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A Systematic Review of the Biomarker S100B: Implications for Sport-Related Concussion Management

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Objective: Elevated levels of the astroglial protein S100B have been shown to predict sport-related concussion. However, S100B levels within an athlete can vary depending on the type of physical activity (PA) engaged in and the methodologic approach used to measure them. Thus, appropriate reference values in the diagnosis of concussed athletes remain undefined. The purpose of our systematic literature review was to provide an overview of the current literature examining S100B measurement in the context of PA. The overall goal is to improve the use of the biomarker S100B in the context of sport-related concussion management.

Data Sources: PubMed, SciVerse Scopus, SPORTDiscus, CINAHL, and Cochrane.

Study Selection: We selected articles that contained (1) research studies focusing exclusively on humans in which (2) either PA was used as an intervention or the test participants or athletes were involved in PA and (3) S100B was measured as a dependent variable.

Data Extraction: We identified 24 articles. Study variations included the mode of PA used as an intervention, sample types, sample-processing procedures, and analytic techniques.

Data Synthesis: Given the nonuniformity of the analytical methods used and the data samples collected, as well as differences in the types of PA investigated, we were not able to determine a single consistent reference value of S100B in the context of PA. Thus, a clear distinction between a concussed athlete and a healthy athlete based solely on the existing S100B cutoff value of 0.1 μg/L remains unclear. However, because of its high sensitivity and excellent negative predictive value, S100B measurement seems to have the potential to be a diagnostic adjunct for concussion in sports settings. We recommend that the interpretation of S100B values be based on congruent study designs to ensure measurement reliability and validity.

Key Words: head injuries, physical activity, exercise mode, analysis techniques

Key Points

- When standardized analytical approaches are applied, measuring peripheral S100B in concussion management is beneficial.
- Determining a single consistent reference value for S100B in the context of physical activity is currently not possible. Thus, repeated assessments of individual baseline values (eg, in the preseason) should be conducted.

Undiagnosed or underreported mild traumatic brain injury (mTBI; concussion) can lead to a number of severe and long-term consequences for athletes, including headache, speech and motion dysfunction, impairment in sensory and cognitive perception, and even death.[1] Early identification of sport-related concussion is therefore essential to prevent poor clinical outcomes, ensure the health and well-being of athletes suffering from mTBI, and optimize postinjury performance. Diagnostic imaging can be used to detect brain damage. However, assessment tools such as computed tomography (CT) scans are often not on site or available at sport events; they may be cost intensive and are not ideal for detecting mTBI as they cannot distinguish subtle changes in brain tissue. Thus, a substantial percentage of sport-related mTBIs go unreported or undiagnosed (or both).[2] Difficulties detecting and diagnosing this injury highlight the need for objective and quick indicators of abnormal cerebral processes after mTBI.

In the assessment of traumatic brain injury, S100B is the most widely investigated biomarker.[3] A 21-kDa protein abundant in the central nervous system (CNS), S100B is predominantly expressed in astrocytes, with a cerebrospinal fluid (CSF) to serum ratio of 18:1.[4] When secreted by astrocytes, S100B has neurotrophic and neuroprotective effects at physiologic nanomolar concentrations. However, higher (micromolar) concentrations of S100B have been shown to be neurotoxic and expressed in astrocytic death.[5,6] After a traumatic brain injury, S100B is released or leaked by the cells of the CNS and enters the peripheral bloodstream by passing through the presumably disrupted blood-brain barrier (BBB). The mechanisms that lead to an increase in the peripheral S100B concentration are still unclear. However, because proteins in general do not easily cross the intact BBB,[7] the mechanism of peripheral

S100B increase might be based on an active or passive release of S100B secreting cells, an alteration in the permeability of the BBB, or a combination of these.

Identifying concussed athletes based on S100B assessment requires accurate reference values. A peripheral concentration of S100B in serum less than the recommended cutoff level of 0.1 μg/L[3,8,9] has been associated with negative CT scans regarding traumatic brain injury with a sensitivity of 96.8% and a specificity of 42.5%.[3] The *sensitivity* of peripheral S100B measurement refers to the ability to correctly identify concussed athletes, and the *specificity* refers to the ability to correctly identify those athletes without mTBI. Accordingly, a peripheral S100B level greater than 0.1 μg/L does not necessarily mean that an athlete is concussed and has to be removed from training and competition, because multiple factors can influence the S100B serum concentration level.[3,10–13] Serum S100B values have been measured across diverse groups, making determination of accurate values challenging. For example, S100B values greater than the recommended 0.1 μg/L have been measured in healthy individuals of both sexes younger than 20 years,[12] in healthy adults of different races,[11] and in patients without head trauma but with extracranial injuries.[13] Identification of the appropriate S100B reference value in sport-related concussion management is further complicated by the fact that various types of physical activities affect S100B concentrations in apparently healthy athletes.[14–24]

The range of variables found to affect S100B reference values raises questions concerning the reliability of this substance as a marker of mTBI in athletes. However, S100B testing in the peripheral bloodstream is very economical (about $20).[25] The blood can be sampled anywhere and anytime by appropriate, certified staff and S100B concentrations can be measured using commercially available kits or sent to a reference laboratory. More importantly, the S100B blood test will provide an indication of whether an athlete requires medical follow-up, depending on the magnitude of the increase. A prompt posttraumatic assessment of S100B adds value to the early diagnostic and prognostic analysis of mTBI.[26] Hence, it is important to establish an individual's S100B level via multiple assessments and compare the value with values for concussed athletes matched on factors such as sex, age, race, and the specific type of physical activity (PA) (eg, sprint versus endurance sport, contact versus noncontact sport) to maximize the interpretation of the peripheral S100B value. Adding the peripheral S100B measurement to existing concussion management could enable trained sport medicine professionals (eg, athletic trainers with phlebotomy technician certification) to identify concussed athletes with much greater expediency and accuracy and aid in monitoring their recovery.[27]

The purpose of our systematic literature review was to provide an overview of the current literature describing S100B measurement in the context of PA. Our primary aim was to synthesize the state-of-the-art knowledge regarding S100B reference values in distinguishing healthy and concussed athletes. Our intention was to categorize peripheral S100B values measured after different types of PA in healthy athletes. We hypothesized that vigorous PA increases peripheral S100B levels beyond the cutoff level of 0.1 μg/L in the absence of mTBI. A second aim of the review was to provide an overview of the diverse theories that explain increases in peripheral S100B concentrations in the context of PA. Furthermore, we discuss the effects of various methodologic factors, including the timing of sample withdrawal, sample processing and analysis, and choice of the analytical technique, all of which have considerable influence on S100B values. Finally, we offer examples and recommendations regarding peripheral S100B measurement in sport-related concussion management.

METHODS

The systematic review was conducted in accordance with the guidelines outlined in the *Cochrane Handbook for Systematic Reviews of Interventions*.[28] Based on these guidelines, we defined the hypothesis, developed criteria for study inclusion and data collection, and made determinations with regard to the presentation and interpretation of the results. Furthermore, 2 researchers used the Physiotherapy Evidence Database (PEDro) scale[29] to rate the methodologic quality of the studies. The PEDro scale is an 11-item scale designed for rating the methodologic quality of randomized controlled trials. Studies scoring 9 or 10 are considered to have *excellent* internal methodological validity; 6 to 8, *good*; 4 or 5, *fair*; and less than 4, *poor*.[30] Initial discrepancies between the reviewers were discussed, and consensus was reached on all PEDro scores.

Search Strategy

We searched the following electronic databases with no date or language limitations: PubMed, SciVerse Scopus, SPORTDiscus, CINAHL, and Cochrane. These databases were searched using the following key words: *S100B*, *S100β*, *S100beta*, *S-100B*, *S-100beta*, *S-100β*, *biomarker*, *assess**, and *diagnos**. The references were imported into the literature-management program Endnote (version X5; Thomson Reuters, Carlsbad, CA). After we eliminated duplicate publications, 2605 potentially relevant abstracts remained.

Study Selection

The search was restricted to studies focusing on (1) humans (2) in which either PA was used as an intervention or physically active individuals or athletes were test participants, and (3) studies involving S100B as the dependent variable. These abstracts were individually evaluated by 2 independent reviewers, both experienced researchers in the field of sports medicine. The title, abstract, and key words of each publication were considered to determine if the inclusion criteria (1–3) were satisfied. The final data reported in the review were based on the reviewers' consensus. A total of 29 abstracts met these criteria. Because methods or measurements were not completely described in the 29 abstracts, we obtained the full-text articles of each to determine if the study should be included in the systematic review. One reviewer completed a full-text article evaluation to assure that all inclusion criteria were met. In the end, 26 articles met the predefined

criteria and were included in our systematic literature review.

Data Extraction

To extract the data, we developed a questionnaire with the following 4 main categories:

1. Filter questions (Is the abstract referring to the biomarker S100B in its function as a diagnostic tool? Is PA or exercise defined as an intervention or an influencing factor of S100B?)
2. Formal information about the articles (year of publication, type of document, type of article, research area, name of journal, address of corresponding author)
3. Information about the protein S100B (term used for S100 calcium binding protein B, measurement unit, cutoff level, details of S100B levels—study results, type of sample tissue, time sample was withdrawn, details of sample processing, main approaches used to explain alterations of S100B levels)
4. Information about the study design (type and details of intervention, method of S100B analysis, details about test participants)

To ensure agreement between the reviewers, we pilot tested the questionnaire on 5 articles included in the systematic review before data extraction began. The initial Holsti coefficient for intercoder reliability[31] was 94%. Discrepancies in data extraction were solved by discussion between the reviewers.

We categorized the results in tables using the original descriptions from reviewed articles (eg, no history of cardiac, cerebrovascular, respiratory disease[32]) or sorted them into rational categories (eg, group *soccer* included, eg, soccer training session with[24,33–36] and without heading[35,36]). The S100B levels were described in the articles using different SI units (μg/L, pg/mL, ng/L, ng/mL), so we converted these to μg/L with 2 decimal places for standardization purposes.

