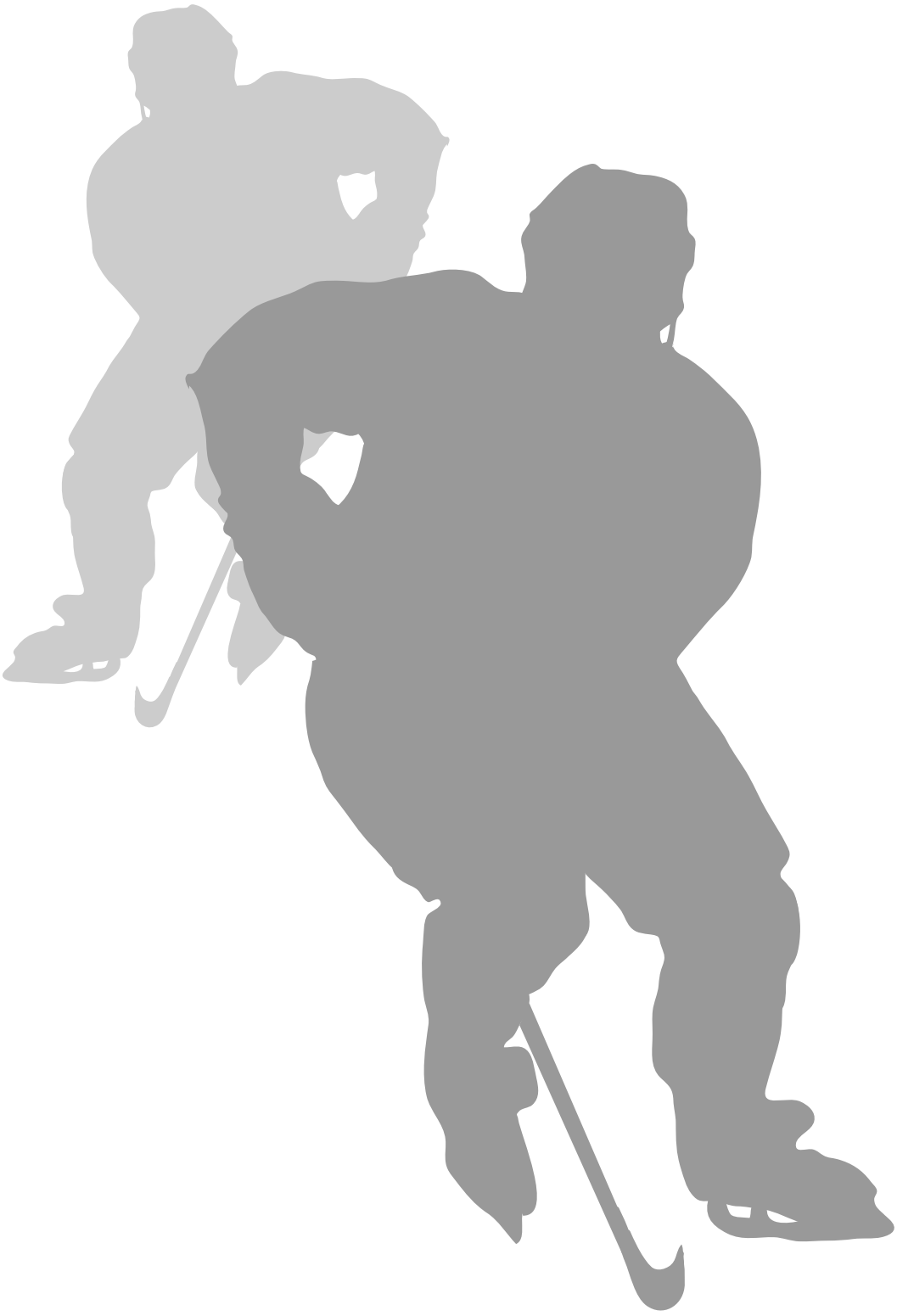
- CSF NFL, a marker for axonal injury, was especially interesting since the concentration was shown to correlate to the amount of head trauma and was normalized at full recovery.

- T-tau in plasma increased significantly in boxers compared to controls after a bout, but there was no correlation with any of the CSF biomarkers.

- Neuropsychological evaluation was not as sensitive as CSF biomarker analysis in detecting small axonal and glial injury.

- ApoE genotype did not influence biomarker concentrations after bout.

- Post-injury biomarker measurements in the cerebrospinal fluid, especially NFL, may give objective information on the severity of axonal damage after a concussion, why longitudinal follow-up samples may be used to monitor subsidence of increased axonal proteins and thereby help athletes decide when they can safely resume training/competing.



