Peripheral Blood and Salivary Biomarkers of Blood–Brain Barrier Permeability and Neuronal Damage: Clinical and Applied Concepts

Damir Janigro, Damian M. Bailey, Sylvain Lehmann, Jerome Badaut, Robin O’Flynn, Christophe Hirtz and Nicola Marchi

1 Department of Physiology Case Western Reserve University, Cleveland, OH, United States,
2 FloTBI Inc., Cleveland, OH, United States,
3 Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Wales, United Kingdom,
4 IRMB, INM, UFR Odontology, University Montpellier, INSERM, CHU Montpellier, CNRS, Montpellier, France,
5 Brain Molecular Imaging Lab, CNRS UMR 5287, INCIA, University of Bordeaux, Bordeaux, France,
6 Cerebrovascular and Glia Research, Department of Neuroscience, Institute of Functional Genomics (UMR 5203 CNRS—U 1191 INSERM, University of Montpellier), Montpellier, France

Within the neurovascular unit (NVU), the blood–brain barrier (BBB) operates as a key cerebrovascular interface, dynamically insulating the brain parenchyma from peripheral blood and compartments. Increased BBB permeability is clinically relevant for at least two reasons: it actively participates to the etiology of central nervous system (CNS) diseases, and it enables the diagnosis of neurological disorders based on the detection of CNS molecules in peripheral body fluids. In pathological conditions, a suite of glial, neuronal, and pericyte biomarkers can exit the brain reaching the peripheral blood and, after a process of filtration, may also appear in saliva or urine according to varying temporal trajectories. Here, we specifically examine the evidence in favor of or against the use of protein biomarkers of NVU damage and BBB permeability in traumatic head injury, including sport (sub)concussive impacts, seizure disorders, and neurodegenerative processes such as Alzheimer’s disease. We further extend this analysis by focusing on the correlates of human extreme physiology applied to the NVU and its biomarkers. To this end, we report NVU changes after prolonged exercise, freediving, and gravitational stress, focusing on the presence of peripheral biomarkers in these conditions. The development of a biomarker toolkit will enable minimally invasive routines for the assessment of brain health in a broad spectrum of clinical, emergency, and sport settings.

Keywords: neurovascular unit, blood biomarkers, saliva, concussion, epilepsy, neurodegeneration, traumatic brain injury, extreme sports

INTRODUCTION: FROM BLOOD–BRAIN BARRIER TO BLOOD–BRAIN DYNAMIC INTERFACE

The blood–brain barrier (BBB) is the complex and finely tuned network of brain capillaries governing the homeostatic exchange of ions, molecules, and cells between the brain and the peripheral blood (1–3). The importance of the BBB in the understanding and diagnosis of neurological disorders and brain health is recognized (4). The notion of BBB has evolved from...
that of a static brain shield to that of a dynamic blood–brain interface where endothelial cells continuously communicate with mural cells (pericytes and smooth muscle) and glia (astrocytes and microglia), located near neurons and spatially assembled to constitute the neurovascular unit (NVU) (Figure 1) (2). A precise layering of cells and extracellular matrixes forms an impermeable wall (Figure 1B). BBB dysfunction has etiologic and diagnostic significance (4), and BBB permeability is a key element of perivascular and neuroinflammation (Figure 1B1) (5, 6). Increased BBB permeability provokes an immediate loss of homeostatic control of ions, ATP, and neurotransmitters levels in the brain, promoting abnormal synaptic transmission or neuronal firing, possibly leading to neurological sequelae (6–13). On the other hand, neuronal activity significantly influences cerebrovascular functions in health and disease conditions (14, 15). Diagnostically and because of increased BBB permeability, peripherally injected imaging contrast agents can access the brain parenchyma while a suite of central nervous system (CNS) proteins (see Table 1) or nucleic acids [circulating free DNA and microRNA; for a review see (44, 45)] can exit into the peripheral blood (Figures 2A–C, 3A–C). Contrast MRI and CT scans are common clinical tools, while monitoring the levels of CNS proteins in peripheral body fluids represents a novel strategy for identifying BBB and neuronal damage (46). Importantly, the NVU connects with specialized brain acellular spaces through which the cerebrospinal and interstitial fluids carry ions, molecules, and proteins across the parenchyma or toward waste clearance pathways (Figure 2B) (47–50). This spatial perivascular and interstitial connectivity is important in the context of contrast-based brain imaging, possibly influencing the availability of biomarkers and their exit trajectories from the CNS (Figure 2; see (46, 51, 52) for a review). Starting from these fundamental concepts, we here examine the evidence supporting the development and the use of specific peripheral biomarker proteins to detect glioneuronal damage and BBB permeability in a plethora of clinical, emergency and sport-related settings.

**FIGURE 1** The dynamic NVU multicellular layout. (A) Within the NVU, the BBB encapsulates a set of unique properties of the microvascular capillary and post-capillary venules. The BBB endothelium lacks fenestrations, is assembled by structured tight junctions (TJs), and expresses luminal or abluminal transporters, altogether finely regulating brain homeostasis for proper neuronal physiology. These endothelial specializations are generated and controlled by precise interactions with pericytes, astrocyte end-feet, and microglial cells, all participating to the NVU. (B) Exploded view to illustrate the varying cellular composition and wall thickness of the intima, media, and adventitia layers. The endothelial basement membrane (BM) embeds pericytes. A second basement membrane is deposited by astrocytes and surrounds the end-feet. At the capillary level, the endothelial and parenchymal basement membranes merge. At the post-capillary venules, the two basement membranes separate to provide a perivascular space that allows for immune cells homing. (B1) Commonly reported pathological modifications leading to BBB permeability or NVU damage. (C) The cerebrovasculature in numbers (A, arteries; V, veins; CTX, cortex; SMC, smooth muscles cells). Proper BBB commences as the deepening cortical arteries (diameter > 100 µm in mice) branch into arterioles (diameter 15–50 µm; wall thickness 5–10 µm) and capillaries (diameter < 10 µm; wall thickness a few µm). Pial vessels have glia limitsans and an anatomically distinguishable Virchow-Robin space (B). Blood flow velocity rates in cortical mouse arterioles and capillaries are 3 and <0.5 mm/s, respectively. Capillary blood flow decreases with cortical depth (down to 0.1 mm/s). Diameter ranges (rodent) and anatomical abundance of glia, Virchow-Robin space, collagens, and mural cells (smooth muscles or pericytes) is provided. The multicellular layering within the tunica media (sign + + + indicates more than three smooth muscle cells at the arteries; + indicates one layer of pericytes at the BBB) is the major determinant of vascular thickness and elasticity. Original images by NM and IGF graphical service.
| Proteins | MW (kDa) | Role as biomarker | Estimated half-life in blood | Usage temporal trajectories | Source | Sampling methods | CNS disease | Reported (and varying) blood baselines | References |
|----------|---------|------------------|-----------------------------|----------------------------|--------|-----------------|------------|--------------------------------------|------------|
| GFAP | 50 | Astrocyte damage or astrogliosis | 48 h (16) | Acute and Subacute (hours–days) (17) | Astrocyte cytoskeleton No clearly reported extra-cranial sources (16) | Venipuncture CSF | TBI Multiple sclerosis AD | Baseline 0.01 ng/ml TBI with negative CT 0.21 ng/ml TBI with positive CT 0.73 ng/ml | (17–21) |
| S100B | 11 | BBB and astrocyte damage, astrogliosis | 2–6 h (22) | Acute (min, hours) (17) | Astrocyte calcium binding protein CNS development Extra-cranial sources [adipocytes (18)] | Venipuncture CSF Urine Saliva | TBI Epilepsy Multiple sclerosis | Pediatric 0.11 ng/ml (23) Adults 0.045 ng/ml (24) (sub)concussion, mTBI: 0.1 ng/ml (25, 26) | (17, 23, 25, 27–29) |
| UCH-L1 | 24 | Neuronal cell damage (33) | 7–9 h (31) | Acute (min, hours) Subacute (days) (17) | Axonal integrity Extra-cranial sources [neuromuscular junction (18)] | Venipuncture CSF | TBI Neurodegeneration | | (19, 21) |
| NSE | 47 | Neuronal cell damage | 30 h (32) | Acute (min, hours) Subacute (days) (17) | Neuron cytoplasmic enolase Detected in blood erythrocytes | Venipuncture CSF | TBI Epilepsy | Adults 6.1 µg/ml (24) | (33, 34) |
| NFL | 68 | Axonal injury, neuronal death | 3 weeks (35) | Subacute days to weeks (17) and chronic | Neuron class IV intermediate filaments of cytoskeleton | Venipuncture CSF | TBI Neurodegeneration | Threshold CSF 386 ng/ml (36) | (37, 38) |
| PDGFRβ | 123 | Pericyte reactivity or damage | na | Subacute (39) | Pericytes–endothelial interface | CSF | Neurodegeneration | See (40) for graphic baseline (115 % increase in CSF between no and mild cognitive impairment) | (39) |
| Tau, pTau | 50–80 | Neuronal or axonal damages, neurodegeneration | 10 h (41) | S majuscle, subacute, and chronic (17) | Neuronal microtubule-associated proteins Aggregates into neurofibrillary tangles | Venipuncture CSF | Neurodegeneration | Blood total and phosphorylated tau, (42) | T-tau control = 65.59 fg/ml P-tau control = 20.85 fg/ml P-tau/T-tau ratio control = 30.94 Total-tau in serum 4.4 pg/ml (24) Threshold CSF P-tau 78 pg/ml (39) | (17, 24, 43) |
PERIPHERAL BIOMARKERS: BASIC CONCEPTS AND FOCUS ON TRAUMATIC BRAIN INJURY