Statistical Analysis

According to the *Cochrane Handbook for Systematic Reviews of Interventions*,[28] 4 critical criteria need to be satisfied to conduct a meta-analysis of existing studies: (1) identification and selection of studies, (2) heterogeneity of results, (3) availability of information, and (4) analysis of the data.[37] Of the articles that met our inclusion criteria, many failed to address at least 2 of the criteria necessary to conduct a meta-analysis, namely heterogeneity of results and availability of information. The dissimilar results among the articles could be attributed to differences in the individual study designs. Furthermore, the lack of information that we intended to extract (eg, details of the study design and actual values for S100B) precluded the possibility of conducting a meta-analysis. Finally, the *Cochrane Handbook* indicates the futility of conducting statistical analyses when the aforementioned criteria have not been satisfied as "meta-analyses of poor quality studies may be seriously misleading."[38]

In addition to assessing the available literature according to the 4 criteria outlined above, we assessed the methodologic quality of the studies using the PEDro scale.[29] This scale has been adopted previously to rate the methodologic quality of randomized controlled trials, which is a common practice in systematic reviews. Three of the PEDro scale items—blinding, concealed allocation, and randomization—have evidence for discriminative validity.[39] As complete blinding of participants is impossible when assessing modalities of PA and exercise and because of deficiencies in the design (eg, no control group) and description of the study methods (eg, no information regarding the allocation of treatment), we rated most articles as *fair* to *poor* on the PEDro scoring scale. Based on the guidelines of the *Cochrane Handbook for Systematic Reviews of Interventions*[28] and the PEDro scores, conducting a meta-analysis was inappropriate for this type of systematic review.

RESULTS

We found widespread nonuniformity in terms of study designs, analytical methods used, data samples collected, and types of PAs examined. Thus, we provide a systematic description of the results regarding the S100B values, types of PA reported, participants, explanatory approach for increased S100B values, time of specimen collection, specimen processing and storage processing, analytical measurement, and methods used for analysis (Table 1).

The S100B Values

The S100B values described in the articles were based on a range of study designs (ie, pre-post design, group comparisons, intervention) and thus, in some studies, there were no pretest or posttest values ascertained, descriptions regarding S100B values were insufficient, or results were expressed in "differences" or "percentages" without showing total values (Table 1). A categorization of S100B values according to the kind of PA was statistically impossible. However, there was a tendency toward significant increases in peripheral S100B values after competitive and vigorous PA in the absence of apparent head injury (see "Mode of PA" and Table 2).

Mode of PA

We identified more than 9 kinds of PA and categorized them according to the descriptions provided in the articles. As mentioned earlier, a trend indicated that vigorous PA might cause significant increases in peripheral S100B values. Conspicuous are those S100B concentrations that exceed concentrations of more than 0.1 μg/L[3,8,9] in the absence of apparent head injury (Table 2).

Participants

Participants were described variously as breath-hold and scuba divers (experienced or professional), race walkers and runners, wrestlers (Greco-Roman, freestyle), soccer players (professional, amateur), basketball and ice hockey players, trained swimmers, boxers (amateur), and as other "professional" athletes and "physically active" individuals (treadmill walking, ergometer bicycling, bungee jumping). The sex of the participants was described in 22 articles, with male participants being investigated disproportionally

(males = 665 versus females = 14). None of the authors provided an explanation for the sex ratio. The age of the participants in the intervention groups ranged from 17 to 52 years. Only 1 article provided information about the race of the participants.[35] In 5 articles, previous concussions and notable findings such as a history of head injury were described.[19,22,34,36,48] Apart from that, either the participants were described as having unremarkable health histories or we could not obtain any information regarding their health status. The sample sizes in the chosen articles varied between n = 1 (case control study[43]) and 535 (prospective cohort study[35]). The sizes of groups exposed to an intervention (any kind of PA) ranged between n = 5[44] and n = 69[35] (Table 3).

Explanations of Increased Peripheral S100B Due to PA

The results of our systematic review revealed diverse approaches to explaining increases in peripheral S100B concentrations in the context of PA. An overview of all main approaches with which the authors explain increases of peripheral S100B in the context of PA is in Table 4. Separately or in combination, the explanatory approaches refer to cerebral and extracerebral sources and active and passive release mechanisms, including a possible passage through the BBB to explain increased peripheral S100B levels (Table 4).

Timing of Sample Collection

Authors of articles with a pre-post study design described the time of sample collection before the intervention as *prior*, *before*, *pre-exercise*, or *at the start of the intervention*,* with few details regarding the exact point of time of blood collection *(–5 and 0 minutes before*,[20] *1 to 5 hours before*,[48] *1 to 2 hours before*,[22] *24 hours before*,[23] *8 to 9 AM at the start*[43]). In 1 study,[20] the investigators monitored S100B values during the intervention at 15-minute intervals. The time of measurement after the intervention was described generally as *postexercise*,[46] *end of*,[45] *after*,[14] *next morning*,[35] or, more precisely, as *immediately following*,[15,47] *right after*,[35] *immediately after*,[19,48] or *15 minutes after* the race.[23] Furthermore, points of time of the blood collection were characterized as fixed intervals (*5*, *10*, *15*, *30*, *60*, *120 minutes after apnea*[45]; *after 20*, *60, 80 minutes of recovery*[18]; *0*, *1*, *3*, *20 hours after the race*[21]; *10 minutes thereafter*[42]; *within 15 minutes after*[44]; *20 minutes after*[16]; *within 1 hour after*[22]; *after 0.53 ± 0.06 hours*, *1.97 ± 0.06 hours*, *4.02 ± 0.07 hours*[34]; *60 and 360 minutes after the heading session*, *64 and 355 minutes after the exercise session*, and *65 minutes after trauma*[36]; and *71 minutes after*[49]). In addition, the details of the time of blood collection were described as *over the course of time*, as well as *after 26 hours*,[43] *48 hours after the end of the race*,[15] *after 5 days*,[43] *days 1 and 10 of experimental testing*,[47] *7 to 10 days after the training session*,[33] *after 2 months of resting*,[33] and *at different altitudes*[32] (see also Table 1).

*References 14–17,19,21–24,34–36,42,44–47,49.

Processing of the S100B Sample

To obtain S100B values, the sample processing consisted of several steps. The different types of samples (blood, saliva, CSF) have to be processed to obtain serum or plasma, which can then be stored or processed further (or both). The articles described different types of sample processing.

Some samples were *allowed to clot cool*[49] or by *keeping them on ice or snow*,[32,46] *at 5°C*,[22] or *at room temperature*[18] *for 30 minutes*,[35] *60 minutes*,[18] or *for 3 hours maximum*.[49] The following steps of centrifugation were described in slightly different terms according to acceleration, temperature, and time. Some of the samples were centrifuged *immediately after the collection*[3,4] or *within 2 hours*[24] *with an adjustment of 900g for 10 minutes*,[14] *1000g for 10 minutes at 4°C*,[16] *3000g for 10 minutes*,[35] or *3000g for 7 minutes*.[23] If the samples were transferred for storage,[44] they were stored either on dry ice[24] or at 0°C in a cooler.[44] In other articles, the procedure of sample processing after obtaining the supernatant was described as an *immediate freezing*[23] or *within 2 hours*,[35] *at –20°C*,[18,20,46,49] *–70°C*,[14,16,23,36] *–78°C*,[19,22,34,42,44] or *–80°C*.[15,24,33,47] In 1 study, the handling of samples was followed "according to standards and brought to the laboratory for further processing."[3,4] Other researchers provided no further information (see also Table 1).

The S100B Analysis

According to the type and size of the sample, different methods and principles of the immunoassay techniques were used to assess S100B levels. The collective title "immunoassay" represents a specific biochemical testing technique that uses antibodies to identify or quantify the presence or concentration of a substance (S100B) in solutions that frequently contain a complex mixture of substances, such as biological fluids (eg, serum, plasma, saliva, CSF; Table 1). The immunoassay uses an antibody, immobilized on a plastic surface, that binds to its specific targets (eg, S100B; antigen-antibody reaction). Another reagent is used to generate a signal from the captured material. The level of signal indicates the concentration of the substance.[50] According to the labels used in the system of immunoassays, the methods described in the selected articles of our systematic review are listed in alphabetic order. Detection limits between 0.005 and 0.02 μg/L were listed as reference values for the analytic methods, and intra-assay and interassay coefficients of variation were determined to be approximately 10% or less (Table 5).

DISCUSSION

Categorization of Sport-Related S100B Reference Values

To support the use of peripheral S100B measurement in sport-related concussion management, appropriate reference values are needed. Thus, we reviewed the current literature in the context of S100B measurement and PA. Our main goal was to categorize peripheral S100B values measured before and after different types of PA in healthy athletes. Given the nonuniformity in the types of sports,