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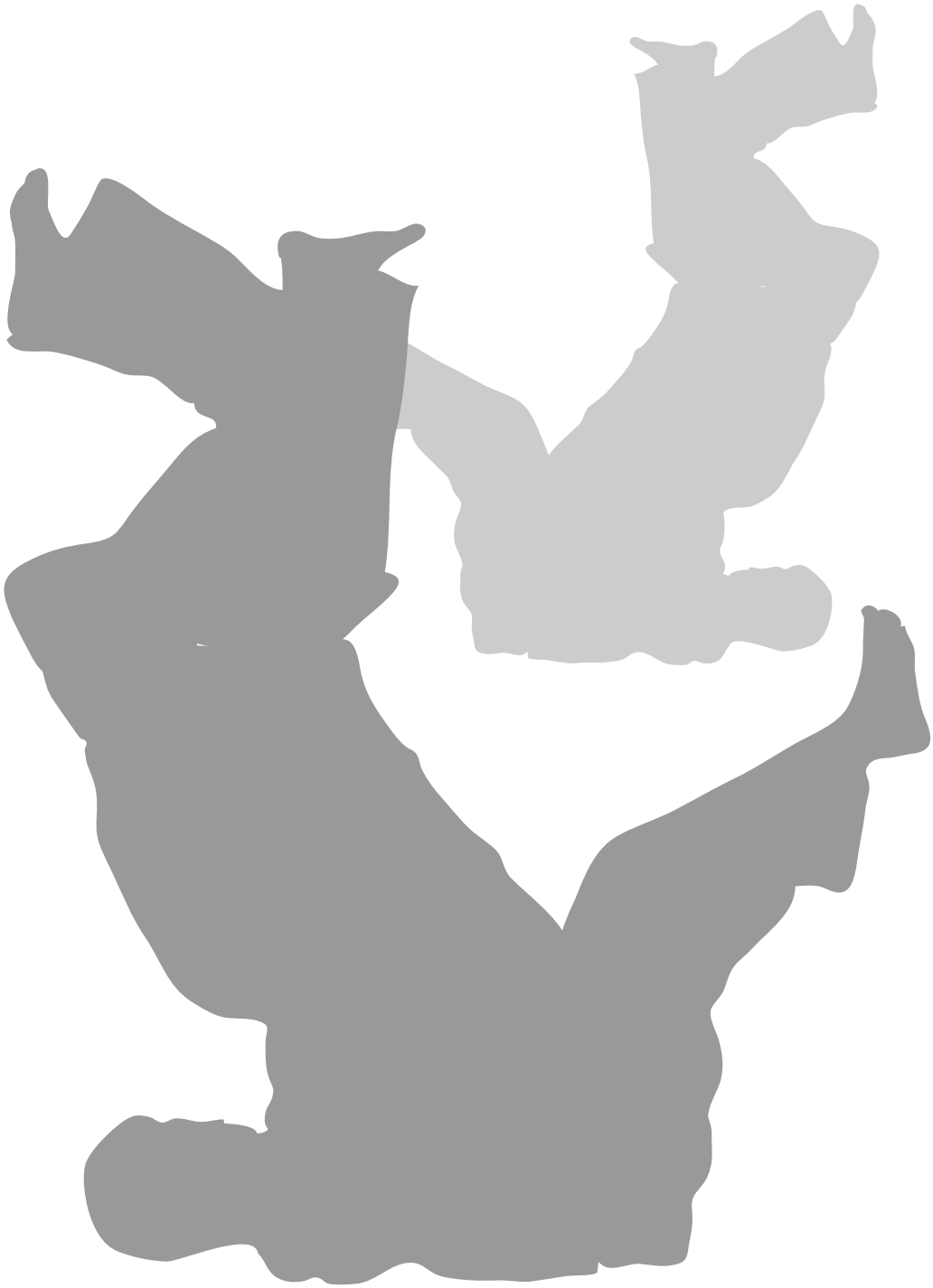
FUTURE PERSPECTIVES

The aim is to perform a long-term follow up of the study participants consisting of boxers and controls included in this thesis.

It is time to diversify the "Return-to-Play guidelines" after concussion to better prevent the risk of recurrent concussions, complications and/or long-term consequences [32,36,180].

Longitudinal, clinical studies on sports-related concussion employing cerebrospinal fluid and blood biomarkers are needed to establish reliable, standardized and quantitative tests to track brain recovery over time.



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REFERENCES

1. DeKosky ST, Blennow K, Ikonomovic MD, Gandy S (2013) Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. Nature reviews Neurology 9: 192-200.
2. Lundqvist E (2009) Swedish Injury Database: Skadehändelser som föranlett akutbesök vid läkarmottagning, 2008. wwwsocialstyrelsense: 1-83.
3. Browne GJ, Lam LT (2006) Concussive head injury in children and adolescents related to sports and other leisure physical activities. Br J Sports Med 40: 163-168.
4. Cohen JS, Gioia G, Atabaki S, Teach SJ (2009) Sports-related concussions in pediatrics. Curr Opin Pediatr 21: 288-293.
5. Steenstrup SE, Bere T, Bahr R (2014) Head injuries among FIS World Cup alpine and freestyle skiers and snowboarders: a 7-year cohort study. Br J Sports Med 48: 41-45.
6. Viano DC, Casson IR, Pellman EJ, Bir CA, Zhang L, et al. (2005) Concussion in professional football: comparison with boxing head impacts--part 10. Neurosurgery 57: 1154-1172; discussion 1154-1172.
7. Jako P (2002) Safety measures in amateur boxing. Br J Sports Med 36: 394-395.
8. Bianco M, Loosemore M, Daniele G, Palmieri V, Faina M, et al. (2013) Amateur boxing in the last 59 years. Impact of rules changes on the type of verdicts recorded and implications on boxers' health. British journal of sports medicine 47: 452-457.
9. Zazryn TR, Finch CF, McCrory P (2003) A 16 year study of injuries to professional boxers in the state of Victoria, Australia. Br J Sports Med 37: 321-324.
10 Sakka L, Coll G, Chazal J (2011) Anatomy and physiology of cerebrospinal fluid. Eur Ann Otorhinolaryngol Head Neck Dis 128: 309-316.
11. Charnas L, Pyeritz RE (1986) Neurologic injuries in boxers. Hosp Pract (Off Ed) 21: 30-31, 34-39.
12. Adams JH, Doyle D, Ford I, Gennarelli TA, Graham DI, et al. (1989) Diffuse axonal injury in head injury: definition, diagnosis and grading. Histopathology 15: 49-59.
13. Topal NB, Hakyemez B, Erdogan C, Bulut M, Koksal O, et al. (2008) MR imaging in the detection of diffuse axonal injury with mild traumatic brain injury. Neurol Res 30: 974-978.
14. Selassie AW, Wilson DA, Pickelsimer EE, Voronca DC, Williams NR, et al. (2013) Incidence of sport-related traumatic brain injury and risk factors of severity: a population-based epidemiologic study. Ann Epidemiol 23: 750-756.
15. Neselius S, Brisby H, Theodorsson A, Blennow K, Zetterberg H, et al. (2012) CSF-biomarkers in Olympic boxing: diagnosis and effects of repetitive head trauma. PloS one 7: e33606.
16. Zetterberg H, Hietala MA, Jonsson M, Andreasen N, Styrud E, et al. (2006) Neurochemical aftermath of amateur boxing. Arch Neurol 63: 1277-1280.