Elevated BBB permeability, or dysfunction, occurs in response to an acute injury (e.g., head trauma, stroke, and status epilepticus) and may be present throughout CNS disease progression (e.g., neurodegeneration, epileptogenesis, and multiple sclerosis), often due to inflammation (5–7, 13). Peripheral biological fluids represent suitable matrices to detect and quantify brain-derived proteins reporting BBB permeability and susceptibility to glio-neuronal damage (27–29, 53). Table 1 provides a list of protein biomarkers and their characteristics, properties, and proposed use in diagnostics. In general, peripheral biomarker proteins must (i) be present in brain interstitial fluids or be released by neurovascular cells into the interstitial or perivascular spaces, reaching the peripheral blood across a leaky BBB or by cerebrospinal fluid (CSF)–blood exchange (Figures 2A, 3A,B); (ii) have a concentration gradient driving passive diffusion (Figure 2C; see (29)); (iii) have a known and appropriate half-life to allow diagnostic interpretation (29) (biomarker half-life in peripheral fluids may impact usefulness in acute vs. long-term settings; see Table 1); and (iv) have a low molecular weight to allow a rapid egress across the damaged barriers or interfaces (19, 25, 27).

The bulk of neurological clinical biomarker literature has often focused on traumatic brain injury (TBI), with a recent emphasis on mild TBI (mTBI) (18, 54). Within this framework, the astrocytic protein S100B (55) has been examined as a peripheral biomarker of BBB permeability and gliosis (Table 1 and Figures 3A,B). Early proof-of-principle studies showed serum S100B levels to rapidly increase in response to a sudden BBB permeability, supporting the hypothesis that perivascular S100B can readily exit the brain (27, 28, 56). S100B was reported to rule out mTBI sequelae in emergency room settings (57), and measurement of blood S100B levels displayed a 99.7% negative predictive value (NPV) (57–60). Further evidence indicated that monitoring S100B after a mTBI could override the need for a CT scan for the identification of intracranial injury, with an excellent NPV (61). However, another study reported no relationship between serum S100B concentration and mTBI severity (62). In sports, S100B blood levels increased immediately after football games as compared to pregame baselines in players experiencing repeated head hits (25, 63). The evidence of a rapid S100B surge in blood after sub concussive hits was confirmed in follow-up studies (63–65). Importantly, extra-CNS sources of S100B were reported, representing a potential confounding factor if timing of blood draws in relation to injury is not adequately controlled and standardized (18, 66). These concerns have been discussed in (23, 67, 68).

The astrocytic glial fibrillary actin protein (GFAP) and the neuronal ubiquitin carboxyl-terminal hydrolase isoenzyme L1 (UCH-L1) are important biomarker candidates for glioneuronal damage (Table 1 and Figures 3A,B). UCH-L1 is also expressed at the neuromuscular junction (69, 70) while the contribution of extracranial sources of GFAP is debated (20, 71, 72). Monitoring of blood GFAP and UCH-L1 levels was used to grade brain injury after TBI. GFAP and UCH-L1 levels were increased in non-concussive and concussive head trauma as compared to body trauma (73, 74). The analysis of blood GFAP (or S100B) levels within 24 h from the head injury was proposed as a means to improve the detection of TBI and to identify patients in need of a subsequent MRI, in addition to routine CT surveillance (75, 76). GFAP and UCH-L1 blood levels were used to rule out intracranial injuries and the need for CT scans, showing high test sensitivity and NPV (21). One study reported no significant difference in blood UCH-L1 between control and players who sustained repetitive head hits (77). Collectively, this evidence points to GFAP as a diagnostic candidate to be used in TBI (33, 54, 71, 72). In two studies (78, 79), however, GFAP and UCH-L1 levels were below the lower limits of quantification or detection (LLOQ or LLOD, respectively) in a percentage of both TBI and trauma control groups, representing a possible concern for estimating NPV (20, 80).

Important biomarkers detecting neuronal damage are myelin basic protein (MBP), neuron-specific enolase (NSE), tau, and neurofilament light chain [NfL; Table 1 and Figures 3A,B; see
Blood MBP levels were unchanged in a pediatric mTBI population as compared to controls. Interestingly, MBP levels remained elevated for up to 2 weeks in case of intracranial hemorrhage (81). NFls are found in axons and have been proposed as biomarkers of axonal damage triggered by mTBI, for example, after an amateur boxing bout (82–84). S100B levels were also increased following amateur boxing (85). Further evidence indicated neurofilament heavy chain increase after mTBI (82). Finally, NSE levels in CSF were shown to be proportional to TBI severity, in the setting of moderate or severe TBI (86–88). NSE in the blood is less investigated due to its presence in erythrocytes (89, 90). Collectively, these data support the further development of biomarker toolkits of TBI, with a special relevance to mild head injury and sport-related (sub)concussions, when emergency and sideline diagnostic solutions need to be readily accessible.

**PHOSPHORYLATED TAU AS AN EMERGING BLOOD BIOMARKER OF ALZHEIMER’S AND NEURODEGENERATIVE DISEASES**

Accumulating evidence points to blood phosphorylated tau as a promising biomarker to improve the diagnosis and staging of and to enable trials in Alzheimer’s disease (AD) subjects. In a cross-sectional study performed in AD patients, phosphorylated tau isoforms were used as diagnostic biomarkers to track disease progression (91). A method measuring attomolar concentrations of tau isoforms in plasma was implemented using stable isotope labeling kinetics and mass spectroscopy. Changes in plasma p-tau, particularly p-tau217, mirrored specific changes in CSF to detect phosphorylation of soluble tau and amyloidosis. No correlation was found between CSF and plasma p-tau202 levels. Plasma p-tau217 level distinguished amyloid-negative from amyloid-positive groups regardless of the cognitive status, indicating that p-tau217 in plasma may be an accurate biomarker of abnormal brain tau metabolism. Furthermore, a longitudinal study of familial AD (presenting pathogenic mutations in PSEN1 or APP genes) included 19 symptomatic and 51 asymptomatic participants where plasma p-tau181 levels were quantified by using a single-molecule array (Simoa) method (92). Elevated plasma p-tau181 concentrations segregated symptomatic mutation carriers from non-carriers. In another cross-sectional study including the Arizona-based neuropathology cohort (37 AD and 47 without AD), the Swedish BioFINDER 2 cohort [121 AD, 178 mild cognitive impairment [MCI], 301 without AD, and 99 other neurological disorders], and a Columbian autosomal-dominant AD kindred (365 PSEN1 E280A mutation carriers and 257 mutation non-carriers), plasma tau phosphorylated at the threonine 217 (p-tau217) was quantified by the Meso Scale Discovery (MSD) assay as a
diagnostic AD biomarker (93). Among 1,402 participants from the three cohorts, plasma p-tau217 discriminated AD from other neurological disorders with higher accuracy compared with plasma p-tau181, plasma NfL, CSF p-tau181, and CSF Aβ42:Aβ40 ratio. A positive correlation between CSF and plasma p-tau217 was found in the Swedish BioFINDER 2 cohort. Finally, a high-sensitivity immunoassay measuring p-tau181 in plasma and serum was developed (94). A positive correlation was reported between plasma and CSF p-tau181 levels, distinguishing Aβ-negative cognitively unimpaired older adults from Aβ-positive older adults and Aβ-positive individuals with MCI.