Table 1. Methods Used to Measure S100B Levels[a] Extended on Next Page

Reference	Physical Activity	Time of Blood Withdrawal	Sampling and Storage
Schulte et al,[40] 2013	LC to examine lactate-induced changes in serum [S100B] without physical activity	Before start LC, after 12 min LC, at end of LC, and 6, 12, 24, and 60 min after LC	Serum obtained by centrifugation and frozen and stored for 1 mo at −40°C until analysis
Schulte et al,[41] 2011	A: Cycling with vibration B: Cycling without vibration Means: time = 25:27 ± 1:30 min, vibration = 20 Hz, amplitude = 4 mm	Rest, immediately before and after test and at 30, 60, and 240 min postexercise	Serum prepared from samples by centrifugation and frozen and stored at −40°C until analysis
Stavrinou et al,[42] 2011	Recreational scuba diving (conservative dive profile; open water) Total: 3 dives in 2 d, 12 h between sessions	Before dive and 10 min after	Samples allowed to clot, centrifuged immediately, 0°C, transferred for storage at −78°C until analysis
Michetti et al,[14] 2011	A: Vigorous training B: Sedentary	Before and after physical activity/stress	Immediately centrifuged (900*g* for 10 min), stored at −70°C
Spiropoulos et al,[15] 2010	A: "Spartathlon" foot race (246 km) mean = 32 h 8 min, Md = 30 h 2 min, range = 25 h 17 min–34 h 43 min B: Sedentary	Before start of race and immediately after and 48 h after end of race	Stored frozen at −80°C until analysis
Bjursten et al,[32] 2010	Hike to 4554 m above sea level	At 1155 m after acclimatization, 3647, 4554, 3647, 1155 m	Cooling with snow, centrifugation (3000*g* for 10 min, within 1 h), stored frozen until analysis
Arslan et al,[16] 2010	A: Local Greco-Roman wrestling competition B: Freestyle wrestling competition (3 × 2 min per match)	Before and 20 min after matches	Centrifugation (1000*g* for 10 min, +4°C), stored at −70°C
Zetterberg et al,[43] 2009	2-mo period of nonparticipation in boxing	At the start, after 2 mo of resting from championship/training boxing	nd
Liner and Andersson,[44] 2009	International breath-hold diving competition (70 m, 90 s) resulted in LOC	Within 15 min after LOC, after 26 h, after 5 d	Handled according to standards and brought to laboratory for further processing
Andersson et al,[45] 2009	A: Voluntary, maximum-duration apnea diving 335 ± 38 (range = 281–403) s B: Resting in supine position	Beginning of recordings, at start and end of apnea, fixed intervals (5, 10, 15, 30, 60, 120 min after apnea)	Samples kept on ice, centrifuged, frozen, and later analyzed
Straume-Naesheim et al,[35] 2008	A: Head impact occurring during a soccer league match B: Regular league match with no recorded head trauma C: High-intensity training session without heading D: Low-intensity training session with heading exercises only	Before the season/training session, right after the match/training session, next morning	Clot (30 min), centrifugation (3000*g* for 10 min), frozen within 2 h until analysis
Stålnacke and Sojka,[34] 2008	A: 5 × Heading soccer ball falling from 18 m B: No heading	Before, after 0.53 ± 0.06 h, 1.97 ± 0.06 h, 4.02 ± 0.07 h	Clot, centrifugation, transport, frozen and stored at −78°C until analysis
Cheuvront et al,[46] 2008	10 d of heat acclimatization (100 min of treadmill walking in 45°C)	Pre-exercise and postexercise, d 1 and d 10 of testing	Serum stored and frozen (−20°C) until analyses

Table 1. Extended From Previous Page and Continued on Next Page

Analysis/Sample Type	Significance	S100B, μg/L	
		Pre	Post
Immunoluminometric assay/ serum	ns	All groups: 0.03 (0.0–0.09)	nd
Luminometric assay/serum	ns	All groups: 0.05 (0.01–0.09)	nd
Immunoluminometric analysis/ serum	ns	Dive 1, 0.06 ± 0.01 Dive 2, 0.06 ± 0.01 Dive 3, 0.06 ± 0.01[b]	Dive 1, 0.11 ± 0.06 Dive 2, 0.07 ± 0.01 Dive 3, 0.07 ± 0.01[b]
Immunoluminometric assay/ saliva	s Pre > post, A > B, $P < .01$	A: Md = 0.75 B: Md = 0.30	A: Md = 2.29 B: Md = 0.83
Electrochemiluminescence immunoassay/serum	s Pre-post $P < .001$	A: 0.13 ± 0.01 B: nd	A: Postrace = 0.29 ± 0.01, 48 h after race = 0.13 ± 0.01 B: nd
Immunometric process/serum	s < .05 versus baseline	Increase from baseline of 122% (SD = 80%), 42% (SD = 27%), 47% (SD = 34%), and 33% (SD = 25%), respectively, at different time points	
ELISA method/serum	s Pre-post A: $P < .001$ B: $P < .01$ ns groups	A: Md 0.04 (0.01–1.26) B: Md 0.03 (0.01–0.14)	A: Md 0.9 (0.05–1.67) B: Md 0.07 (0.03–0.17)
Biochip array technique/serum	ns	A: 0.07 (0.03–0.24) B: 0.07 (0.02–0.13)	
Electrochemiluminescence immunoassay/serum	Case report	0.1 after 15 min LOC 0.1 after 26 h 0.05 after 5 d	
Immunoradiometric assay/ serum	s Pre-post A: <.05 B: ns	nd	Average pre-post A: Δ 37% B: nd
Electrochemiluminescence assay/serum	s Pre-post all groups nd A and B had higher increases than C and D, $P < .001$	All groups 0.05 (0.02–0.11) BL (n = 49), 0.05 (0.03–0.09)–0.06 (0.03–0.11)	Δ B1: A and B: 0.06 (0.05–0.07) versus C and D: 0.03 (0.02–0.03) 33.9% elevated B1 >0.12 (after match with/without head impacts)
Immunoluminometric assay/ serum	ns	A: 0.16 ± 0.13 B: 0.16 ± 0.08	0.5 h after A: 0.11 ± 0.02 B: 0.11 ± 0.04 2 h after A: 0.1 ± 0.03 B: 0.1 ± 0.04 4 h after A: 0.11 ± 0.04 B: 0.1 ± 0.04
ELISA/serum	ns	Total range = 0.05–0.12	Δ d 1: 0.02 ± 0.04 Δ d 10: 0.01 ± 0.03

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Table 1. Continued From Previous Page and Extended on Next Page

Reference	Physical Activity	Time of Blood Withdrawal	Sampling and Storage
Zetterberg et al,[33] 2007	Heading (ball kicked from distance of 30 m) A: 10 times B: 20 times	CSF and serum: 7–10 d after training session	CSF: stored at −80° until analysis Serum: nd
Schulpis et al,[17] 2007	A: Forced training, α-tocopherol (200 mg/24 h os) supplement for 30 d B: Forced training	Pre and post forced training before and after α-tocopherol supplement	nd
Watson et al,[18] 2006	NF cycling in a climatic chamber (35.0°C ± 0.5°C), 55% $\dot{V}O_2$peak for 6 × 15 min without replacement of sweat loss	During final minute of each exercise period (pre, post 20, 60, 80 min, recovery)	Clot at room temperature (60 min), centrifuged to yield serum, stored at −20°C until analysis
Stålnacke et al,[19] 2006	2 Competitive soccer games	Before and immediately after game	Clot, centrifugation, stored at −78°C until analysis
Saenz et al,[47] 2006	Boston Marathon (69°F – 70°F), mean finishing time (250 ± 33 min)	Before and immediately after race	Aliquots of samples frozen within 1 h of collection at −80°C
Watson et al,[20] 2005	Sitting in water tank for 30 min immersed to neck (39.0°C ± 0.1°C) and "W" cycling in a climate chamber (35°C ± 0.3°C), 60% $\dot{V}O_2$peak for 60 min	2 samples (−5 and 0 min), during exercise at 15-min intervals	Centrifuged to yield serum; kept frozen at −20°C until analysis
Stålnacke et al,[48] 2004	2 Competitive soccer games	1–5 h before, immediately after game	Clot, centrifugation, transport, frozen, stored at −78°C until analysis
Hasselblatt et al,[21] 2004	Münster Marathon, Md 4:12 (range = 2:56–4:55) h	Before and 0, 1, 3, 20 h after race	nd
Stålnacke et al,[22] 2003	Competitive games of Swedish Elite Ice Hockey League and Swedish Elite Basketball League	1–2 h before (ie, just before warming up) and within 1 h after game	Clot (5°C), centrifugation, frozen and kept at −78°C until analysis
Mussack et al,[36] 2003	A: Controlled heading Md 55 (range = 49–63) min B: Normal exercise Md 61 (58–66) min C: TBI CCT+ visible brain damage D: TBI CCT− without visible brain damage	A: Before and 60, 360 min after heading session B: Before and 64, 355 min after exercise session C, D: 65 min after trauma	Samples processed to serum, supernatants frozen in aliquots at −70°C until batch evaluation
Dietrich et al,[23] 2003	7600-m swimming race, XIII Travessio do Pontal de Tapes, March 2002 (107.6 ± 3.4 min)	24 h before and 15 min after race	Serum obtained by centrifugation (3000*g* for 7 min), immediately frozen and stored at −70°C
Woertgen et al,[49] 2002	Bungee jump, 50 m, 2.8*g*	Before, immediately after, 71 min after jump	Samples were cooled for 3 h maximum, serum frozen and stored at −20°C until analysis
Otto et al,[24] 2000	A: Boxing competition (5 × 2 min) B: Boxing sparring bouts (3–5 × 2 min) C: 25-km race (98–142 min) D: Cross-country jogging (10 km, 55–75 min) E: Exhaustive running/sprint (3× as fast as possible for 2 min) F: Exhaustive ergometer cycling (3× as fast as possible for 2 min), 200–375 W G: Headers (20× heading back a 450-g football 7.5 m without jumping)	Before and within 15 min after each exercise	Samples spun down within 2 h and serum stored first on dry ice and finally at −80°C

Abbreviations: BL, baseline; B1, within 1 h after match/game training session; CCT, cranial computed tomography; CI, confidence interval; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; LC, lactate clamp; LOC, loss of consciousness; Md, median; nd, no details; NF, non-fluid; ns, not significant; os, by mouth; s, significant; TBI, traumatic brain injury; W, warm; Δ, difference.

[a] The type of physical activity, the time of blood withdrawal and S100B analysis techniques, and the sample types are described using the descriptions and terms from reviewed articles. The Significance column indicates if the differences within the S100B data were statistically significant and provides further information. S100B data are given as mean ± SD and/or (range) and in μg/L with 2 decimal places (conversion as needed) unless otherwise indicated.

[b] Mean (SD) calculated based on the data in the article.

[c] Data not represented here.