17 Barkhoudarian G, Hovda DA, Giza CC (2011) The molecular pathophysiology of concussive brain injury. Clinics in sports medicine 30: 33-48, vii-iii.
18. Laurer HL, Bareyre FM, Lee VM, Trojanowski JQ, Longhi L, et al. (2001) Mild head injury increasing the brain's vulnerability to a second concussive impact. Journal of neurosurgery 95: 859-870.
19. Guskiewicz KM, McCrea M, Marshall SW, Cantu RC, Randolph C, et al. (2003) Cumulative effects associated with recurrent concussion in collegiate football players: the NCAA Concussion Study. JAMA 290: 2549-2555.
20. Slobounov S, Slobounov E, Sebastianelli W, Cao C, Newell K (2007) Differential rate of recovery in athletes after first and second concussion episodes. Neurosurgery 61: 338-344; discussion 344.
21. Field M, Collins MW, Lovell MR, Maroon J (2003) Does age play a role in recovery from sports-related concussion? A comparison of high school and collegiate athletes. J Pediatr 142: 546-553.
22. Omalu BI, Bailes J, Hammers JL, Fitzsimmons RP (2009) Chronic Traumatic Encephalopathy, Suicides and Parasuicides in Professional American Athletes: The Role of the Forensic Pathologist. Am J Forensic Med Pathol.
23. Omalu BI, Hamilton RL, Kamboh MI, DeKosky ST, Bailes J Chronic traumatic encephalopathy (CTE) in a National Football League Player: Case report and emerging medicolegal practice questions. J Forensic Nurs 6: 40-46.
24. Kumar V, Kinsella LJ Healthy brain aging: effect of head injury, alcohol and environmental toxins. Clin Geriatr Med 26: 29-44.
25. McCrory P, Meeuwisse WH, Aubry M, Cantu B, Dvorak J, et al. (2013) Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport held in Zurich, November 2012. British journal of sports medicine 47: 250-258.
26. McCrea M, Guskiewicz K, Randolph C, Barr WB, Hammeke TA, et al. (2013) Incidence, clinical course, and predictors of prolonged recovery time following sport-related concussion in high school and college athletes. J Int Neuropsychol Soc 19: 22-33.
27. McCrory P, Meeuwisse W, Johnston K, Dvorak J, Aubry M, et al. (2009) Consensus statement on Concussion in Sport--the 3rd International Conference on Concussion in Sport held in Zurich, November 2008. J Sci Med Sport 12: 340-351.
28. Register-Mihalik JK, Guskiewicz KM, McLeod TC, Linnan LA, Mueller FO, et al. (2013) Knowledge, attitude, and concussion-reporting behaviors among high school athletes: a preliminary study. J Athl Train 48: 645-653.
29. Echlin PS, Johnson AM, Riverin S, Tator CH, Cantu RC, et al. (2010) A prospective study of concussion education in 2 junior ice hockey teams: implications for sports concussion education. Neurosurg Focus 29: E6.
30. Kushi H, Saito T, Sakagami Y, Ohtsuki J, Tanjoh K (2009) Acute subdural hematoma because of boxing. J Trauma 66: 298-303.
31. Boden BP, Tacchetti RL, Cantu RC, Knowles SB, Mueller FO (2007) Catastrophic head injuries in high school and college football players. Am J Sports Med 35: 1075-1081.
32. Thomas M, Haas TS, Doerer JJ, Hodges JS, Aicher BO, et al. (2011) Epidemiology of sudden death in young, competitive athletes due to blunt trauma. Pediatrics 128: e1-8.
33. John N, Leach JL, Rachana T, Mangano FT (2009) Traumatic aneurysm of the occipital artery secondary to paintball injury. Clin Neurol Neurosurg 111: 105-108.
34. Cantu RC (1998) Second-impact syndrome. Clin Sports Med 17: 37-44.
35. McCrory P, Davis G, Makdissi M (2012) Second impact syndrome or cerebral swelling after sporting head injury. Curr Sports Med Rep 11: 21-23.
36. Weinstein E, Turner M, Kuzma BB, Feuer H (2013) Second impact syndrome in football: new imaging and insights into a rare and devastating condition. J Neurosurg Pediatr