Furthermore, at the BBB, the low-density receptor-related protein 1 (LRP1) plays an important role in regulating cerebrovascular permeability (95). sLRP1, a truncated soluble form of LRP1, freely circulates in plasma, and it sequesters unbound Aβ in the peripheral circulation (96). Plasma sLRP1 levels are significantly reduced in AD patients, and sLRP1 binding to Aβ is disrupted by oxidation (96, 97). Impaired sLRP1-mediated binding of plasma Aβ was suggested as an early biomarker for MCI preceding AD-type dementia (97). In summary, this evidence supports the further development of tau-based blood biomarkers as an accessible test for the screening and diagnosis of AD within the spectrum of cognitive impairments and dementia.

PERIPHERAL BIOMARKERS OF BBB PERMEABILITY AND SEIZURE CONDITIONS

The use of blood biomarkers extends to epilepsies, a cluster of diseases where BBB damage represents an etiological or a contributing pathophysiological player (98–100). A first study (67) demonstrated that blood S100B is elevated at seizure onset and after seizures, in support of the hypothesis that BBB damage may trigger a seizure (7, 101–103). A systematic review analyzed 18 studies and a total of 1,057 subjects, indicating that epileptic patients displayed elevated S100B blood levels as compared to controls (104). Meta-regression analyses showed that gender and mean age can impact serum S100B levels (104). Another study correlated MRI T1 peri-ictal imaging to blood S100B in drug-resistant epileptic patients, confirming the increase in BBB permeability during a seizure (105). Increased S100B blood levels were reported in pediatric temporal lobe epilepsy, with blood samples obtained 30 min after a complex partial seizure (106).

Children suffering from intractable focal epilepsy displayed elevated blood S100B levels as compared to controls (107). One study included 39 patients suffering from simple febrile seizures and age- and sex-matched controls, showing no S100B differences between groups when assessed immediately after seizures (108). These findings were corroborated in a follow-up study, (109) with the conclusion that febrile seizures are relatively harmless to the developing brain. Currently, a clinical trial is investigating whether S100B, as well as other protein biomarkers, increase in blood after a first generalized seizure could be used to predict first-to-chronic seizure conversion in adult subjects (https://clinicaltrials.gov/ct2/show/NCT02424123). Moreover, NSE elevations were reported in blood over time in patients affected by temporal lobe and extratemporal lobe epilepsies (110). Finally, recent evidence indicates miRNA in blood, or body fluids, as potential biomarkers indicating neurovascular and neuroinflammatory modifications occurring in specific forms of epilepsies [see (111–113) for comprehensive topic reviews]. In summary, blood biomarkers could represent a surrogate method of clinical electroencephalographic explorations to examine damage and brain neurophysiology in epileptic patients.

IMAGING BBB PERMEABILITY AND BRAIN DAMAGE: IS THE INTEGRATION WITH BLOOD BIOMARKERS POSSIBLE?

Available evidence supports the prospective use of blood biomarkers to detect NVU damage in acute and chronic neurological conditions. In this context, can peripheral biomarkers replace brain imaging? This is an important question especially if one considers the logistics (scarce imaging availability in rural areas and emergency, sport, and combat settings) and economic advantages that come with peripheral biomarkers, notwithstanding the complications associated with radiation exposure (e.g., CT scan). As a result, the diagnostic equivalence of blood biomarkers and enhanced MRI or CT scans (114–119) is being investigated. Accumulating evidence has shown that miTBI represents an optimal clinical arena to study the usefulness of imaging and peripheral biomarkers, also fulfilling an urgent clinical need (120–122). Neuroimaging techniques [CT scan (61)] show limitations for the diagnosis of miTBI patients (122, 123). Importantly, blood levels of GFAP, tau, and NfL were higher in patients with TBI-related findings on CT as compared to subjects presenting with normal CT, where the only significant predictor of damage was GFAP (124). Combining the biomarkers tau, NfL, and GFAP showed a good discriminatory power for detecting MRI abnormalities, even in miTBI patients with a normal CT (124). Furthermore, peak serum S100B levels negatively correlated with resting-state brain connectivity and behavioral outcomes in miTBI to severe TBI cases (125). S100B has proven its high NPV to rule out intracranial bleeding in patients after miTBI. However, its specificity for brain parenchyma structural lesions remains debated, and MRI is required for a specific explanation of clinical symptoms (76, 126, 127). Positron emission tomography (PET) and radiolabeled biomarkers were tested along with blood biomarkers. The [18F]AV1451 (flortaucipir) tau ligand was detected at the white/gray matter junction in frontal, parietal, and temporal brain regions, a typical localization of chronic traumatic encephalopathy (CTE) and tauopathy in veterans. Elevated levels of NfL were also reported in plasma (43). Finally, TBI is associated with inflammation as blood levels of IL6, TNFα, and VEGF were increased in CT- and MRI-positive patients as compared to controls (126).

Importantly, newer brain imaging approaches are being tested. Proton magnetic resonance spectroscopy (1H-MRS) represents an emerging neuroimaging modality to track the
metabolic changes occurring after TBI (128, 129). Spectroscopy can predict changes of key metabolites such N-acetylaspartate (NAA), a marker of neuronal loss (130), and its early decrease associates with long-term poor outcomes in clinical pediatric mTBI and moderate TBI (130). Experimentally, spectroscopy modifications post injury were linked to altered astrocyte metabolism (131). Brain structural changes observed using diffusion tensor imaging were correlated to astrocyte dysfunction and astrogliosis at early (1–7 days) and late (60 days) time points after injury (132, 133). Tractography provides an opportunity for measuring structural alterations in the white matter that are not detected by conventional structural MRI (134). Magnetic encephalography has also been proposed to study mTBI damage, in addition to being used for post-traumatic stress disorders (135, 136). Collectively, these data underscore the need for integrating the temporal and quantitative profiles of emerging imaging read-outs with the dynamics of peripheral biomarker of NVU damage. These studies will allow us to fully understand whether blood biomarkers can reliably act as surrogates for brain imaging.

SALIVA AS A BIOMARKER MATRIX: GENERAL CONCEPTS

Another key cellular “barrier” can be exploited for diagnostic purposes, namely, the salivary glands and gingival vessels, both interfacing with the peripheral blood (Figures 4A, B) (53, 137–144). While plasma and serum are considered as classic biofluids for assessment of systemic biomarkers, saliva is being increasingly viewed as a matrix with a high diagnostic value (141, 145). Saliva collection is economical, safe and can be performed without the assistance of specialized health care personnel, allowing for point-of-injury (POI) sampling. Saliva lacks cellular and soluble components (e.g., coagulation cascade). As the leakage of brain-derived biomarkers in saliva undergoes a process of biological filtration (53, 137, 146), the use of saliva does not require separation steps that are an obstacle to the development of POI blood tests (138, 139). Human saliva is a clear, slightly acidic (pH 6.0–7.0) heterogeneous biofluid composed of water (99%), proteins (0.3%), and inorganic substances (0.2%) (147). Saliva contains enzymes, hormones, antibodies, nucleic acids, antimicrobial constituents, and cytokines (148), which accumulate in salivary glands and are secreted into the oral cavity through acinar cell ducts (149). Available protocols indicate that saliva samples can be stored short term at room temperature and long term at −20°C or −80°C without significant protein degradation, similar to serum or plasma samples (150, 151). Relevant information inherent to the preparation and the technical handling of saliva samples can be found in (150, 152–154).

The whole saliva (WS) proteome, when compared with the plasma proteome, displays a larger proportion (14.5%) of low-molecular-weight proteins (<20 kDa), in contrast to only 7% for the plasma proteome (154). The highest fraction of proteins found in WS ranges from 20 to 40 kDa, whereas the 40–60 kDa range is the largest fraction for plasma. This is consistent with selective permeability between blood and saliva for low-molecular-weight proteins. Five diagnostic alphabets are outlined in saliva, including proteome (153, 155), transcriptome
(156, 157), microRNA (158), metabolome (159), and microbiome (160). Saliva is used by clinical laboratories for the detection of secretory IgA antibodies, for the analysis of salivary cortisol and hormones, and for genetic purposes (161–163).

**SALIVARY BIOMARKERS OF NVU DAMAGE: A NEW DIAGNOSTIC OPPORTUNITY?**

The salivary proteome has been characterized in CNS disease conditions, such as schizophrenia, bipolar disorders, and genetic disorders including Down's syndrome and Wilson disease (164). An overview of biomarkers identified in saliva for the diagnosis of neurodegenerative diseases such as AD, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis is provided in (165). Inflammatory biomarkers (e.g., IL-1β, TNF-α, and IL-6) have been quantified in saliva (166).