Analysis/Sample Type	Significance	S100B, μg/L	
		Pre	Post
Electrochemoluminescence immunoassay/serum, CSF	ns	None	A: Md 0.06 (0.03–0.12) B: Md 0.04 (0.01–0.07) C: Md 0.04 (0.03–0.06)
Immunoluminometric assay/ serum	s Pre-post $P < .001$ Groups post $P < .001$	A: 0.11 ± 0.03 B: 0.12 ± 0.02	A: 0.28 ± 0.06 B: 0.18 ± 0.04
ELISA/serum	s Pre-post $P < .001$	0.09 ± 0.02	0.20 ± 0.06
Immunoluminometric assay/ serum	s Pre-post $P = .000$	0.11 ± 0.05	0.18 ± 0.11
Biosite Triage stroke panel/ plasma	ns	0.06 ± 0.14	0.06 ± 0.12
ELISA/serum	s Pre-post $P = .02$	0.07 ± 0.05	Δ 0.12 ± 0.10
Immunoluminometric assay/ serum	s Pre-post $P < .001$	0.06 ± 0.03	0.12 ± 0.04
Luminometric assay/serum	s Pre-post $P < .001$	nd	Δ 0.05
Immunoluminometric assay/ serum	s Pre-post A: $P = .00004$ B: $P = .001$	A: 0.22 ± 0.04 B: 0.22 ± 0.04	A: 0.30 ± 0.11 B: 0.30 ± 0.10
Immunoluminometric assay/ serum	s Pre-post in B after age stratification[c] Pre in A, B after age stratification[c] C versus D Groups A versus B C versus D	A: Md 0.15 (0.08–0.25) B: Md 0.10 (0.03–0.14) C: Md 0.62 (0.46–1.02) D: Md 0.10 (0.04–0.27)	A: Md 0.18 (0.11–0.27) B: Md 0.11 (0.06–0.20) C: Md 0.32 (0.19–0.61) D: Md 0.08 (0.04–0.17)
Monoclonal immunoluminometric assay/ serum	s Pre-post $P < .001$	0.07 ± 0.02 (0.01–0.25)	0.11 ± 0.02 (0.01–0.3)
Radioimmunoassay/serum	ns	0.22 ± 0.02 (0.2–0.26)	Post 0.22 ± 0.03 (0.19–0.28) After 71 min 0.23 ± 0.02 (0.2–0.27)
Immunoluminometric assay/ serum	Significant increase after boxing and running disciplines but not after cycling or heading (P: nd provided)	Mean (Md) all groups 0.04 (0.04) (0.01–0.17)	Δ Mean (Md), (range) A: 0.12 (0.09), (0–0.26) B: 0.04 (0.03), (0–0.12) C: 0.07 (0.06), (0–0.21) D: 0.04 (0.03), (0–011) E: 0.02 (0.02), (0–0.07) F: 0 (0), (−0.04–0.06) G: 1.3 (0), (−0.02–0.01)

analytical methods used, and data samples collected, as well as the diversity of participants investigated, we were not able to determine a single consistent reference value of S100B in the context of PA. Thus, a clear distinction between concussed and healthy athletes based on the existing S100B cutoff value of 0.1 μg/L[3,8,9] remains unclear.

In the following sections, we will provide a comprehensive theory of increased peripheral S100B levels after PA, including a focus on release mechanisms, sources of S100B, and renal elimination. Furthermore, we will focus on the effects of different methodologic approaches on S100B values, as well as the interpretation of peripheral S100B increases in clinical practice. We will draw

Table 2. Modes of Physical Activity in Studies of S100B Concentrations[a]

Mode of Physical Activity	Details	S100B > 0.1 μg/L	
		Pretest	Posttest
Basketball	Forced basketball training with α-tocopherol supplementation[17]	↑	↑
	and competitive games of the Swedish Elite Basketball League[22]	↑	↑
Cycling	Prolonged cycling with and without replacement of sweat loss[18]		↑
	Prolonged cycling under temperate and warm conditions[20]		↑
	Exhaustive ergometer cycling[24,41]		
Diving	Competitive[43] and voluntary breath-hold diving[45]		
	Recreational scuba diving[42]		
Hiking/running/walking	Ascent at 4554 m[32]		
	"Spartathlon" foot race[15]	↑	↑
	Boston Marathon[47]		
	Münster Marathon[21]		
	25-km race, cross-country jogging, and exhaustive sprints[24]		
	Prolonged treadmill walking[46]		
Ice hockey	Competitive games of the Swedish Elite Ice Hockey League[22]	↑	↑
Martial arts	Competitive wrestling[16]		
	Competitive boxing[24,43]		↑
Soccer	Soccer league matches[19,35,48]		↑
	Soccer training session with heading[24,33–36]		
	Soccer training session without heading[35,36]		
Swimming	7600-m swimming race[23]		↑
Other	Bungee jumping,[49] unspecified vigorous training,[14] lactate clamp in sitting participants[40]		

[a] The different modes of physical activities are sorted in rational categories, listed in alphabetical order (left column), and described in further detail (middle column). The right column indicates with an arrow (↑) if S100B concentrations in the peripheral blood unequivocally exceeded the cutoff level of 0.1 μg/L.

conclusions based on the results and, finally, offer recommendations regarding the use of peripheral S100B measurement in sport-related concussion management.

A Comprehensive Overview of S100B-Increasing Mechanisms and Sources

The results of our systematic review indicate that PA can lead to an acute significant increase in mean peripheral S100B concentration,[14,15,17–20,22–24,36,48] exceeding the cutoff value of 0.1 μg/L,[3,8,9] in the absence of apparent head trauma. In addition to acute alterations in S100B concentrations, involvement in intense PA under competitive and stressful conditions might also modify S100B baseline values. In professional sportsmen, Michetti et al[14] and Stålnacke et al[22] found noticeably high S100B baseline concentrations that exceeded the upper limit for a normal healthy adult population.

We identified different approaches to explain the increased S100B levels during PA in the peripheral bloodstream and combined them into 1 comprehensive theory. We considered cerebral and extracerebral sources, active and passive release mechanisms, possible passage through the BBB or blood-CSF barrier, and renal elimination (Figure).[51]

Cerebral S100B Release. Based on the findings of our systematic review, we speculate that an elevated S100B level is a sign of cerebral cell death and represents the passive release of S100B from damaged neurons or glial cells (or both, including those from the BBB) without any stimulated active release into the extracellular compartment.[58,59] Alternatively, elevated S100B may also be the result of a pathophysiologic cascade that exerts a neurotoxic effect and leads to secondary brain tissue damage.[5,59,60] Because S100B is most abundant in astroglial tissue,[60] cerebral brain damage (eg, trauma, ischemia, disease, intoxication) can lead to a peripheral increase in S100B levels.[3] Some researchers[21,22,36] suggested that chronic vibration or an acute axial impact (or a combination) related to PA (eg, running, jumps, heading) might be a source for cell damage and thus an explanation for the rise in S100B during PA. Contrary to this theory, a missing correlation between a serum increase in S100B and acceleration–deceleration events in ice hockey indicates that a mechanical impact might not be a cause of enhanced release of cerebral blood into the peripheral bloodstream.[22] Although various theories explain increases in serum S100B under pathophysiologic conditions such as concussion, researchers concur on the need for further study of the causes, mechanisms, and consequences of these increases.

Vigorous PA cannot be regarded as a pathologic condition per se that leads to cell damage. Therefore, an increase in peripheral S100B after sports need not necessarily result from leakage of the protein out of damaged cells. Mental and metabolic stress, as occurs in competitive sports, increases peripheral S100B levels in the absence of apparent brain injury.[59,61–64] In an experimental study, Agawa et al[61] found serum serotonin levels increased during challenging exhaustive exercise. Teradaira et al[64] showed similar results by exposing sitting students to a stress model using visual display terminals; they noted increased plasma concentrations of serotonin. Because of the features of the enzyme kinetics, Agawa et al[61] and Teradaira et al[64] concluded that increased levels of peripheral serotonin, among other markers, appeared to be induced by mental stress. One approach to explain stress-induced increased peripheral S100B levels is serotonin-induced release of intraglial S100B due to activation of cerebral 5-HT_{1A} receptors.[65] This receptor activation

stimulates the expression and release of S100B, which, accompanied by an alteration in the BBB during stressful conditions,[18,20,66] might be a physiologic up-regulation to initiate S100B's neuroprotective effects. Sport competitions with mentally challenging situations are closely connected to metabolic stress. Both aerobic and anaerobic exercise can induce a state of oxidative stress that leads to the physical reaction of an up-regulation in endogenous antioxidant defenses.[61] Gerlach et al[62] found support for the theory of S100B up-regulation under severe metabolic stress conditions such as hypoxia. They suggested that the secretion of S100B up to nanomolar concentrations is an early astroglial response to metabolic injury that initiates neuroprotective and neurotrophic effects.[67] Furthermore, S100B is elevated and released into the blood circulation in a variety of CNS disorders that are accompanied by inflammatory signaling by activating advanced glycation end products.[68] Is the release of S100B an effect of the condition and thus a passive release by damaged inflammatory cells or rather the cause, revealing its dose-dependent neuroprotective function? Furthermore, cell death to eliminate dysfunctional inflammatory cells might also be a source of S100B release.[69] Increased release after PA as well as in athletes' baseline values could be related to physiologic regulatory mechanisms and might be a sign for a low-grade systemic inflammation in professional and competitive conditions of sports.[14,22,69]

Passage of S100B Through the BBB. The release mechanisms discussed in the previous paragraph describe a release of S100B in the brain. Increased release of S100B from brain cells does not necessarily lead to increased peripheral levels of S100B because of the protective function of the BBB. This barrier regulates the homeostatic, nutritive, and immune environments of the CNS and the exchange of molecules between the CNS and peripheral bloodstream. Proteins with molecular weights up to 600 Da are known to cross the BBB.[7] As a 21-kDa protein, S100B cannot pass the intact BBB passively by diffusion, nor are there any indications of an active-transport mechanism in any direction.[58]

To enter the peripheral bloodstream, cerebral S100B has to cross the altered BBB with increased permeability. The permeability might be increased because of passive mechanisms such as sport-related BBB damage.[5] Shear stress in terms of mechanical impact, axial vibrations, accelerations–decelerations, inconspicuous falls, collisions, or simple jumping or heading may be involved in sport-related head trauma.[70] This type of mechanical stress can affect endothelial physiology and the formation of the tight junctions,[71] and thus the permeability of the BBB, allowing S100B to pass through the BBB into the peripheral blood flow.

Another explanation for facilitated passage of S100B through the BBB is the effect of vigorous PA.[66,72,73] Sharma et al[72] proposed a mechanism by which PA up-regulates serotonin levels under severe stressful conditions (ie, forced swimming of rats in a water maze). Serotonin binds to the 5-HT_2 receptors, increasing BBB permeability.[72] In parallel, serotonergic activation and the modulation of the serotonin receptor 5-HT_{1A} promote increased expression of astroglial S100B (see "Cerebral S100B Release").[74] Additionally, initial results from a study by Fontes-Ribeiro et al[73] suggest a slight increase in BBB permeability by decreasing the occludin protein levels of the BBBs' tight junctions during acute, intense PA. Dysfunction of the BBB can expose the brain to hazardous molecules and pathologic organisms, significantly affecting normal brain function.