11: 331-334.
37. Smith DH, Hicks R, Povlishock JT (2013) Therapy development for diffuse axonal injury. J Neurotrauma 30: 307-323.
38. Smith DH, Nonaka M, Miller R, Leoni M, Chen XH, et al. (2000) Immediate coma following inertial brain injury dependent on axonal damage in the brainstem. J Neurosurg 93: 315-322.
39. Hahnel S, Stippich C, Weber I, Darm H, Schill T, et al. (2008) Prevalence of cerebral microhemorrhages in amateur boxers as detected by 3T MR imaging. AJNR Am J Neuroradiol 29: 388-391.
40. Park SJ, Hur JW, Kwon KY, Rhee JJ, Lee JW, et al. (2009) Time to recover consciousness in patients with diffuse axonal injury : assessment with reference to magnetic resonance grading. J Korean Neurosurg Soc 46: 205-209.
41. Luo J, Nguyen A, Villeda S, Zhang H, Ding Z, et al. (2014) Long-term cognitive impairments and pathological alterations in a mouse model of repetitive mild traumatic brain injury. Front Neurol 5: 12.
42. Pellman EJ, Viano DC, Tucker AM, Casson IR, Committee on Mild Traumatic Brain Injury NFL (2003) Concussion in professional football: location and direction of helmet impacts-Part 2. Neurosurgery 53: 1328-1340; discussion 1340-1321.
43. Teasdale G, Jennett B (1974) Assessment of coma and impaired consciousness. A practical scale. Lancet 2: 81-84.
44. Stalhammar D, Starmark JE, Holmgren E, Eriksson N, Nordstrom CH, et al. (1988) Assessment of responsiveness in acute cerebral disorders. A multicentre study on the reaction level scale (RLS 85). Acta Neurochir (Wien) 90: 73-80.
45. McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, et al. (2009) Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J Neuropathol Exp Neurol 68: 709-735.
46. Martland H (1928) Punch Drunk. JAMA 91: 1103-1107.
47. Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, et al. (2000) Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. Neurology 55: 1158-1166.
48. Salib E, Hillier V (1997) Head injury and the risk of Alzheimer's disease: a case control study. Int J Geriatr Psychiatry 12: 363-368.
49. Stein TD, Alvarez VE, McKee AC (2014) Chronic traumatic encephalopathy: a spectrum of neuropathological changes following repetitive brain trauma in athletes and military personnel. Alzheimers Res Ther 6: 4.
50. McCrory P, Meeuwisse WH, Kutcher JS, Jordan BD, Gardner A (2013) What is the evidence for chronic concussion-related changes in retired athletes: behavioural, pathological and clinical outcomes? Br J Sports Med 47: 327-330.
51. Roses AD (1996) Apolipoprotein E in neurology. Current opinion in neurology 9: 265-270.
52. Zhou W, Xu D, Peng X, Zhang Q, Jia J, et al. (2008) Meta-analysis of APOE4 allele and outcome after traumatic brain injury. Journal of neurotrauma 25: 279-290.
53. Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, et al. (1997) Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. JAMA 278: 136-140.
54. Tang MX, Maestre G, Tsai WY, Liu XH, Feng L, et al. (1996) Effect of age, ethnicity, and head injury on the association between APOE genotypes and Alzheimer's disease. Annals of the New York Academy of Sciences 802: 6-15.
55. O'Meara ES, Kukull WA, Sheppard L, Bowen JD, McCormick WC, et al. (1997) Head

injury and risk of Alzheimer's disease by apolipoprotein E genotype. American journal of epidemiology 146: 373-384.
56. Mayeux R, Ottman R, Maestre G, Ngai C, Tang MX, et al. (1995) Synergistic effects of traumatic head injury and apolipoprotein-epsilon 4 in patients with Alzheimer's disease. Neurology 45: 555-557.
57. Jagoda AS (2010) Mild traumatic brain injury: key decisions in acute management. Psychiatr Clin North Am 33: 797-806.
58. Putukian M (2011) The acute symptoms of sport-related concussion: diagnosis and on-field management. Clin Sports Med 30: 49-61, viii.
59. ngebrigtsen T, Romner B, Kock-Jensen C (2000) Scandinavian guidelines for initial management of minimal, mild, and moderate head injuries. The Scandinavian Neurotrauma Committee. J Trauma 48: 760-766.
60. Kurca E, Sivak S, Kucera P (2006) Impaired cognitive functions in mild traumatic brain injury patients with normal and pathologic magnetic resonance imaging. Neuroradiology 48: 661-669.
61. Guskiewicz KM (2003) Assessment of postural stability following sport-related concussion. Curr Sports Med Rep 2: 24-30.
62. Parker TM, Osternig LR, P VAND, Chou LS (2006) Gait stability following concussion. Med Sci Sports Exerc 38: 1032-1040.
63. Schneiders AG, Sullivan SJ, Gray AR, Hammond-Tooke GD, McCrory PR (2010) Normative values for three clinical measures of motor performance used in the neurological assessment of sports concussion. J Sci Med Sport 13: 196-201.
64. Collins MW, Grindel SH, Lovell MR, Dede DE, Moser DJ, et al. (1999) Relationship between concussion and neuropsychological performance in college football players. JAMA : the journal of the American Medical Association 282: 964-970.
65. Echemendia RJ, Putukian M, Mackin RS, Julian L, Shoss N (2001) Neuropsychological test performance prior to and following sports-related mild traumatic brain injury. Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine 11: 23-31.
66. Hinton-Bayre AD, Geffen G (2002) Severity of sports-related concussion and neuropsychological test performance. Neurology 59: 1068-1070.
67. Rohling ML, Binder LM, Demakis GJ, Larrabee GJ, Ploetz DM, et al. (2011) A meta-analysis of neuropsychological outcome after mild traumatic brain injury: re-analyses and reconsiderations of Binder et al. (1997), Frencham et al. (2005), and Pertab et al. (2009). The Clinical neuropsychologist 25: 608-623.
68. Wall SE, Williams WH, Cartwright-Hatton S, Kelly TP, Murray J, et al. (2006) Neuropsychological dysfunction following repeat concussions in jockeys. Journal of neurology, neurosurgery, and psychiatry 77: 518-520.
69. Heilbronner RL, Bush SS, Ravdin LD, Barth JT, Iverson GL, et al. (2009) Neuropsychological consequences of boxing and recommendations to improve safety: a National Academy of Neuropsychology education paper. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists 24: 11-19.
70. Lezak MD (1984) Neuropsychological assessment in behavioral toxicology--developing techniques and interpretative issues. Scandinavian journal of work, environment & health 10 Suppl 1: 25-29.
71. Porter MD (2003) A 9-year controlled prospective neuropsychologic assessment of amateur boxing. Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine 13: 339-352.