The deployment of POI salivary tests represents an opportunity for the detection of time-sensitive brain injuries (139–141, 167, 168). NSE was shown as a possible diagnostic salivary biomarker for neuronal damage in patients post stroke (169). Saliva samples have been analyzed for S100B levels, pro-inflammatory factors, and microRNAs in the settings of TBI (168, 170, 171). In particular, S100B levels in saliva were elevated in children post TBI (171). In another pilot study, 15 adult patients with suspected TBI and 15 control subjects were studied. Average salivary S100B level was 3.9-fold higher than blood S100B level, regardless of the presence of pathology [S100B]saliva correlated positively with [S100B]serum, and salivary S100B levels were as effective in differentiating TBI patients from control subjects as serum levels (172).

In an attempt to further accentuate the diagnostic significance of salivary testing, we reviewed the literature to obtain potential blood-to-saliva ratios for a number of proteins (Figure 4C). This search was directed to proteins that are not secreted by salivary glands. These proteins can access the salivary fluid by pericellular capillary leak, primarily the crevicular fluid. Importantly, it is currently unknown whether the steady-state permeability of the blood-to-saliva protein diffusion is preserved even at times when the BBB is breached due to brain insults. Literature references were used to examine insulin (173, 174), EGF (175), HGH (19, 176), S100B (18, 54–56, 177–180), adiponectin (181), prostate-specific antigen (PSA) (182), and cytokines (183). To our knowledge, there are no reports of salivary BDNF or NFL levels. All retrieved values were plotted to outline the theoretical cutoff properties of salivary filtration (Figure 4C). Large molecules (e.g., IgG) can be present in saliva owing to active secretion or local production.

Finally, we examined whether blood-to-saliva biomarkers' passage could be empirically predicted or modeled (153). Available data indicate that saliva is not a diluted substitute for the determination of plasma protein levels, as indicated by the incoherent plasma and saliva proteomes (152). Therefore, understanding the kinetic protein passage from blood to saliva is difficult. In the past, a model describing the passage of biomarkers from the brain into the peripheral blood was proposed (27–29). A physiologically based pharmacokinetic model can be used to describe the distribution of drugs and small molecules in body fluids (184). This computational approach can estimate the extent and time course of salivary biomarkers originating from the brain, offering the likelihood of a protein in saliva to be blood-borne (185). The physiologically based pharmacokinetic model used to describe the distribution of brain-derived biomarkers in blood was expanded to include an idealized salivary gland receiving its vascular supply from the external carotid. The venous output was mimicked according to the properties of jugular vein branches. To approximate the combined contribution of transcellular and paracellular pathways of protein extravasation across capillary endothelial cells and salivary gland epithelia, the following equation was used to calculate Js, the transfer of protein from blood to saliva:

\[
Js = Jv R + (Cp – Cb) / \text{PS} \tag{1}
\]

where \(Js\) (mol/min) is the mass transfer from blood to saliva, \(Jv\) (ml/min) is the blood flow to the salivary gland, \(R\) is the reflectance of the vascular wall, \(Cp\) (mol/L) is the concentration of biomarker in the serum, \(Cb\) (mol/L) is the concentration of biomarker in the saliva, and \(P\) and \(S\) refer to permeability (cm/s) and surface of exchange (cm²), respectively. The value of reflectance has no dimension and has a range from one (no passage of protein) to 0 (protein passage dictated by diffusion alone). The value of reflectance is derived from pore radius and molecular radius. To estimate PS, we used \(PS = Jv / Cb\) with salivary flow at 1 ml/min and \(Cb\) and \(Cp\) at 2.5 and 61.5 mg/ml, respectively. These values were derived by measurements and transfer of albumin levels from blood and saliva. The equation can be greatly simplified by fitting experimental data to confirm their accuracy. Once this is done, the predictors of passage of a given protein are primarily related to its molecular size (vascular wall reflectance) and the presence of a gradient for passage from blood to crevicular fluid. For (1), note that if the reflectance tends toward 1 (large molecular weight), the first term equals zero, thus leaving only the permeability of the capillary wall and the osmotic gradient as variables. Considering that permeability also depends on molecular size, a cutoff for extravasation seems to be mostly related to the size of the permeating protein. By using other computational models, it was shown that the physicochemical properties of proteins were the main predictors of presence in saliva. Among several properties, molecular size was the most relevant (185, 186).

It is important to underscore that the use of saliva samples comes with confounding factors. For instance, gingivitis or periodontal disease can affect the identification and quantification of proteins. It has been shown that submandibular saliva flow rates are lower in AD patients as compared to controls (187), possibly impacting the proportion of proteins detectable (188). In summary, fully defining the qualitative and quantitative characteristics of salivary biomarkers in physiological and neuropathological conditions is important to develop non-invasive point of care applicable to NVU screening.
PUSHING THE BBB LIMITS: RELEVANCE OF PERIPHERAL BLOOD BIOMARKERS IN HUMAN MODELS OF EXTREME BRAIN PHYSIOLOGY

Here, we focus on extreme sport settings that can be exploited as ‘human’ models to study BBB permeability, neuronal damage, and hemodynamic modifications in a controlled spatiotemporal manner. We review the evidence supporting the use of blood biomarkers to detect neurovascular modifications associated with extremes of cerebral blood flow (Figure 5A). These models share similar pathophysiological features unified by the cerebral formation of free radicals, associated reactive oxygen/nitrogen species (ROS/RNS), and impaired cerebral autoregulation (CA).

EXERCISE, CEREBROVASCULAR REGULATION, AND BLOOD BIOMARKERS

Evidence indicates that moderate-intensity continuous training (MICT) and corresponding improvements in cardiopulmonary fitness (CRF) can increase cerebral perfusion and vasoactivity across the human life span (192, 193), translating into a lower risk of stroke mortality and dementia (194, 195). The primary mechanisms include accelerated neurogenesis, in particular of the hippocampal dentate gyrus (196); reduction in β-amyloid (197); neuro-oxidative inflammatory nitrosative stress (198); proprioceptive adaptations incurred by movements that require sustained mental effort (199); increased brain-derived neurotrophic factor that modulates brain plasticity by promoting neuritic outgrowth and synaptic function (200); and improved BBB integrity and bolstering of tight junctions (201). More recently, high-intensity interval training (HIIT) has emerged as a more time-efficient model of exercise that can potentially promote superior improvements in CRF and cerebrovascular adaptation (191). However, this type of exercise characterized by high-flow/high-arterial-pressure transmission poses unique challenges for the brain with emerging evidence suggesting that an acute bout of HIIT could increase BBB permeability in the absence of neuronal injury (e.g., increased blood S100B and no NSE changes), subsequent to a free radical-mediated impairment in dynamic CA that persists into the recovery period (202) (Figure 5B).

HIGH-ALTITUDE MOUNTAINEERING, FREEDIVING, NVU DYNAMICS, AND BLOOD BIOMARKERS

High-altitude (HA) mountaineering (Figure 6A) and freediving (Figure 6B) represent unique physiological models to study severe arterial hypoxemia (O₂ lack) and hypocapnia/hypercapnia (CO₂ lack/excess) in ‘extreme’ athletes who consistently operate at, or very close to, the limits of human consciousness (189, 212). Diffusion-weighted magnetic resonance imaging has identified increases in brain volume, T₂ relaxation time (T₂-rt), and apparent diffusion coefficients (ADCs) in healthy participants acutely exposed to hypoxia, taken to reflect extracellular vasogenic edematous brain swelling (205, 206). These changes were pronounced in the splenium and genu of the corpus callosum, the likely consequence of a unique vascular constitution. Densely packed horizontal fibers characterized by short arterioles that lack adrenergetic tone likely render it more susceptible to hyperperfusion edema in the setting of hypoxic cerebral vasodilatation and/or autoregulatory impairment (205, 206). Local sampling of CSF and arterial–jugular venous blood concentration gradients of biomarkers including S100B indicated that BBB disruption is likely minor and linked to increased free radical formation (207, 216).