Furthermore, Watson et al[18,20] reported an increase in serum S100B concentration after prolonged exercise in a warm environment (relative humidity = 56% ± 5% and 60% ± 5%). Here, S100B is discussed as a peripheral marker of BBB integrity. Shrinking of BBB cells due to heat stress concomitant with a loss of body fluid could be a mechanism by which the protein S100B passes through and leaks into the peripheral bloodstream. Total body fluid loss from increased sweating and deficient fluid ingestion during intense or prolonged exercise may lead to shrinkage of barrier endothelial cells, which can cause a temporary gap in the tight junctions and reduce the barrier integrity. In addition to the ways exercise may contribute to increased BBB permeability (eg, hyperthermia, central serotonergic neurotransmission), Watson et al[18,20] stated that exercise in a warm environment may lead to a loss of body fluid (sweat), resulting in plasma hyperosmolality and a shift of fluids from the interstitial and intracellular spaces. Extracellular osmolality can influence the volume of the brain[75] and possibly the permeability of the BBB.[18] Thus, the possibility that an osmotic fluid shift across the BBB into the periphery due to sweating in warm conditions (eg, sauna) causes the washout of cerebral S100B has to be taken into consideration when using the S100B measurement in the diagnosis of concussed athletes.

Even though most of the current literature refers to S100B as a marker of BBB permeability, the passage of S100B from the brain to the peripheral bloodstream is not specifically related to the BBB. The barrier between the blood and the CSF is made up of leaky capillaries, allowing a passage for proteins.[5,76] Hence, these leaks might be a passage for S100B to move from the CNS to the peripheral bloodstream and increase S100B concentrations. Nevertheless, Marchi et al[77] concluded that the transthyretin monomer is a more appropriate candidate marker for blood-CSF barrier alterations, as this protein is primarily localized in choroid plexus CSF. However, S100B seems to be a better marker for indicating alterations in the permeability of the BBB.

Noncerebral S100B Release. Whereas the previous section highlighted the release of S100B from cerebral sources and the passage through physiologic barriers such as the BBB and the blood-CSF barrier, we now focus on the contribution of S100B from peripheral sources**.** Activation or exercise-induced damage of peripheral tissue containing S100B will likely lead to an increase in serum S100B.[78] Several sources, apart from the brain, could contribute to the serum S100B content by active or passive release. Immunoassays and mRNA quantification of cells have identified adipocytes, erythrocytes, chondrocytes, lymphocytes, bone marrow cells, melanocytes, testes, heart, and aorta as S100B-expressing cells. The contribution of S100B from adipose tissue due to lipolysis or muscle cell membrane ruptures seems to be most plausible.[21,80–83] Because of the low brain specificity of the protein S100B, a debate has arisen during the last decade as to the origin of serum S100B and its release. Several lines of evidence suggest that S100B serum concentrations can be significantly affected by

Table 3. Participants in Studies of Physical Activity and S100B Concentrations[a] Continued on Next Page

Reference	Participant Groups	Sex and No.	Clinical History	Age, y
Schulte et al,[40] 2013	A: Exercise science students B: CG: no	M 8	Participants displaying cerebral and neurophysiologic problems excluded from study after medical examination	25.8 ± 4.1
Schulte et al,[41] 2011	A: Healthy nonsmoker B: CG: no	M 12	No noteworthy medical history, especially with respect to neurologic problems	25.3 ± 1.6
Stavrinou et al,[42] 2011	A: Experienced healthy divers B: CG: no	M 5	No history	37.2 (23–42)
Michetti et al,[14] 2011	A: Professional sportsmen B: CG: Healthy controls	A: M 25 B: M 50	Negative history for nervous system diseases/infections and neurodegenerative disorders	A: 23 ± 3 B: 24 ± 2
Spiropoulos et al,[15] 2010	A: Runners/healthy race walkers B: CG: sedentary healthy men	A: M 10 B: M 10	No history of recent infection or other disease	A: 42.8 ± 1.4 (33–53) B: 42.2 ± 10.4 PR: nd
Bjursten et al,[32] 2010	A: Experienced healthy hikers B: CG: no	? 7	No history of cardiac, cerebrovascular, respiratory disease; 3 participants developed acute mountain sickness 3 y earlier	42–51 PR: nd
Arslan et al,[16] 2010	A: Greco-Roman wrestlers B: Freestyle wrestlers	A: M 15 B: M 16	Excluded: smoking, chronic illness, musculoskeletal problems, alcohol intake, any medication	A: Md 19 (19–30) B: Md 20 (19–26) PR: nd
Zetterberg et al,[43] 2009	A: Healthy amateur boxers B: CG: Healthy controls	A: M 44 B: M 23	A: No comorbid conditions/ medications; bouts: 840 (200–2000) B: No TBI/combative sports	A: 19 (17–28) B: 28 (19–50) PR: nd
Liner and Andersson,[44] 2009	A: Healthy professional breath-hold diver B: History: no	M 1	Pre: nd Post: LOC	Total: 23 PR: nd
Andersson et al,[45] 2009	A: Healthy trained breath-hold divers B: CG: limited experienced divers	A: M 8, F 1 B: M 5, F 1	Healthy participants, no LOC at end of apnea	Total: 31 (7) PR: nd
Straume-Naesheim et al,[35] 2008[b]	Norwegian professional soccer players A: Head impact B: Match control C: High-intensity exercise D: Heading	A: ? 69 B: ? 56 C: ? 48 D: ? 46	A: Total: 228 head impacts from 352 matches, 13 time-loss injuries, 7 concussions B: No recorded head trauma during matches C, D: nd	A: 28.1 (22.5–35.0) B: 26.2 (19.0–33.0) C: 26.1 (18.5–33.6) D: 26.1 (18.4–33.7)
Stålnacke and Sojka,[34] 2008	Soccer players A: Heading B: No heading	A: M 10 B: M 9	Total: No. of previous concussions 0–6	Total: 22 ± 8 PR: nd
Cheuvront et al,[46] 2008	A: Healthy volunteers B: CG: no	M 9	No medications, otherwise healthy on physical examination	19 (18–21) PR: nd
Zetterberg et al,[33] 2007	Healthy amateur soccer players A: 10× heading B: 20× heading C: CG: healthy, nonathletes	A: M 10 B: M 13 C: M 9	All were healthy and none showed any signs of focal neurologic injury	A: Md 26 (19–32) B: Md 23 (20–28) C: Md 24 (22–27) PR: nd
Schulpis et al,[17] 2007	A: Basketball players B: CG: no	M 10	nd	18.5 ± 0.6 PR: nd
Watson et al,[18] 2006	A: Physically active males B: CG: no	M 8	None accustomed to exercising in warm environment at time of study	25.8 ± 6.5 PR: nd
Stålnacke et al,[19] 2006	A: 4 Elite Soccer League teams B: CG: no	F 44	No. of previous concussions 1.4 ± 1.9 (0–5) for n = 26	23 ± 3 PR: nd
Saenz et al,[47] 2006	A: Marathon runners B: CG: no	F 6 M 29	No cardiovascular diseases	49 ± 7 PR: nd

Table 3. Continued From Previous Page

Reference	Participant Groups	Sex and No.	Clinical History	Age, y
Watson et al,[20] 2005	A: Active healthy men B: CG: no	M 7	Excluded: participants with history of metabolic disease or psychiatric illness	25.7 ± 5.0 PR: nd
Stålnacke et al,[48] 2004	A: 4 Elite Soccer League teams B: CG: no	M 28	No. of previous concussions: 0–7, no concussions during study	26 ± 5 PR: nd
Hasselblatt et al,[21] 2004	A: Runners B: CG: no	F 4 M 14	Neither head injury nor other neurologic or medical problem encountered throughout or after race	39 ± 8 PR: nd
Stålnacke et al,[22] 2003	A: 2 Ice hockey teams B: 2 Basketball teams	A: M 26 B: M 18	No. of previous concussions: 0–4	A: 28 ± 4 B: 25 ± 4 PR: nd
Mussack et al,[36] 2003	Amateur soccer players A: Heading group B: Exercise group C: TBI CCT+ D: TBI CCT−	A: M 61 B: M 58 C: M 20 D: M 61	Conspicuous findings in A: 49/61 B: 38/58	A: Md 15.3 (14.8–16.4) B: Md 15.9 (15.0–16.8) C: Md 41.8 (32.3–61.1) D: Md 37.1 (27.6–53.5) PR: nd
Dietrich et al,[23] 2003	A: Trained swimmers B: CG: no	M 16	Independent of any traumas caused by axial vibration of brain	25.4 ± 2.2 PR: nd
Woertgen et al,[49] 2002	A: Healthy patients B: CG: no	M 8 F 3	No LOC, no neurologic deficit after jump	28 (16–45) PR: nd
Otto et al,[24] 2000	A: Amateur boxers B: Amateur boxers C: Volunteers D: Volunteers E: Volunteers F: Athletes G: Sportsmen	A: M 10 B: M 13 C: M 11 D: M 12 E: M 12 F: M 12 G: M 12	nd	A/B: 17–40 (Md 20) C: 20–44 (Md 32) D: 23–52 (Md 30) E: 25–52 (Md 30) F: 23–52 (Md 28.5) G: 22–52 (Md 26) PR: nd

Abbreviations: ?, sex not specified; CCT, cranial computed tomography; CG, control group; F, females; LOC, loss of consciousness; M, males; Md, Median; nd, no details; PR, participants' races; TBI, traumatic brain injury.

[a] Age data are given as mean ± SD and/or (range) unless otherwise indicated.