72. Maruff P, Thomas E, Cysique L, Brew B, Collie A, et al. (2009) Validity of the CogState brief battery: relationship to standardized tests and sensitivity to cognitive impairment in mild traumatic brain injury, schizophrenia, and AIDS dementia complex. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists 24: 165-178.
73. Schatz P, Maerlender A (2013) A two-factor theory for concussion assessment using ImPACT: memory and speed. Arch Clin Neuropsychol 28: 791-797.
74. Broglio SP, Macciocchi SN, Ferrara MS (2007) Sensitivity of the concussion assessment battery. Neurosurgery 60: 1050-1057; discussion 1057-1058.
75. Cole WR, Arrieux JP, Schwab K, Ivins BJ, Qashu FM, et al. (2013) Test-retest reliability of four computerized neurocognitive assessment tools in an active duty military population. Arch Clin Neuropsychol 28: 732-742.
76. Bigler ED, Rosa L, Schultz F, Hall S, Harris J (1989) Rey-Auditory Verbal Learning and Rey-Osterrieth Complex Figure Design performance in Alzheimer's disease and closed head injury. Journal of clinical psychology 45: 277-280.
77. Wright MJ, Schmitter-Edgecombe M, Woo E (2010) Verbal memory impairment in severe closed head injury: the role of encoding and consolidation. Journal of clinical and experimental neuropsychology 32: 728-736.
78. Himanen L, Portin R, Isoniemi H, Helenius H, Kurki T, et al. (2006) Longitudinal cognitive changes in traumatic brain injury: a 30-year follow-up study. Neurology 66: 187-192.
79. Gallagher C, Burke T (2007) Age, gender and IQ effects on the Rey-Osterrieth Complex Figure Test. Br J Clin Psychol 46: 35-45.
80. Benton AL, Hamsher Kd, Sivan AB, editors (1994) Multilingual aphasia examination Iowa City: AJA Associates, Inc.
81. Ross TP (2003) The reliability of cluster and switch scores for the Controlled Oral Word Association Test. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists 18: 153-164.
82. Curtis KL, Thompson LK, Greve KW, Bianchini KJ (2008) Verbal fluency indicators of malingering in traumatic brain injury: classification accuracy in known groups. Clin Neuropsychol 22: 930-945.
83. Malek-Ahmadi M, Small BJ, Raj A (2011) The diagnostic value of controlled oral word association test-FAS and category fluency in single-domain amnestic mild cognitive impairment. Dementia and geriatric cognitive disorders 32: 235-240.
84. Baillargeon A, Lassonde M, Leclerc S, Ellemberg D (2012) Neuropsychological and neurophysiological assessment of sport concussion in children, adolescents and adults. Brain injury : [BI] 26: 211-220.
85. Wechsler D (1981) Wechsler Adult Intelligence Scale – Revised. Psychological Corporation, New York.
86. Haut MW, Kuwabara H, Leach S, Arias RG (2000) Neural activation during performance of number-letter sequencing. Applied neuropsychology 7: 237-242.
87. Kane MJ, Engle RW (2002) The role of prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: an individual-differences perspective. Psychonomic bulletin & review 9: 637-671.
88. Rosen VM, Bergeson JL, Putnam K, Harwell A, Sunderland T (2002) Working memory and apolipoprotein E: what's the connection? Neuropsychologia 40: 2226-2233.
89. Mayers LB, Redick TS, Chiffriller SH, Simone AN, Terraforte KR (2011) Working memory capacity among collegiate student athletes: effects of sport-related head contacts,