Some mountaineers, notably those who ascend (too) rapidly to altitudes above 2,500 m and thus not adequately acclimatized, can develop acute mountain sickness (AMS), a primary disorder of the CNS characterized by headache that is associated with, if not the primary trigger for, other vegetative symptoms (217). Traditionally, AMS has been considered a mild form of HA cerebral edema (HACE, the most malignant of all HA illnesses, oftentimes proving fatal) with a common pathophysiology of intracranial hypertension subsequent to vasogenic edematous brain swelling at opposing ends of a clinical continuum. An increase in intracranial pressure (ICP) could potentially result in the mechanical stimulation of pain-sensitive unmyelinated fibers that reside within the trigeminal–vascular system, triggering the symptoms of a headache (218). This makes intuitive sense in light of an early study that identified an increased T₂ signal in the white matter of mountaineers with moderate to severe AMS in whom clinical HACE had not yet developed (no ataxia or altered consciousness) (219). However, follow-up MRI studies consistently failed to support this concept, with no clear relationships observed between hypoxia-induced increases in brain volume or T₂-rt and cerebral AMS scores (206, 220). Indeed, the only defining morphological feature that distinguishes the AMS brain from its healthy counterpart is a selective attenuation in the ADC signal taken to reflect intracellular (cytotoxic) edema that likely coexists with extracellular vasogenic edema (206, 220). Attenuation of the ADC signal likely reflects fluid redistribution from within the extracellular space, as intracellular (astrocytic) swelling proceeds without any additional increment in brain volume, edema, or ICP (221). The underlying causes and temporal sequence are unknown, perhaps a reflection of ion pump suppression subsequent to (free radical-mediated) downregulation of Na⁺/K⁺-ATPase activity (211). More recent evidence suggests that a functional impairment in cerebral ‘venous outflow’ at the level of the transverse venous sinus may prove the unifying risk factor for AMS (222).

Freediving (Figure 6B) offers yet another remarkable model of severe arterial hypoxemia (189, 212). The static apnea world record currently stands at an impressive 11 min 35 s held by Stéphane Mifsud. However, unlike mountaineers, apnea results in severe hypercapnia, further compounding the cerebral hyperemic stimulus (Figure 6B), with freedivers also having to contend with the additional challenge of
FIGURE 5 | Challenges for the exercising human brain: applicability of NVU biomarkers. (A) Evolutionary ‘drive-for-size’ with exponential increases in estimated brain mass observed in fossil hominids. Note the structural complexities and corresponding biochemical demands that define the ‘modern’ human brain, highlighting its limited energy reserves in the form of oxygen \( \text{O}_2 \), glucose, and adenosine triphosphate (ATP) in the face of extraordinarily high rates of neuronal metabolism. This renders the human brain exquisitely sensitive to anoxia and ischemia, and thus, it has developed a sophisticated armory of mechanisms that collectively defend \( \text{O}_2 \) homeostasis. Calculations cited and figures modified from (189). (B) Physical exercise poses unique challenges for the human brain with perfusion typically characterized by preferential redistribution to the phylogenetically ‘older’ regions subserved by the posterior circulation (typical B-mode Doppler images illustrated). This makes teleological sense given that it is one of the most primitive neuroanatomical regions of the human brain, which has remained highly conserved across vertebrate evolution housing (almost exclusively) all the major cardiovascular and respiratory control centers essential for the integrated regulation of autonomic nervous control (190). However, this can come at a cost, with emerging evidence indicating that high flow/pressure and systemic/cerebral formation of free radicals and oxidative inactivation of nitric oxide (oxidative–nitrosative (OXNOS) stress) contribute to impaired cerebral autoregulation and BBB disruption. The latter is confirmed through proportional extravasation of brain-specific proteins, including S100B, in the absence of structural tissue damage. BBB permeability can cause extracellular vasogenic edema resulting in a regional \( \text{O}_2 \) diffusion limitation, with the potential to adversely affect cerebral bioenergetics and cognition. This is relevant to patients already suffering from impaired cerebral autoregulation/autonomic dysfunction, including older adults, notwithstanding patients diagnosed with diabetes, hypertension, stroke, and AD (191) (original images by DMB).

More recent, direct approaches have taken advantage of sampling arterial–jugular venous blood and combining regional measurements of CBF during the course of an apnea in champion freedivers (213, 214). Despite no detectable \( \text{O}_2 \) gradient across the brain, a truly remarkable observation, CDO\( _2 \) subsequent to increased perfusion was well maintained even at PaO\( _2 \)'s as low as 23 mmHg (Figure 6B). Similar to the aforementioned acute hypoxia study (207), apnea was associated with a net trans-cerebral outflow of free radicals and S100B (in the absence of any local gradients in NSE or MBP) that may reflect minor BBB permeability due to the combination of hemodynamic (increased intracranial
pressure) and molecular (increased free radical formation) stress in the absence of neuronal damage (214). Rather than consider this simply as a damaging maladaptive response, vasogenic edematous brain swelling may prove the adaptive phenotypical response in the hypoxia-tolerant human brain (211, 225).

**GRAVITATIONAL STRESS, CEREBROVASCULAR REGULATION, AND BLOOD BIOMARKERS**

Alterations in gravitational fluid pressure gradients caused by the microgravity of orbital spaceflight and hypergravity...
associated with takeoff and landing pose unique physiological challenges for the astronaut brain. Recent interest has focused on the complex pathophysiology underlying a constellation of debilitating neurological, ophthalmological, and neurovestibular symptoms, known collectively as spaceflight-associated neuro-ocular syndrome (SANS) (226). At the cellular level, microgravity has been associated with a loss of cytoskeletal integrity through dissociation of actin and tubulin bundles (227), and evidence obtained using animal models suggests that BBB disruption may occur during the early phases of unloading induced by suspension or microgravity (228) and during hypergravity induced by prolonged centrifugation (229, 230). In a recent study (215), parabolic flight (PF), a ground-based spaceflight analog, was used as a human model to induce rapidly alternating shifts in central blood volume during repeated exposures to microgravity (0 Gz) interspersed with hypergravity (1.8 Gz) (231) to explore how altered CBF impacts the NVU (232) (Figure 6C).

Blood flow to the posterior cerebral circulation (vertebral arteries) was selectively elevated during the most marked gravitational differential from microgravity to hypergravity. Posterior hyperperfusion was associated with a free radical-mediated reduction in nitric oxide bioavailability (oxidative–nitrosative stress) and selective increases in blood S100B and GFAP that persisted following return to microgravity, whereas blood biomarkers of neuronal–axonal damage (NSE, NFL, UCH-L1, and tau) remained stable (215). These findings suggest that the cumulative effects of repeated gravitational transitions may promote minor BBB damage due to the combined effects of hemodynamic-molecular stress. While we appreciate that PF is an entirely different stimulus dominated by hypergravity, these findings provide important mechanistic insight to help understand the neurological risks associated with prolonged microgravity during spaceflight, given that increased BBB permeability directly impacts neuronal function, predisposing to neurological sequelae and brain disease (6).

**BLOOD BIOMARKERS OF NVU DAMAGE: AVAILABLE ANALYTICAL TOOLS, LIMITATIONS, AND CONTROVERSIES**

No single ideal peripheral biomarker exists; rather, a suite of biomarkers could have a significant diagnostic impact. In recent years, innovative methods for biomarker detection have been implemented (Table 2). Reaching high sensitivity has several advantages, particularly in the context of neurological settings. Foremost is the ability to detect biomarkers such as NfL, Tau, or GFAP that are readily present in the CSF and in low concentrations in the blood. New technology has enabled the quantification of brain-derived protein biomarkers in blood, getting one step closer to a minimally invasive diagnosis of brain damage and neurodegenerative processes. Furthermore, high-sensitivity methods use microliter quantities of biofluid, allowing the quantification of several analytes and multiplex measurement. As an example, we here provide NfL, GFAP, and tau serum baseline levels as measured in our laboratory using Simoa (Table 2). We include specific LLOD and LLOQ values relative to our particular experience. Obviously, this new technology presents limitations. A shortcoming of high-sensitivity assay resides in the fact that Research Use Only (RUO) kits are not able to provide, yet, a level of robustness and precision that one would expect for a clinical *in vitro* diagnostics (IVD) use. To date, the impact of analytic interference is not sufficiently investigated. Therefore, the expectations formulated following cohort-based studies need confirmations in large preclinical studies and multicentric clinical trials.

Although the use of blood biomarkers of BBB or neuronal damage is appealing, a number of clinical stumbling blocks currently limit full applicability. The usefulness of blood biomarkers in a given human depends on the availability of reference values, correcting for age, ethnicity, kidney function, and body mass index (29). Adequateness of the blood sampling schedule and availability of baseline controls are crucial for a reliable biomarker outcome. Sample readiness before and after pathological events (e.g., inpatient seizure monitoring and head trauma as in contact sports) provides the optimal framework to calculate biomarker differential in the same individual and within a controlled time frame (24, 25). Availability of *ad hoc* baseline samples (e.g., specific enrollments for sport events and military personnel) represents a robust method enabling personalized medicine.