[b] Percentage of participants who were Norwegian or Scandinavian: A = 79.7, B = 87.8, C = 81.3, D = 80.4. This is the only study for which details about participants' race or ethnicity were available.

extracerebral sources. On the one hand, researchers have shown increases in S100B concentrations and thus tendencies toward false-positive values of S100B after large extracranial injuries[13] and multiple trauma[84] (see review by Gang and Gang[78]). On the other hand, an investigation of 200 participants by Pham et al[10] did not reveal a significant contribution of S100B expressed in adipocytes to peripheral S100B levels. Given the expression of S100B in adipocytes, Pham et al[10] studied the relationship between individuals' fat content and S100B levels by determining body mass index (BMI). However, determining individuals' fat content by BMI calculation has to be regarded critically. The BMI is a formula based upon an individual's weight and height. The National Institutes of Health have acknowledged major shortcomings of the BMI calculation as an individual's body fat—particularly in an athlete or athletic individual—may be overestimated.[85]

Despite the fact that S100B is most abundant in cerebral tissue, previous authors have shown[80,83] that contributions to the serum increases might originate from extracerebral sources (ie, melanocytes, erythrocytes, fat cells, testes, heart, and aorta). In sum, further studies are needed to clarify whether increased peripheral S100B reflects the damage to brain tissue or an opening of the BBB or whether the physiologic side effects of PA increase the release of S100B by cerebral or extracerebral sources.

Renal Elimination. In addition to sources and mechanisms that contribute to an increase in S100B in the peripheral bloodstream, this protein is also subject to renal elimination and thus a down-regulating mechanism. The S100B protein is metabolized and eliminated mainly via degradation in the proximal tubules of the kidney. Peripheral S100B concentrations need between 25 minutes[86] and 132 minutes[87] to fall to half their value as measured at the beginning of the time period (half-life). The flow rate of filtered fluid through the kidney (glomerular filtration rate) is not related to the half-life of urinary S100B protein.[88] Thus, alteration of the glomerular filtration rate by PA has no influence on the clearance of urinary S100B protein in sport-related mTBI. Consequently, peripheral S100B concentrations would be chronically increased because of renal dysfunction. Depending on the exercise mode and intensity, PA might be a risk factor for renal failure due to severe dehydration from excessive sweating,[89] especially in combination with nonsteroidal anti-inflammatory drugs.[90]

The Influence of Human Biology on S100B Baseline Values

As discussed previously, PA, the methodologic approach regarding the choice of sample, the sample-processing procedures, and the analytical techniques used could affect

Table 4. Explanations of Increased Peripheral S100B Due to Physical Activity[a]

Reference	Theory
Schulte et al,[40] 2013	Variations in brain activity according to serotonergic activity after intensive physical activity
	Muscular origin of S100B after damage due to mechanical mechanisms that force BBB alterations
Schulte et al,[41] 2011	Various sports involving rigorous mechanical impact on the head
	Physical activity to exhaustion/after competition for approximately 2 h
Stavrinou et al,[42] 2011	Cumulative neural tissue damage by subclinical neurotrauma
Michetti et al,[14] 2011	Physiologic up-regulation or inflammatory cascades under condition of stress and/or physical activity
	Acute/chronic hypoxia conditions
Spiropoulos et al,[15] 2010	Exercise-induced inflammation
Bjursten et al,[32] 2010	Compromised integrity of BBB by mental stress or hypoxic conditions
Arslan et al,[16] 2010	Injury of cerebral or extracerebral tissue
Zetterberg et al,[33] 2007; Zetterberg et al,[43] 2009	Brain injury caused by heading in soccer/amateur boxing
Liner and Andersson,[44] 2009	Impaired integrity of central nervous system: BBB disruption not necessarily related to either neuronal or glial brain damage
Andersson et al,[45] 2009	Temporary opening of BBB
Straume-Naesheim et al,[35] 2008	Activities with high intensity and/or number of headers
Stålnacke and Sojka,[34] 2008; Stålnacke et al,[19] 2006; Stålnacke et al,[48] 2004; Stålnacke et al,[22] 2003	Game-associated activities and events
	No. of headers and other trauma events
	Direct head trauma (heading) and acceleration/deceleration of body without head trauma (falls, collisions, jumps, etc)
	Opening/increased permeability of BBB during exercise
Cheuvront et al,[46] 2008	Exercise heat strain, alterations in BBB integrity
Schulpis et al,[17] 2007	Release from muscle and nerves induced by training
Watson et al,[18] 2006; Watson et al,[20] 2005	Alterations in the integrity of BBB due to exercise-induced hyperosmolality/prolonged exercise in a warm environment
Saenz et al,[47] 2006	Systemic inflammatory response to exertional rhabdomyolysis
	Subclinical central nervous system damage
Hasselblatt et al,[21] 2004	Extracranial release: skeletal muscle tissue
Mussack et al,[36] 2003	Transient release due to brain damage after heading
	Whole-body stress during regular exercise
	Extracranial release: fat, skin, skeletal muscle tissue
Dietrich et al,[23] 2003	Variations in physiologic brain activity: increased serotonin acting in 5-HT_{1A} receptors
	Peripheral sources: lipolysis
Woertgen et al,[49] 2002	Brain tissue damage after acceleration and deceleration/shear force
Otto et al,[24] 2000	Mechanical impact to the brain

Abbreviation: BBB, blood-brain barrier.

[a] In the cases of multiple authors and publications, the explanatory approaches were condensed.

the assessment of S100B values. Furthermore, several factors in human biology, such as age and sex, may influence S100B baseline levels in the peripheral bloodstream without PA. Most of the participants in the reviewed studies were described as physically active individuals of both sexes and of different ages. Few if any details were provided regarding participants' ethnic backgrounds or race.

The results of our review showed a conspicuous male-to-female ratio (based on 22 articles) of 48:1, and therefore a clear underrepresentation of females. None of the authors provided a rationale for the sex ratio. The tendency to examine male participants and exclude female participants in S100B research reflects a gender bias within the broader natural sciences and reveals an ongoing failure to address sex differences or similarities in study design and analysis, leading to a gender bias in research.[91] Discrepant information exists regarding the influence of sex on peripheral S100B values. No statistically significant differences between males and females were found in healthy individuals aged 18 to 65 years,[92] 18 to 80 years,[93] male and female term neonates, children, or adults up to 70 years.[12] Additionally, Stålnacke et al[48,94] confirmed that S100B in the serum of adult soccer players increased equally in both sexes. However, Gazzolo et al[95] found significant sex differences when S100B concentrations were correlated with age. The S100B concentrations in the blood of female pediatric patients monitored from birth to 15 years of age differed significantly from those in male patients of the same age, suggesting that brain maturation in the pediatric period differs by sex, as it does in the intrauterine and adult periods.

The ages of the participants in the intervention groups ranged from 17 to 52 years. Gazzolo et al[95] described a negative correlation between blood S100B protein concentrations and gestational age, with higher concentrations in neonates. This correlation was not apparent in individuals older than 20 years of age.[12,95] Additionally, Wiesmann et al[92] found a weak correlation between decreasing concentration and increasing age, with no significant differences between age groups. The findings of increased S100B values in children during the first year of life and in adolescence could indicate alterations in BBB permeability[36] or possible neurotrophic effects of S100B as a cytokine at physiologic concentrations to induce and support brain maturation and neuronal outgrowth.[6] How-

Table 5. Analytical Techniques for Measuring S100B Concentrations[a]

Analytical Technique	Procedure	Validation Characteristics
Biochip array technique[43]	The biochip array technique was used in 1 of the reviewed studies. The multi-analyte approach by biochip array technology is based upon ELISA principles and contains discrete regions of antibodies specific for different proteins. Thus, the device allows simultaneous quantification of the different substances in a single serum sample.[51]	Intra-assay and interassay CV: $\leq$10%[43]
Biosite Triage Stroke Panel[47]	The Biosite Triage Stroke Panel was described in the methods of 1 article. This assessment panel is a point-of-care fluorescence immunoassay for the rapid quantitative measurement of brain natriuretic peptide and fibrin degradation products containing D-dimer, MMP-9, and S100B in EDTA-anticoagulated whole blood or plasma specimens. The test results are presented as a multimarker index value in the assessment and diagnosis of stroke.[52]	Biosite Triage Stroke Panel CV: 15%[47]
Electrochemiluminescence immunoassay[15,33,35,43]	In 4 publications, the method of electrochemiluminescence immunoassays used to analyze the concentration of S100B was described. The technique is based on a solid-phase, streptavidin-coated microparticle and electro-chemiluminescence technology. The label used in the system enables extremely stable reagents compared with enzyme conjugates.[53]	Roche Diagnostics CV for all biochemical analyses were $<$10%[33] Roche Elecsys; Roche Diagnostics, F. Hoffmann-La Roche Ltd, Basel, Switzerland Lower detection limit: 0.005 μg/L[35] Roche S100 reagent kit; Roche Diagnostics GmbH, Mannheim, Germany: Measurement range: 0.02–39 μg/L Total imprecision (CV total) at 0.2 μg/L: 1.29%[43]
ELISA[16,18,20,46]	The technique of ELISA was mentioned 4 times in the methods of reviewed studies. This technique is commonly performed in ''wet labs'' using antibodies labeled with an enzyme marker. Alterations in biological activity of the enzyme are a result of the enzyme-antibody-antigen reaction, which is proportional to the concentration of the antigen.[54]	Bio Vendor, Modrice, Czech Republic Lowest detection limit: 0.005 μg/L Intra-assay CV for 0.41 μg/L: 4.5% Interassay CV for 0.47 μg/L: 3.1%[16] Sangtec Medical, Bromma, Sweden: Intra-assay CV: 3.5–7.2%[17,18,20] Fujirebio Diagnostics AB, Göteborg, Sweden: Intra-assay CV: 11%[46]
Immunoluminometric assay[14,17,19,21–24,34,36,42,48] Immunoradiometric assay[45,48]	The use of luminometric assays was illustrated in 11 publications; it is thus the most common technique for S100B assessment. Detection of S100B in body fluids by luminometry is based upon counting the emission of light by a substance not resulting from heat of light via phototubes.[55] Depending on the principle and technique, different types of luminescence are applied (eg, immunoluminometric assay for S100B detection). Immunoluminometric assays are based on labeling and detecting with either chemiluminescent or bioluminescent molecules to identify substances such as S100B in biological fluids.[56] The application of immunoradiometric assay was described in 2 articles. Immunoradiometric assay is a form of radioimmunoassay in which a specific antibody is added directly to the test antigen (eg, S100B). Radioimmunoassays measure antigen-antibody reactions using a radioactively labeled substance to measure the binding of the unlabeled substance to a specific antibody or other receptor system.[54] The radioimmunoassay technique is extremely sensitive and extremely specific but requires specialized equipment and licensing.	Sangtec Medical, Bromma, Sweden Detection limit: 0.02 μg/L[14,22,34,42,48] Measuring range: 0.02–30 μg/L[17] Intra-assay CV: 5.5% Interassay CV:10.1%[14] Intra-assay CV: 6.4% at 0.11 μg/L, 2.8% at 1.6 μg/L, and 3.6% at 18.4 μg/L Interassay CV:11% at 0.11 μg/L, 3.7% at 1.6 μg/L, and 3.2% at 18.4 μg/L[34] LIA-mat Sangtec 100, Bromma, Sweden Intra-assay CV: 5.5% at 0.28 μg/L Interassay CV: 10.1% Detection limit: 0.2 μg/L[24] LIA-mat, Byk-Sangtec 100, Dietzenbach, Germany Detection limit: 0.02 μg/L CV: 5%[23] CV: 9.5%[49] Sangtec 100, DiaSorin, Dietzenbach, Germany Detection limit: 0.02 μg/L[21,40] Measuring range: 0.02–30 μg/L[40] CV: $<$5%[40]

Abbreviations: CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay.