concussions, and working memory demands. Journal of clinical and experimental neuropsychology 33: 532-537.
90. Cornell DG, Roberts M, Oram G (1997) The Ray-Osterrieth Complex Figure Test as a neuropsychological measure in criminal offenders. Arch Clin Neuropsychol 12: 47-56.
91. Cicerone KD, Azulay J (2002) Diagnostic utility of attention measures in postconcussion syndrome. Clin Neuropsychol 16: 280-289.
92. Reitan RM, Wolfson D (1992) Neuropsychological evaluation of older children. Tucson, AZ: Neuropsychology Press.
93. Atchison TB, Sander AM, Struchen MA, High WM, Jr., Roebuck TM, et al. (2004) Relationship between neuropsychological test performance and productivity at 1-year following traumatic brain injury. The Clinical neuropsychologist 18: 249-265.
94. Lange RT, Iverson GL, Zakrzewski MJ, Ethel-King PE, Franzen MD (2005) Interpreting the trail making test following traumatic brain injury: comparison of traditional time scores and derived indices. Journal of clinical and experimental neuropsychology 27: 897-906.
95. Perianez JA, Rios-Lago M, Rodriguez-Sanchez JM, Adrover-Roig D, Sanchez-Cubillo I, et al. (2007) Trail Making Test in traumatic brain injury, schizophrenia, and normal ageing: sample comparisons and normative data. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists 22: 433-447.
96. Sleimen-Malkoun R, Temprado JJ, Berton E (2013) Age-related dedifferentiation of cognitive and motor slowing: insight from the comparison of Hick-Hyman and Fitts' laws. Front Aging Neurosci 5: 62.
97. Colcombe SJ, Kramer AF, Erickson KI, Scalf P, McAuley E, et al. (2004) Cardiovascular fitness, cortical plasticity, and aging. Proc Natl Acad Sci U S A 101: 3316-3321.
98. Kontos AP, Covassin T, Elbin RJ, Parker T (2012) Depression and Neurocognitive Performance After Concussion Among Male and Female High School and Collegiate Athletes. Archives of physical medicine and rehabilitation.
99. Covassin T, Moran R, Wilhelm K (2013) Concussion symptoms and neurocognitive performance of high school and college athletes who incur multiple concussions. Am J Sports Med 41: 2885-2889.
100. Haaland KY, Temkin N, Randahl G, Dikmen S (1994) Recovery of simple motor skills after head injury. Journal of clinical and experimental neuropsychology 16: 448-456.
101. Prigatano GP, Borgaro SR (2003) Qualitative features of finger movement during the Halstead finger oscillation test following traumatic brain injury. Journal of the International Neuropsychological Society : JINS 9: 128-133.
102. Murelius O, Haglund Y (1991) Does Swedish amateur boxing lead to chronic brain damage? 4. A retrospective neuropsychological study. Acta Neurol Scand 83: 9-13.
103. Mittal S, Wu Z, Neelavalli J, Haacke EM (2009) Susceptibility-weighted imaging: technical aspects and clinical applications, part 2. AJNR Am J Neuroradiol 30: 232-252.
104. Kou Z, Gattu R, Kobeissy F, Welch RD, O'Neil BJ, et al. (2013) Combining biochemical and imaging markers to improve diagnosis and characterization of mild traumatic brain injury in the acute setting: results from a pilot study. PLoS One 8: e80296.
105. Vagnozzi R, Signoretti S, Cristofori L, Alessandrini F, Floris R, et al. (2010) Assessment of metabolic brain damage and recovery following mild traumatic brain injury: a multicentre, proton magnetic resonance spectroscopic study in concussed patients. Brain 133: 3232-3242.

106. Sasaki T, Pasternak O, Mayinger M, Muehlmann M, Savadjiev P, et al. (2014) Hockey Concussion Education Project, Part 3. White matter microstructure in ice hockey players with a history of concussion: a diffusion tensor imaging study. J Neurosurg 120: 882-890.
107. Engelhardt S, Patkar S, Ogunshola OO (2014) Cell-specific blood-brain barrier regulation in health and disease: a focus on hypoxia. Br J Pharmacol 171: 1210-1230.
108. Shahim P, Darin N, Andreasson U, Blennow K, Jennions E, et al. (2013) Cerebrospinal fluid brain injury biomarkers in children: a multicenter study. Pediatr Neurol 49: 31-39 e32.
109. Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, et al. (2013) Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS One 8: e75091.
110. Lycke JN, Karlsson JE, Andersen O, Rosengren LE (1998) Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 64: 402-404.
111. Axelsson M, Malmestrom C, Nilsson S, Haghighi S, Rosengren L, et al. (2011) Glial fibrillary acidic protein: a potential biomarker for progression in multiple sclerosis. J Neurol 258: 882-888.
112. Zurek J, Fedora M (2012) The usefulness of S100B, NSE, GFAP, NF-H, secretagogin and Hsp70 as a predictive biomarker of outcome in children with traumatic brain injury. Acta neurochirurgica 154: 93-103; discussion 103.
113. Kuhle J, Leppert D, Petzold A, Regeniter A, Schindler C, et al. (2011) Neurofilament heavy chain in CSF correlates with relapses and disability in multiple sclerosis. Neurology 76: 1206-1213.
114. Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, et al. (2013) Plasma tau levels in Alzheimer's disease. Alzheimers Res Ther 5: 9.
115. Bulut M, Koksal O, Dogan S, Bolca N, Ozguc H, et al. (2006) Tau protein as a serum marker of brain damage in mild traumatic brain injury: preliminary results. Adv Ther 23: 12-22.
116. Liliang PC, Liang CL, Weng HC, Lu K, Wang KW, et al. (2010) Tau proteins in serum predict outcome after severe traumatic brain injury. J Surg Res 160: 302-307.
117. Honda M, Tsuruta R, Kaneko T, Kasaoka S, Yagi T, et al. Serum Glial Fibrillary Acidic Protein Is a Highly Specific Biomarker for Traumatic Brain Injury in Humans Compared With S-100B and Neuron-Specific Enolase. J Trauma.
118. Sundstrom T, Wester K, Enger M, Melhuus K, Ingebrigtsen T, et al. (2013) [Scandinavian guidelines for the acute management of adult patients with minimal, mild, or moderate head injuries]. Tidsskr Nor Laegeforen 133: E1-6.
119. Kiechle K, Bazarian JJ, Merchant-Borna K, Stoecklein V, Rozen E, et al. (2014) Subject-specific increases in serum S-100B distinguish sports-related concussion from sports-related exertion. PLoS One 9: e84977.
120. Constantinescu R, Zetterberg H, Holmberg B, Rosengren L (2009) Levels of brain related proteins in cerebrospinal fluid: an aid in the differential diagnosis of parkinsonian disorders. Parkinsonism & related disorders 15: 205-212.
121. Schlaepfer WW, Lynch RG (1977) Immunofluorescence studies of neurofilaments in the rat and human peripheral and central nervous system. J Cell Biol 74: 241-250.
122. Ganesalingam J, An J, Bowser R, Andersen PM, Shaw CE (2013) pNfH is a promising