As examined so far, the appearance of NVU proteins in blood is reported for neurodegenerative diseases (91, 94), brain tumors (115, 117), TBI (25, 233), neurologic manifestations of systemic disease (234), psychiatric diseases, and seizures (21, 53, 235). Peripheral biomarkers have an excellent NPV to rule out disease(s) but have a poor positive predictive value (PPV) to identify a specific pathological condition (27, 28, 46, 53, 236–238). Another concern is the potential contamination related to extra-CNS sources of protein biomarkers. For example, S100B could be derived from adipose tissue with levels directly depending on body mass index (239). A study excluded the impact of adipose tissue on S100B serum levels (23).

**TABLE 2A | Available biomarker detection tools.**

| Novel diagnostic technology | Providers |
|----------------------------|-----------|
| Electrochemiluminescent immunoassay | Meso scale discovery |
| Single-molecule array immunoassay | Simoa® (Quanterx) |
| Immunomagnetic reduction | MagQu Co |
| Proximity extension assay | Olink |
| Immunocapture mass spectrometry | Thermo Fisher, Shimadzu, Agilent, AB Sciex, Waters |

**TABLE 2B | Examples of analytical parameters.**

| NFL | GFAP | Tau |
|-----|------|-----|
| Control baseline levels | 8 pg/ml | 54 pg/ml | 0.35 pg/ml |
| Limit of detection (LOD) | 0.10 pg/ml | 0.22 pg/ml | 0.02 pg/ml |
| Limit of quantification (LOQ) | 0.24 pg/ml | 0.47 pg/ml | 0.05 pg/ml |
Elevated serum S100B was reported in patients presenting with extracranial pathology (240), such as polytrauma and burns (66).

Another important question is whether peripheral biomarkers have a prognostic value for the development of long-term brain pathology. Currently, there is no collective agreement on whether an unhealthy BBB may already exist, and could be diagnosed, in an otherwise apparently healthy brain (241, 242). However, recent evidence indicates that subjects presenting early cognitive impairment had preexisting BBB damage. The platelet-derived growth factor receptor beta (PDGFRβ), Table 1 (243, 244) shedding from perivasculal pericytes was proposed as a biomarker of BBB integrity anticipating and predicting neurodegeneration (39, 243). A high-sensitivity method for detecting pericyte injury quantifying PDGFRβ in CSF was recently proposed (245). This method could be extended to study brain pericyte–endothelial damage in neurodegenerative disorders. Moreover, repetitive head hits during contact sports [American football (25)] were shown to associate with recurrent BBB permeability and S100B increases in blood. Players experiencing recurrent BBB permeability presented higher serum reactive autoantibodies, with a possible correlation with cognitive defects (25). The clinical significance of repeated BBB damage in sports is currently debated, with evidence pointing to a role in accelerated neurodegeneration (73). Moreover, total tau in blood was reported as a biomarker of axonal damage in hockey (24). Tau and amyloid monitoring in CSF is undergoing validation processes for dementia and AD (246, 247).

OUTLOOK AND FINAL REMARKS

Using peripheral biomarkers to monitor BBB permeability could extend to clinical cases where opening of the BBB is necessary to enhance drug penetration into the brain (13) or when re-establishment of physiological BBB tightness is justified to treat brain diseases (248, 249). Emerging evidence supports a holistic approach to tackle CNS diseases, where neuronal and cerebrovascular contributors of diseases are synchronously targeted. An increasing number of BBB-repairing molecules are currently being tested [for review, see (5, 6)], targeting NVU cells and neuroinflammation. Importantly, BBB biomarker and repairing strategies could become important in the settings of acute or chronic peripheral diseases (infections, metabolic or inflammatory) where immunity and inflammation negatively impact BBB permeability and, consequentially, synaptic transmission (5, 7, 250).

In conclusion, the NVU represents a modern and integrated entry point for the investigations of brain functions, and a continuous technological advancement will be instrumental to improve our ability to link NVU damage with diagnostics. The field of biomarkers of NVU damage, or dysfunction, is expanding together with the use of omic techniques and machine-learning routines for the discovery of signatures of acute or chronic disease conditions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article-supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

NM coordinated this effort, generated most of the figures, table, and contributed or wrote all parts. DJ focused on salivary biomarkers and provided parts of figures. DB focused on extreme conditions and biomarkers and providing relevant figures. JB and NM focused on imaging. SL, RO’F, and CH focused on salivary biomarkers and revised applicability of biomarkers. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Liebner S, Dijkstraen RM, Reiss Y, Plate KH, Agalliu D, Constantin G. Functional morphology of the blood-brain barrier in health and disease. Acta Neuropathol. (2018) 135:311–36. doi: 10.1007/s00401-018-1815-1
2. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis. (2010) 37:13–25. doi: 10.1016/j.nbd.2009.07.030
3. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurol. (2006) 7:41–53. doi: 10.1038/nrn1824
4. Banks WA. From blood-brain barrier to blood-brain interface: new opportunities for CNS drug delivery. Nat Rev Drug Discov. (2016) 15:275–92. doi: 10.1038/nrd.2015.21
5. Giannoni P, Claeyssen S, Noe F, Marchi N. Peripheral routes to neurodegeneration: passing through the blood-brain barrier.

Front Aging Neurosci. (2020) 12:3. doi: 10.3389/fnagi.2020.00003
6. Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV. Blood-brain barrier: from physiology to disease and back. Physiol Rev. (2019) 99:21–78. doi: 10.1152/physrev.00050.2017
7. Marchi N, Granata T, Janigro D. Inflammatory pathways of seizure disorders. Trends Neurosci. (2014) 37:55–65. doi: 10.1016/j.tins.2013.11.002
8. Obermeier B, Verma A, Ransohoff RM. The blood-brain barrier. Handb Clin Neurol. (2016) 133:39–59. doi: 10.1016/B978-0-444-63432-0.00003-7
9. Arango-Lievano M, Boussadia B, De Terdonck LDT, Gault C, Fontanaud P, Lafont C, et al. Topographic reorganization of cerebrovascular mural cells under seizure conditions. Cell Rep. (2018) 23:1045–59. doi: 10.1016/j.celrep.2018.03.110
10. Giannoni P, Badua I, Dargazanli C, De Maudave AE, Klement W, Costalat V, et al. The pericyte-glia interface at the blood-brain barrier. Clin Sci (Lond). (2018) 132:361–74. doi: 10.1042/CS20171634
11. Librizzi L, de Cutis M, Janigro D, Runcz L, de Bock F, Barbier EL, et al. Cerebrovascular heterogeneity and neuronal excitability. *Neurosci Lett.* (2018) 667:75–83. doi: 10.1016/j.neulet.2017.01.013

12. Pollak TA, Drndarski S, Stone JM, David AS, McGuire P, Abbott NJ. The brain–blood barrier in psychosis. *Lancet Psychiatry.* (2018) 5:79–89. doi: 10.1016/S1876-0557(17)30148-2

13. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol.* (2018) 14:133–50. doi: 10.1038/nrneurol.2017.188

14. Marchi N, Hallene KL, Kight KM, Cucullo L, Moddel G, Bingaman W, et al. Significance of MDR1 and multiple drug resistance in human epieplptic brain. *BMC Med.* (2004) 2:37. doi: 10.1186/1741-7015-2-37

15. Pulido RS, Munji RN, Chan TC, Quirk CR, Weiner GA, Weger BD, et al. Neuronal activity regulates blood-brain barrier efflux transport through endothelial circadian genes. *Neuron.* (2020) 108:937–52.e937. doi: 10.1016/j.neuron.2020.09.002

16. Diaz-Arrastia R, Wang KK, Papa L, Sorani MD, Yue JK, Puccio AM, et al. Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glibal fibrillary acidic protein. *J Neurotrauma.* (2014) 31:19–25. doi: 10.1089/neu.2013.3040

17. Wang KK, Yang Z, Zhu T, Shi Y, Rubenstein R, Tyndall JA, et al. An update on diagnostic and prognostic biomarkers for traumatic brain injury. *Expert Rev Mol Diagn.* (2018) 18:165–80. doi: 10.1080/14737159.2018.1428089