[a] Typical validation characteristics in regards to the analytical procedure are accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range. Information about these characteristics is provided only if mentioned in the reviewed articles.

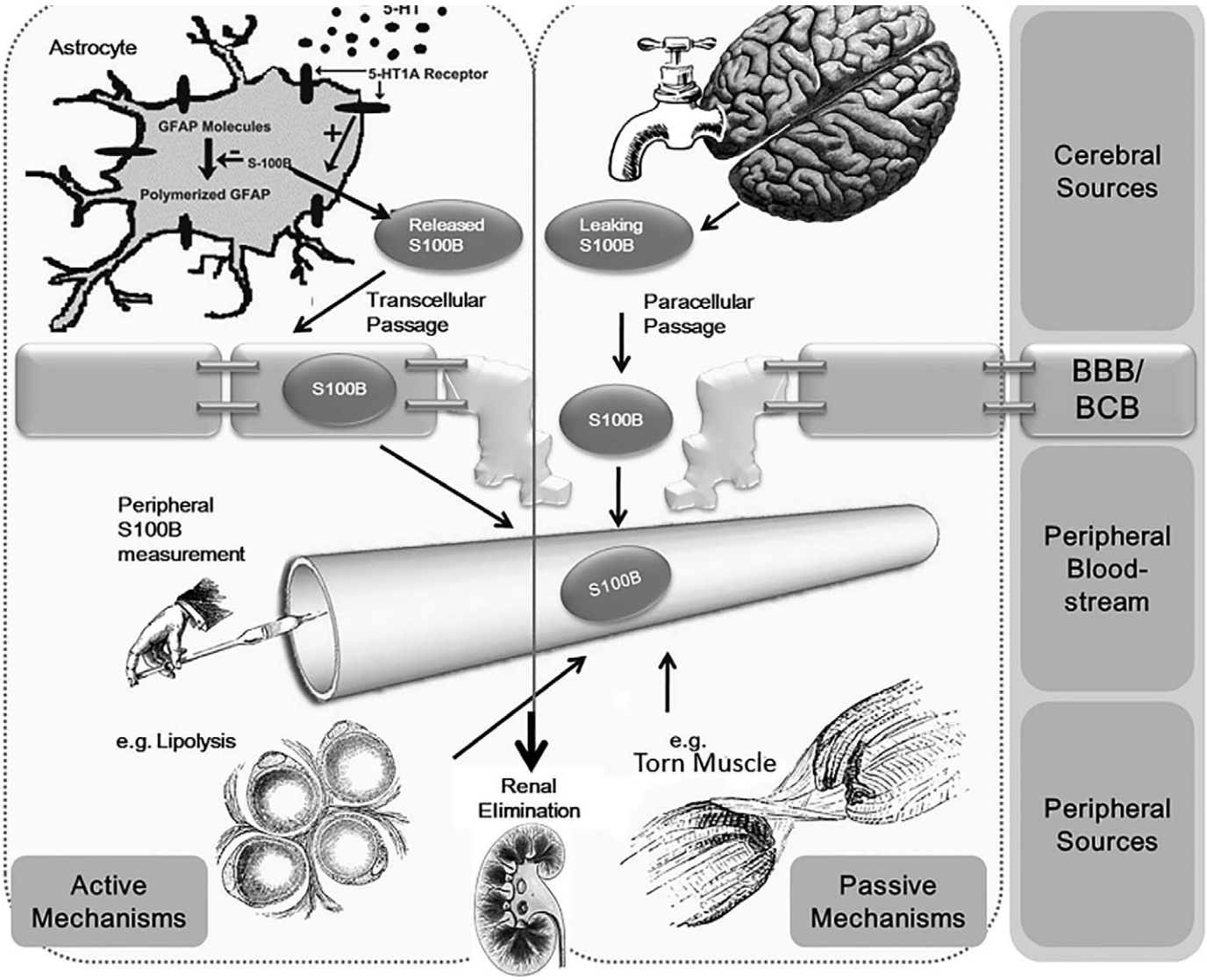

Figure. Sport-related S100B increases. Comprehensive overview with possible release mechanisms and sources that might lead to peripheral S100B increases in the context of physical activity. Once S100B is released from cerebral sources, it has to pass the blood-brain barrier (BBB) to enter the peripheral bloodstream.[57] Additionally, peripheral sources might contribute to an increase in peripheral S100B levels. In each case, active and passive mechanisms might be involved in the cascade. From http://etc.usf.edu/clipart. Abbreviations: BCB, blood-cerebrospinal fluid barrier; GFAP, glial fibrillar acidic protein.

ever, Einav et al[93] found no correlation between S100B concentration and age. These inconsistent findings suggest that S100B baseline concentrations decrease up to the age of 20 years but do not vary beyond that point.

Information about the citizenship of the participants was provided in only 1 article,[35] so we were unable to draw conclusions regarding the race or the skin color of athletes. Athletes of different races have similar densities of melanocytes, whereas the differences in skin color are reflected by melanocytic activity. As S100B is also expressed by melanocytes in selected elements of normal skin,[96] athletes' skin color might also influence S100B baseline concentration in the peripheral bloodstream. Ben Abdesselam et al[11] investigated serum S100B concentrations in 136 healthy individuals divided into 3 groups according to race (the authors offered no further details about the group classification) into group A (Asian), B (black), and C (Caucasian). Healthy adult individuals in groups A and B had higher serum S100B concentrations than group C. As differences in skin color are reflected by melanocytic activity and melanocytes from dark-skinned individuals have a higher metabolic activity than those of fair-skinned individuals,[97] the differences in baseline peripheral S100B concentrations might be due to the different levels of metabolic activity reflected by skin color.

Together, these results indicate that there may be an influence of PA, age, sex, and athlete skin color on S100B baseline values in the peripheral blood and that these are important factors for correctly interpreting athletes' S100B values to assess and manage concussion. These factors need to be understood for adults as well as for children, with a special focus on the transition at approximately age 20 years, before S100B is routinely measured to aid in concussion management. Hence, baseline values as a point of reference should be assessed on a regular basis.

The Effects of Different Methodologic Approaches on S100B Values

To measure the S100B concentration, a variety of methodologic aspects must be considered regarding the timing of the sample collection, sample type, and sample processing, as well as the analytic technique used. In combination, the range of options is huge and differs in reliability, validity, and economic cost.

Timing of Sample Collection. The results of our systematic review provided few details regarding the appropriate time of sample withdrawal. Authors using a pre-post study design described the time of sample withdrawal postexercise as a maximum of 24 hours before the intervention and inconsistently postexercise. Variations in the time between an S100B-level–increasing event and collection of the sample can also be expected to influence the accuracy of the diagnosis and determination of injury severity in sport-related concussion. The half-life of S100B has been shown to be 25 minutes,[86] whereas studies of patients with mTBI demonstrated half-lives of 97 minutes[98] and 132 minutes.[87] However, these investigators did not discuss the possibility of impairment of renal function, which would slow the elimination rate. Furthermore, increased release of S100B has been found in cell-culture models within 15 minutes of injury.[99] Assuming that the half-life of S100B is less than half an hour, the time elapsed between a potential concussion and sample collection is likely to affect the accuracy of diagnosis of sport-related concussion based on peripheral S100B measurement. A peripheral S100B level $\leq$0.1 μg/L within 3 to 4 hours of injury predicts a CT scan that is negative for mTBI, but the measurement is more accurate if taken within the first 30 minutes after a potential injury, based on the short half-life of this protein.[3,8,9] Ideally, sport-related concussion assessment includes a combination of self-reported symptoms, postural control, and neurocognitive function.[100] Experience has shown that it takes less than 30 minutes to administer and assess a combined test battery (eg, Immediate Post-Concussion Assessment and Cognitive Testing [ImPACT], Standardized Assessment of Concussion [SAC], Balance Error Scoring System [BESS]) using an established process.[101,102] In the context of this assessment (before or after), an additional blood draw to measure S100B would be practical and add value to the early diagnostic and prognostic analysis of sport-related concussion.

Sample and Sample Processing. The protein S100B can be detected in diverse biological fluids such as CSF, blood components (serum, plasma), urine, saliva, amniotic fluid, and even human milk.[59] We identified 4 different types of S100B samples (serum, plasma, saliva, CSF) that have been investigated and various methods for processing samples (time, centrifugation, temperature). None of the articles provided a precise indication regarding the storage time of the frozen sample.