biomarker for ALS. Amyotrophic lateral sclerosis & frontotemporal degeneration 14: 146-149.
123. Tortelli R, Ruggieri M, Cortese R, D'Errico E, Capozzo R, et al. (2012) Elevated cerebrospinal fluid neurofilament light levels in patients with amyotrophic lateral sclerosis: a possible marker of disease severity and progression. Eur J Neurol 19: 1561-1567.
124. Boylan KB, Glass JD, Crook JE, Yang C, Thomas CS, et al. (2013) Phosphorylated neurofilament heavy subunit (pNF-H) in peripheral blood and CSF as a potential prognostic biomarker in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 84: 467-472.
125. Erenler AK, Yardan T, Duran L, Baydin A (2013) Usefulness of heart-type fatty acid binding protein in the emergency department. J Pak Med Assoc 63: 1176-1181.
126. Ohrfelt A, Andreasson U, Simon A, Zetterberg H, Edman A, et al. (2011) Screening for new biomarkers for subcortical vascular dementia and Alzheimer's disease. Dement Geriatr Cogn Dis Extra 1: 31-42.
127. Pelsers MM, Hanhoff T, Van der Voort D, Arts B, Peters M, et al. (2004) Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. Clin Chem 50: 1568-1575.
128. Gao X, Chen J (2009) Conditional knockout of brain-derived neurotrophic factor in the hippocampus increases death of adult-born immature neurons following traumatic brain injury. Journal of neurotrauma 26: 1325-1335.
129. Salehi Z, Mashayekhi F (2009) Brain-derived neurotrophic factor concentrations in the cerebrospinal fluid of patients with Parkinson's disease. J Clin Neurosci 16: 90-93.
130. Weinstein G, Beiser AS, Choi SH, Preis SR, Chen TC, et al. (2014) Serum brain-derived neurotrophic factor and the risk for dementia: the Framingham Heart Study. JAMA Neurol 71: 55-61.
131. Zetterberg H, Tanriverdi F, Unluhizarci K, Selcuklu A, Kelestimur F, et al. (2009) Sustained release of neuron-specific enolase to serum in amateur boxers. Brain Inj 23: 723-726.
132. Puchades M, Hansson SF, Nilsson CL, Andreasen N, Blennow K, et al. (2003) Proteomic studies of potential cerebrospinal fluid protein markers for Alzheimer's disease. Brain research Molecular brain research 118: 140-146.
133. Qiang JK, Wong YC, Siderowf A, Hurtig HI, Xie SX, et al. (2013) Plasma apolipoprotein A1 as a biomarker for Parkinson disease. Ann Neurol 74: 119-127.
134. Kay AD, Petzold A, Kerr M, Keir G, Thompson E, et al. (2003) Alterations in cerebrospinal fluid apolipoprotein E and amyloid beta-protein after traumatic brain injury. J Neurotrauma 20: 943-952.
135. Horsburgh K, McCarron MO, White F, Nicoll JA (2000) The role of apolipoprotein E in Alzheimer's disease, acute brain injury and cerebrovascular disease: evidence of common mechanisms and utility of animal models. Neurobiology of aging 21: 245-255.
136. Blennow K, Hesse C, Fredman P (1994) Cerebrospinal fluid apolipoprotein E is reduced in Alzheimer's disease. Neuroreport 5: 2534-2536.
137. Brenner M (2014) Role of GFAP in CNS injuries. Neurosci Lett.
138. Rosengren LE, Lycke J, Andersen O (1995) Glial fibrillary acidic protein in CSF of multiple sclerosis patients: relation to neurological deficit. J Neurol Sci 133: 61-65.
139. Takano R, Misu T, Takahashi T, Sato S, Fujihara K, et al. (2010) Astrocytic damage is far more severe than demyelination in NMO: a clinical CSF biomarker study. Neurology 75: 208-216.
140. Storoni M, Verbeek MM, Illes Z, Marignier R, Teunissen CE, et al. (2012) Serum GFAP levels in optic neuropathies. J Neurol Sci 317: 117-122.

141. Astrand R, Unden J, Romner B (2013) Clinical use of the calcium-binding S100B protein. Methods Mol Biol 963: 373-384.
142. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, et al. (2009) S100B's double life: intracellular regulator and extracellular signal. Biochimica et biophysica acta 1793: 1008-1022.
143. Petzold A, Jenkins R, Watt HC, Green AJ, Thompson EJ, et al. (2003) Cerebrospinal fluid S100B correlates with brain atrophy in Alzheimer's disease. Neurosci Lett 336: 167-170.
144. Graham MR, Myers T, Evans P, Davies B, Cooper SM, et al. (2011) Direct hits to the head during amateur boxing is associated with a rise in serum biomarkers for brain injury. International journal of immunopathology and pharmacology 24: 119-125.
145. Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, et al. (1998) Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. J Cell Biol 143: 777-794.
146. Drechsel DN, Hyman AA, Cobb MH, Kirschner MW (1992) Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. Mol Biol Cell 3: 1141-1154.
147. Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K (1994) Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. Proc Natl Acad Sci U S A 91: 5562-5566.
148. Blennow K, Hampel H, Weiner M, Zetterberg H Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol 6: 131-144.
149. Kay AD, Petzold A, Kerr M, Keir G, Thompson E, et al. (2003) Alterations in cerebrospinal fluid apolipoprotein E and amyloid beta-protein after traumatic brain injury. Journal of neurotrauma 20: 943-952.
150. Bulut M, Koksal O, Dogan S, Bolca N, Ozguc H, et al. (2006) Tau protein as a serum marker of brain damage in mild traumatic brain injury: preliminary results. Advances in therapy 23: 12-22.
151. Kavalci C, Pekdemir M, Durukan P, Ilhan N, Yildiz M, et al. (2007) The value of serum tau protein for the diagnosis of intracranial injury in minor head trauma. The American journal of emergency medicine 25: 391-395.
152. Than ME, Coburger I, Hoefgen S (2014) The structural biology of the amyloid precursor protein APP - a complex puzzle reveals its multi-domain architecture. Biol Chem.
153. van Duijn CM, Tanja TA, Haaxma R, Schulte W, Saan RJ, et al. (1992) Head trauma and the risk of Alzheimer's disease. American journal of epidemiology 135: 775-782.
154. iccini A, Russo C, Gliozzi A, Relini A, Vitali A, et al. (2005) beta-amyloid is different in normal aging and in Alzheimer disease. The Journal of biological chemistry 280: 34186-34192.
155. Graham DI, Gentleman SM, Nicoll JA, Royston MC, McKenzie JE, et al. (1996) Altered beta-APP metabolism after head injury and its relationship to the aetiology of Alzheimer's disease. Acta neurochirurgica Supplement 66: 96-102.
156. Olsson A, Csajbok L, Ost M, Hoglund K, Nylen K, et al. (2004) Marked increase of beta-amyloid(1-42) and amyloid precursor protein in ventricular cerebrospinal fluid after severe traumatic brain injury. Journal of neurology 251: 870-876.
157. Jordan BD (1996) Acute and chronic brain injury in United States National Team Soccer Players. Am J Sports Med 24: 704-705.
158. Kaste M, Kuurne T, Vilkki J, Katevuo K, Sainio K, et al. (1982) Is chronic brain damage in boxing a hazard of the past? Lancet 2: 1186-1188.

159. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelso C (1996) Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem 67: 2013-2018.
160. Rosengren LE, Wikkelso C, Hagberg L (1994) A sensitive ELISA for glial fibrillary acidic protein: application in CSF of adults. J Neurosci Methods 51: 197-204.
161. Olsson A, Vanderstichele H, Andreasen N, De Meyer G, Wallin A, et al. (2005) Simultaneous measurement of beta-amyloid(1-42), total tau, and phosphorylated tau (Thr181) in cerebrospinal fluid by the xMAP technology. Clin Chem 51: 336-345.
162. Zetterberg H, Andreasson U, Hansson O, Wu G, Sankaranarayanan S, et al. (2008) Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease. Archives of neurology 65: 1102-1107.
163. Zetterberg H, Mortberg E, Song L, Chang L, Provuncher GK, et al. (2011) Hypoxia Due to Cardiac Arrest Induces a Time-Dependent Increase in Serum Amyloid beta Levels in Humans. PloS one 6: e28263.
164. Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, et al. (2010) Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nature biotechnology 28: 595-599.
165. Nylen K, Ost M, Csajbok LZ, Nilsson I, Blennow K, et al. (2006) Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. Journal of the neurological sciences 240: 85-91.
166. Daneman M, Carpenter PA (1980) Individual differences in working memory and reading. . Journal of Verbal Learning and Verbal Behavior 4: 450-466.
167. Loring DW, Bauer RM (1990) Testing the limits: cautions and concerns regarding the new Wechsler IQ and Memory scales. Neurology 74: 685-690.
168. Vallat-Azouvi C, Weber T, Legrand L, Azouvi P (2007) Working memory after severe traumatic brain injury. Journal of the International Neuropsychological Society : JINS 13: 770-780.
169. Reitan RM (1994) Ward Halstead's contributions to neuropsychology and the Halstead-Reitan Neuropsychological Test Battery. Journal of clinical psychology 50: 47-70.
170. Hannay HJ (1986) Experimental techniques in human neuropsychology. New York: Oxford University Press.
171. Raftery AE, Madigan, D, Hoeting, J. A. (1997) Bayesian Model Averaging for Linear Regression Models. Journal of the American Statistical Association: 179-191.
172. Franz G, Beer R, Kampfl A, Engelhardt K, Schmutzhard E, et al. (2003) Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. Neurology 60: 1457-1461.
173. Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C, et al. (2011) The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. Journal of neurochemistry.
174. Hasselblatt M, Mooren FC, von Ahsen N, Keyvani K, Fromme A, et al. (2004) Serum S100beta increases in marathon runners reflect extracranial release rather than glial damage. Neurology 62: 1634-1636.
175. Hayakata T, Shiozaki T, Tasaki O, Ikegawa H, Inoue Y, et al. (2004) Changes in CSF S100B and cytokine concentrations in early-phase severe traumatic brain injury. Shock 22: 102-107.
176. Kavalci C, Pekdemir M, Durukan P, Ilhan N, Yildiz M, et al. (2007) The value of serum tau protein for the diagnosis of intracranial injury in minor head trauma. Am J Emerg

Med 25: 391-395.
177. Shahim P, Tegner Y, Wilson DH, Randall J, Skillback T, et al. (2014) Blood Biomarkers for Brain Injury in Concussed Professional Ice Hockey Players. JAMA Neurol.
178. McCrea M, Hammeke T, Olsen G, Leo P, Guskiewicz K (2004) Unreported concussion in high school football players: implications for prevention. Clin J Sport Med 14: 13-17.
179. Sullivan S, Friess SH, Ralston J, Smith C, Propert KJ, et al. (2013) Behavioral deficits and axonal injury persistence after rotational head injury are direction dependent. J Neurotrauma 30: 538-545.
180. Blennow K, Hardy J, Zetterberg H (2012) The neuropathology and neurobiology of traumatic brain injury. Neuron 76: 886-899.

DIAGNOSIS AND MONITORING OF SPORT-RELATED CONCUSSION

A STUDY IN AMATEUR BOXERS

UNIVERSITY OF GOTHENBURG