18. Kawata K, Liu CY, Merkel SF, Ramírez SH, Tierney RT, Langford D. Blood biomarkers for brain injury: what are we measuring? *Neurosci Biobehav Rev.* (2016) 68:680–73. doi: 10.1016/j.neubiorev.2016.05.009

19. Mondello S, Kobeissy F, Vestri A, Hayes RL, Kochanek PM, Berger RP. Serum concentrations of ubiquitin C-terminal hydrolase-L1 and glibal fibrillary acidic protein after pediatric traumatic brain injury. *J Neurotrauma.* (2017) 18:772–9. doi: 10.1089/jn.2015.032031-X

20. Thelin EP, Nelson DW, Bellander B-M. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. *Acta Neurochirurgica.* (2017) 159:209–25. doi: 10.1007/s00701-016-3046-3

21. Pham N, Fazio V, Cucullo L, Teng Q, Biberthaler P, Bazarian JJ, et al. Sources of S100B do not affect serum levels. *PLoS ONE.* (2014) 9:e105200. doi: 10.1371/journal.pone.0105200

22. Shahim P, Zettergren H, Vegnner B, Blennow K. Serum neurofilament light protein (NfL) in severe traumatic brain injury patient biofluids. *J Neurosurg.* (2018) 128:861–70. doi: 10.1093/jns/lny094

23. Bogoslovsky T, Gill J, Jeromin A, Davis C, Diaz-Arrastia R. Fluid biomarkers of traumatic brain injury and intended context of use. *Diagnóstics (Basel).* (2016) 6:10.3390/diagnostics6040037

24. Gillick K, Rooney K. Serial NSE measurement identifies non-survivors following out of hospital cardiac arrest. *Resuscitation.* (2018) 128:24–30. doi: 10.1016/j.resuscitation.2018.04.010

25. Shahin P, Zettergren H, Vegnner B, Blennow K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. *Neurology.* (2017) 88:1788–94. doi: 10.1212/WNL.000000000003912

26. Gresele MM, Bertzkeven H, Shaw G. Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis. *Multi Scler.* (2011) 17:31506. doi: 10.11155/2011/31506

27. Yuan A, Rao MV, Veerrana Nixon RA. Neurofilaments and Neurofilament Proteins in Health and Disease. *Cold Spring Harb Perspect Biol.* (2017) 9. doi: 10.1101/cshperspect.a018309

28. Lee Y, Lee BH, Yip W, Chou P, Yip BS. Neurofilament proteins as prognostic biomarkers in neurological disorders. *Curr Pharm Des.* (2020) 25:4560–9. doi: 10.2174/1381612825666191210154355

29. Nation DA, Sweeney MD, Montagne A, Sagare AP, D’Orazio LM, Pachicano M, et al. Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat Med.* (2019) 25:270–6. doi: 10.1038/s41591-018-0297-y

30. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood–brain barrier breakdown in the aging human hippocampus. *Neuron.* (2015) 85:296–302. doi: 10.1016/j.neuron.2014.12.032

31. Randall J, Mörtberg E, Provuncher GK, Fournier DR, Duffy DC, Robertson S, et al. Tau proteins in serum predict neurological outcome after hypoxic brain injury from cardiac arrest: results of a pilot study. *Resuscitation.* (2013) 84:351–6. doi: 10.1016/j.resuscitation.2012.07.027

32. Rubenstein R, Chang B, Yue JK, Chiu A, Winkler EA, Puccio AM, et al. Comparing plasma phospho tau, total tau, and phospho tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol.* (2017) 74:1063–72. doi: 10.1001/jamaneurol.2017.0655

33. Dickstein DL, De Gasperi R, Gama Sosa MA, Perez-Garcia C, Short JA, Sosa H, et al. Brain and blood biomarkers of tauopathy and neuronal injury in humans and rats with neurobehavioral syndromes following blast exposure. *Mol Psychiatry.* (2020) 10.1038/s41380-020-0674-z

34. Bhomia M, Balakathiresan NS, Wang KK, Papa L, Maheshwari RK. A Panel of Serum MiRNA Biomarkers for the Diagnosis of Severe to Mild Traumatic Brain Injury in Humans. *Sci Rep.* (2016) 6:28148. doi: 10.1038/srep28148

35. Papa L, Slobounov SM, Breiter HC, Walter A, Bream T, Seidenberg P, et al. Elevations in MicroRNA biomarkers in serum are associated with measures of concussion, neurocognitive function, and subconcussive trauma over a single national collegiate athletic association division i season in collegiate football players. *J Neurotrauma.* (2019) 36:1343–51. doi: 10.1089/neu.2018.6072

36. Zhang J, Puvvada V, Janigro D. Biomarkers of traumatic brain injury and their relationship to pathology. In: Laskowitz D, Grant G, editors. *Translational Research in Traumatic Brain Injury.* Boca Raton, FL: CRC Press/Taylor and Francis Group (2016).

37. Abbott NJ, Pizzo ME, Preston JE, Janigro D, Thorne RG. The role of brain barriers in fluid movement in the CNS: there is a ‘gymnastic’ system? *Acta Neuropathol.* (2018) 133:387–407. doi: 10.1007/s00401-018-1182-4

38. Bludny SB, Barrand MA. Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. *Fluids Barriers CNS.* (2014) 11:26. doi: 10.1186/2045-8118-11-26

39. Nicholson C, Harbetova S. Brain extracellular space: the final frontier of neuroscience. *Biophys J.* (2017) 113:2133–42. doi: 10.1016/j.bpj.2017.06.052
64. Meier TB, Huber DL, Bohorquez-Montoya L, Nitta ME, Savitz J, Teague TK, Unden L, Calcagnile O, Unden J, Reinstrup P, Bazarian J. Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. J Neurotrauma. (2013) 30:657–70. doi: 10.1089/neu.2012.2439

65. McMahon PJ, Pancyrovski WM, Yue JK, Puccio AM, Inoue T, Sorani MD, et al. Measurement of the glial fibrillary acidic protein and its breakdown products GFAP-BD biomarker for the detection of traumatic brain injury compared to computed tomography and magnetic resonance imaging. J Neurotrauma. (2015) 32:527–33. doi: 10.1089/neu.2014.3635

66. Bogoslovsky T, Wilson D, Chen Y, Hanlon D, Gill J, Jeromin A, et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid beta up to 90 days after traumatic brain injury. J Neurotrauma. (2017) 34:66–73. doi: 10.1089/neu.2015.4333

67. Jeter CB, Hergenroeder GW, Hylin MJ, Redell JB, Moore AN, Dash PK. Biomarkers for the diagnosis and prognosis of mild traumatic brain injury/concussion. J Neurotrauma. (2013) 30:657–70. doi: 10.1089/neu.2012.2439

68. Bohmer AE, Oses JP, Schmidt AP, Peron CS, Krebs CL, Oppitz PP, et al. Neuropen-specific enolase, S100B, and glial fibrillary acidic protein as diagnostic markers for traumatic brain injury. J Neurotrauma. (2013) 30:657–70. doi: 10.1089/neu.2012.2439
125. Thompson WH, Thelin EP, Liša A, Bellander BM, Fransson P. Functional
resting-state fMRI connectivity correlates with serum levels of the S100B
protein in the acute phase of traumatic brain injury. Neuromiage Clin. (2016)
12:1004–12. doi: 10.1016/j.nicl.2016.05.005

126. Edwards KA, Pattison CL, Guedes VA, Peyer J, Moore C, Davis T, et al. Inflammatory cytokines associate with neuroimaging after acute mild traumatic brain injury. Front Neurol. (2020)
11:348. doi: 10.3389/fneur.2020.00348

127. Gozt A, Licari M, Halstrom A, Milbourn H, Lydiard S, Black A, et al. Towards the development of an integrative, evidence-based suite of indicators for the prediction of outcome following mild traumatic brain injury: results from a pilot study. Brain Sci. (2020) 10. doi: 10.3390/brainsci1010020

128. Harris JL, He YW, Choi IY, Lee P, Berman NE, Swerdlow RH, et al. Altered
neurochemical profile after traumatic brain injury: (1)H-MRS biomarkers of pathological mechanisms. J Cereb Blood Flow Metab. (2012) 32:2122–34. doi: 10.1038/jcbfm.2012.114

129. Xu S, Zhao J, Racz J, Shi D, Roys S, Fiskum G, et al. Early microstructural
changes following controlled cortical impact injury in rat: a magnetic resonance imaging and spectroscopy study. J Neurotrauma. (2011) 28:2091–102. doi: 10.1089/neu.2010.1739