Baseline saliva samples seem to contain higher concentrations of S100B (test = 0.75 μg/L, control = 0.30 μg/L)[14] compared with serum (0.12–0.14 μg/L)[11] or plasma (0.05 μg/L)[92] concentrations in normal healthy adults. However, blood (plasma, serum) and CSF S100B levels are reliable biomarkers to predict outcomes in patients with mTBI (see review by Michetti et al[59]). Plasma is the liquid, cell-free component of whole blood, whereas serum is also free of fibrinogen and other clotting factors. Both are extracted by centrifugation, but plasma is prepared by collection in a tube containing an anticoagulant. Common anticoagulants that are used in clinical and laboratory practice are EDTA, heparin, and citrate. Tort et al[103] evaluated the influence of anticoagulants (EDTA, heparin, citrate) on plasma and serum S100B levels. When anticoagulants were used, plasma levels of S100B were higher than serum levels. However, heparin plasma samples were highly correlated with serum samples. Thus, they recommended using heparin plasma when an anticoagulant is required.[103]

Improper technique for blood collection can lead to hemolysis: the membranes of the erythrocytes rupture and release their hemoglobin into the blood plasma. Based on their results, Beaudeux and colleagues[104] concluded that hemolysis might be a cause of increased serum levels of neuron-specific enolase but not of S100B. In 2008, however, Pfeifer et al[105] showed significant increases in both S100B and neuron-specific enolase. Thus, because the prediction of mTBI is more accurate when a panel of complementary biomarkers is used,[106] hemolyzed samples should not be analyzed as they might produce false-positive results.

With regard to storage time, unfortunately, authors of the studies we reviewed provided few if any details on which to base recommendations for further research. When details about the storage temperature were provided, most of the samples were stored at temperatures between −70°C and −80°C. Raabe et al[107] analyzed S100B serum samples (Liaison assay; Byk-Sangtec Diagnostica, Dietzenbach, Germany) immediately and after 4, 8, 12, and 24 hours, stored at room temperature, at 4°C or frozen, and defrosted after 24 hours. They found no effect of storing temperatures or time periods. Similarly, Ikeda et al[108] noted unaltered serum concentrations 7 months after venipuncture and storage at −70°C. However, Müller et al[109] showed that S100B was not stable in frozen serum over 6 years stored at −20°C; values increased during long-term storage. Hence, serum samples can be stored without temperature concerns even overnight and serum S100B concentrations will be unaffected.[109] Long-term storage over years, however, is not recommended.

Techniques for Analyzing S100B. Müller et al[109] found that the choice of analytical method might also play a significant role in determining S100B levels. Results from the Liaison Sangtec 100 (enzyme-linked immunosorbent assay) and Elecsys S100 (Roche Diagnostics, Mannheim, Germany) immunoassays were not interchangeable: S100B concentrations were higher measured using the Liaison Sangtec 100 test.[109] Einav et al[93] also found that values measured by the enzyme-linked immunosorbent assay method (Liaison Sangtec 100) tended to be higher than those measured by the Elecsys S100, particularly when S100B levels exceeded 0.7 μg/L. Levels of S100B exceeding 0.7 μg/L could be interpreted as indicating brain injury.[93] Consequently, the use of different analytical methods is not recommended. Furthermore, test-specific cutoff values for commercial kits are needed to make S100B measurement more effective for sport-related concussion management.

Typical validation characteristics for analytical procedures are accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range. The *accuracy*, also called *trueness* of an analytical procedure, describes "the

Table 6. Best Practices in Sample Patients with Sport-Related Concussion[a]

	Case 1	Case 2
Individual symptoms of soccer player after head trauma	Disorientation	Disorientation, blurred vision, dizziness, headache
Loss of consciousness	30 s	5.5 min
S100B levels 1 h after head trauma	0.5 mg/L	0.12 mg/L
Interpretation	Strong indication of brain tissue injury	Extremely low risk of intracranial lesion
Recommendations	Further investigation or examination with, eg, computed tomography Special attention: increased risk for long-term persisting symptoms	High probability for good outcome

[a] Modified according to Stålnacke et al.[48]

closeness of agreement between the value that is accepted, either as a conventional true value or an accepted reference value, and the value found."[110] Whereas *accuracy* refers to the true value, *precision* describes the repeatability or intra-assay precision (the precision under the same operating conditions), the intermediate precision (precision within a laboratory), and the reproducibility (precision between laboratories) of the measurement. The *specificity* of a test is the ability to assess the substance unequivocally in the presence of other components that are expected to be present. The smallest amount of the substance in a sample that can be detected is called the *detection limit* of an individual analytical procedure. This amount need not be quantified as an exact value. The smallest amount of the substance that can be quantitatively determined with suitable precision and accuracy is called the *quantitation limit* of the assay. The *linearity* of an analytical procedure refers to the test results that are directly proportional to the levels of the substance in the sample within the upper and lower concentration (*range*) for which the analytic procedure has been demonstrated to have suitable levels of precision, accuracy, and linearity.[110]

The articles we reviewed list no information about accuracy, specificity, quantitation limit, or linearity. However, adequate information was provided for the measuring range, including the lower detection limit, and the precision, expressed as the coefficient of variation. A detection limit up to 0.02 μg/L seems to be a common reference value for the analytic methods that are currently available. Additionally, intra-assay and interassay coefficients of variation were determined to be approximately 10% or less (Table 5). For most analytes, a coefficient of variation less than 5% represents acceptable performance. Coefficients of variation up to 10% may be acceptable, but those exceeding 10% are rarely acceptable, except at very low concentrations.[111]

Interpreting Peripheral S100B Increases

The interpretation of S100B values is difficult, as there are no clear, unambiguous reference values that take into account all of the influences discussed previously, especially in the presence of PA. To reliably predict an athlete's diagnosis and outcome, the S100B post–traumatic-event values should be compared with individual baseline values using the same analytical approach. How peripheral S100B measurement could provide appropriate information for the management of sport-related concussion is illustrated by 2 cases of patients with concussion who had loss of consciousness (Table 6).[48]

According to several grading scales that are routinely used for concussion,[112] it could be tempting to conclude that the player in case 2 has a more severe type of mTBI than the player in case 1. The S100B concentrations are above the average in case 1, whereas that in case 2 is still within the range of healthy adult individuals.[11] This indicates a high risk for injured brain tissue only in the player case 1 and requires further investigation (eg, CT) and special attention regarding decision making about returning to training or game play because of the increased risk for long-term, persistent symptoms.

CONCLUSIONS

After an isolated head injury, S100B levels of less than the current cutoff value of 0.1 μg/L[3,8,9] have been associated with CT scans that are negative for mTBI.[3,113] As such, a peripheral S100B concentration less than 0.1 μg/L indicates that the patient likely did not suffer an mTBI (high negative predictive value).[114,115] Although the conflicting results make it complicated to interpret S100B values in the context of sport-related mTBI, the excellent negative predictive value of changes in S100B levels allows the possibility of brain injury to be excluded.[116,117] However, peripheral S100B measurement in athletes based on a general cutoff level of 0.1 μg/L must be evaluated critically. Competitive and vigorous PA, in addition to intraindividual variability, may affect peripheral S100B levels, which may affect the interpretation of S100B levels among an athletic population. Accordingly, repeated assessment of reference values for each athlete is required over the course of the athlete's career. Based on the results of our systematic review (for overview, see "Recommendations"), we believe that the measurement of peripheral S100B can add value to the early diagnostic and prognostic analysis of sport-related concussion. The peripheral S100B concentration can be available within an hour of blood sampling[118] and costs around $20,[25] making this assessment tool in sport-related concussion management affordable for most in school and mass and professional sports.

The S100B protein has gained a role as a complementary specific index of early diagnosis and prognosis in the management of mTBI or concussion associated with sports. To establish reliable, valid S100B reference values for use in the management of sport-related concussion, more studies are needed to clarify the details of S100B increases in athletes under different conditions. Unraveling the mechanism of S100B neurotoxicity and assessment of the therapeutic effects of S100B protein are promising research directions for achieving the optimal clinical treatment of

traumatic brain injury. Because S100B alone is not diagnostic for sport-related mTBI, additional eligible biomarkers (eg, neuron-specific enolase) need to be identified to assemble a promising panel of biomarkers to differentiate among various types and levels of brain injury. Furthermore, the implementation of point-of-care devices with clinically acceptable quality (ie, high sensitivity and specificity for the tool measuring S100B levels) that can detect multiple biomarkers in a timely manner would facilitate sport-related concussion assessment on site and provide the information needed for appropriate treatment and return-to-play decisions. Even though no blood-based on-site tests are currently approved to diagnose concussion, significant efforts are underway to develop such a device. Studies conducted by the US Army indicate that multiplex assays are under development to measure blood-based concussion markers on the battlefield that can provide results in less than 1 hour.[119] According to the official Army home page,[120] this trial was expected to be finished at the end of 2013 and was designed for approval by the Food and Drug Administration.

RECOMMENDATIONS

With respect to peripheral S100B measurement in patients with sport-related concussion, we recommend the following:

- Do not use S100B as a single diagnostic tool for concussion management at this point in time.
- Repeated assessment of each athlete's S100B reference values is required over the course of the athlete's career.
- Use standardized analytical approaches (eg, sample type, sample processing, analytical method, storage temperature, and time) to allow comparison of S100B values.
- When an anticoagulant is required, use heparin plasma.
- Avoid hemolysis of samples.
- If a sample must be stored, the temperature should be between room temperature and 4°C. Storage overnight is possible without affecting S100B serum levels (LIAISON Sangtec 100 assay). Avoid long-term storage of samples at temperatures greater than −70°C.
- Samples may be collected as part of the daily clinical routine without time constraints. Serum S100B values below 0.1 μg/L within 3 to 4 hours of injury are associated with a low risk of obvious neuroradiologic changes.
- Based on S100B's short half-life, a sample collected within the first 30 minutes after sport-related concussion is most accurate.
- An elevated serum S100B concentration after a game is typically less than the concentration noted shortly after a concussion.
- A serum S100B level of less than 0.1 μg/L within 3 to 4 hours of injury predicts a CT scan that is negative for mTBI.
- A serum S100B value above 0.1 μg/L after injury is cause for concern and the need for further testing and treatment should be assessed.
- A serum S100B level greater than 2.5 μg/L may mean the athlete is at high risk for disability after head trauma.
- An altered serum S100B baseline value may be due to the athlete's age (>20 years) or medical history (eg, previous concussion, medications, intoxication).
- Male and female athletes up to age 15 years may demonstrate differences in serum S100B baseline values.
- Athletes of different races and ethnicities may demonstrate differences in serum S100B baseline values.
- Renal dysfunction decreases the rate of S100B elimination and leads to increased peripheral concentrations.

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