130. Holhousier B, Pivonka-Jones J, Nichols JG, Oyoyo U, Tong K, Ghosh N, et al. Longitudinal metabolite changes after traumatic brain injury: a prospective pediatric magnetic resonance spectroscopic imaging study. J Neurotrauma. (2019) 36:1352–60. doi: 10.1080/2018.5919

131. Wright DK, Trezise J, Kamnaksh A, Bekdash R, Johnston LA, Schubert S, et al. Ultra-deep sequencing and long-term disorders in juvenile mild closed head injury. Adv Clin Chem. (2011) 51:469–80. doi: 10.1007/BF03168453

132. Rodriguez-Grande B, Obenaus A, Ichkova A, Aussudre J, Bessy T, Ordidge R, et al. Behavioral, blood, and magnetic resonance imaging markers in the diagnosis of post-traumatic stress disorder and metabolic syndrome in diabetic patients. Indian J Dent Res. (2016) 27:388–91. doi: 10.4103/0970-9290.191887

133. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. (2001) 85:162–9. doi: 10.1016/s0022-0345(00)38004-4

134. Grassl N, Kulak LA, Pichler G, Geyer PE, Hong S, Schubert S, et al. Saliva as a viable biomarker of systemic inflammation. Clin Biochem. (2018) 61:469–80. doi: 10.1016/j.clinbiochem.2018.04.006

135. Bastin P, Mainz D, Grunz D. Salivary cortisol testing: preanalytic and analytic aspects. Ann Biol Clin (Paris.). (2018) 76:393–405. doi: 10.1684/abc.2018.1355

136. Sonesson M, Hamberg K, Wallengren ML, Matsson L, Ericson D. Salivary IGF I in mild-gain saliva of children, adolescents, and young adults. Eur J Oral Sci. (2011) 119:15–20. doi: 10.1111/j.1600-0722.2010.00794.x

137. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU, Role of salivary biomarkers in oral cancer detection. Adv Clin Chem. (2018) 86:23–70. doi: 10.1016/bse.accc.2018.05.002

138. Hirtz C, Chevalier F, Centeno D, Egea JC, Rossignol M, Sommerer N, et al. Complexity of the human whole saliva proteome. J Physiol Biochem. (2005) 61:659–80. doi: 10.1017/S0976-9668(05)70219-x

139. Hirtz C, Vialaret J, Nowak N, Galtung HK, Vestad B, Ovstebo R, Thiede B, Rusthen S, et al. Novel approaches for bioinformatic analysis of salivary RNA sequencing data for development. Bioinformatics. (2018) 34:1–8. doi: 10.1093/bioinformatics/btx504

140. Hirtz C, Vialaret J, Nowak N, Galtung HK, Vestad B, Ovstebo R, Thiede B, Rusthen S, et al. Identification of potential saliva and tear biomarkers in primary Sjogren’s syndrome, utilising the extraction of extracellular vesicles and proteomics analysis. Arthritis Res Ther. (2017) 19:14. doi: 10.1186/s13075-017-1228-x

141. Hirtz C, Chevalier F, Centeno D, Egea JC, Rossignol M, Sommerer N, et al. Complexity of the human whole saliva proteome. J Physiol Biochem. (2005) 61:659–80. doi: 10.1017/S0976-9668(05)70219-x

142. Hirtz C, Vialaret J, Nowak N, Gabella A, Deville de Periere D, Rehn JL, et al. Inflammatory cytokines associated with neuroimaging after acute mild traumatic brain injury. Front Neurol. (2020)
11:348. doi: 10.3389/fneur.2020.00348

143. Patel N, Belcher J, Thorpe NR, Spiteri MA. Measurement of C-reactive protein, procalcitonin and neutrophil elastase in saliva of COPD patients and healthy controls: correlation to self-reported wellbeing parameters. Respir Res. (2015) 16:62. doi: 10.1186/s12931-015-0219-1

144. Pay JB, Shaw AM. Towards salivary C-reactive protein as a viable biomarker of systemic inflammation. Clin Biochem. (2018) 68:1–8. doi: 10.1016/j.clinbiochem.2019.04.006

145. Loo YA, Yan W, Ramachandran P, Wong DT. Comparative human salivary and plasma proteomes. J Dent Res. (2010) 89:1016–23. doi: 10.1177/0022034510380041

146. Dezayee ZM, Al-Nimer MS. Saliva C-reactive protein as a biomarker of metabolic syndrome in diabetic patients. Indian J Dent Res. (2016) 27:388–91. doi: 10.4103/0970-9290.191887

147. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. (2001) 85:162–9. doi: 10.1016/s0022-0345(00)38004-4

148. Grassl N, Kulak LA, Pichler G, Geyer PE, Hong S, Schubert S, et al. Saliva as a viable biomarker of systemic inflammation. Clin Biochem. (2018) 61:469–80. doi: 10.1016/j.clinbiochem.2018.04.006

149. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU, Role of salivary biomarkers in oral cancer detection. Adv Clin Chem. (2018) 86:23–70. doi: 10.1016/bse.accc.2018.05.002
164. Castagnola M, Scarano E, Passali GC, Messana I, Cabras T, Iavarone F, et al. Salivary biomarkers and proteomics: future diagnostic and clinical utilities. *Acta Otorhinolaryngol Ital.* (2017) 37:94–101.

165. Schepici G, Silvestro S, Trubiani O, Bramanti P, Mazzon E. Salivary biomarkers: future approaches for early diagnosis of neurodegenerative diseases. *Bmc Neurosci.* (2018) 19:1004245.

166. Slavish DC, Graham-Engeland JE, Smyth JM, Engeland CG. Salivary markers of inflammation in response to acute stress. *Brain Behav Immun.* (2015) 44:253–69. doi: 10.1016/j.bbi.2014.08.008.

167. Wei F, Wong DT. Point-of-care platforms for salivary diagnostics. *Chin J Dent Res.* (2012) 15:7–15.

168. Di Pietro V, Porto E, Raguza M, Barbagallo C, Davies D, Forcione M, et al. Salivary MicroRNAs: diagnostic markers of mild traumatic brain injury in contact-sport. *Front Mol Neurosci.* (2018) 11:290. doi: 10.3389/fnmol.2018.00290.

169. Al-Rawi NH, Atiyah KM. Salivary neuron specific enolase: an indicator for neuronal damage in patients with ischemic stroke and stroke-prone patients. *Clin Chim Lab Med.* (2009) 47:1519–24. doi: 10.1515/clm.2009.345.

170. Johnson JJ, Loeffert AC, Stokes J, Olympia RP, Bramley H, Hicks SD. Association of salivary microrna changes with prolonged concussion symptoms. *JAMA Pediatr.* (2018) 172:65–73. doi: 10.1001/jamapediatrics.2017.3884.

171. Yeung C, Bhatia R, Bhattarai B, Sinha M. Role of salivary biomarkers in prediction of inflammation in response to acute stress. *Dent Res.* (2012) 15:7–15.

172. Slavish DC, Graham-Engeland JE, Smyth JM, Engeland CG. Salivary markers of inflammation in response to acute stress. *Brain Behav Immun.* (2015) 44:253–69. doi: 10.1016/j.bbi.2014.08.008.

173. Markopoulos AK, Belazi M, Drakoulakos D, Petrou-Americanou C, Sioulis A, Sakellari D, et al. Epidermal growth factor in saliva and serum of patients with cyclosporin-induced gingival overgrowth. *J Periodontal Res.* (2001) 36:88–91. doi: 10.1034/j.1600-0765.2001.360204.x.

174. Sanchez Garcia P, de Portugal Alvarez J, Alonso Gutierrez D, Cruz Hernandez JJ [Determination of insulin in saliva and its correlation with plasma insulin. Assessment of the possible participation++ of the salivary glands in the production of the hormone]. *An Med Interna.* (1989) 65–9.

175. Rantonen PJ, Penttila I, Meurman JH, Savolainen K, Narvanen S, Helenius T. Growth hormone and cortisol in serum and saliva. *Acta Odontol Scand.* (2000) 58:299–303. doi: 10.1080/119288000300521763.

176. Gazzolo D, Puchinotta F, Bashir M, Aboulgar H, Said HM, Iman I, et al. Neurological abnormalities in full-term asphyxiated newborns with prolonged anoxia: a comparison of detectable levels and correlations in a depressed and healthy adolescent sample. *Brain Behav Immun.* (2013) 34:164–75. doi: 10.1016/j.bbi.2013.08.010.

177. Janigro et al. Peripheral Biomarkers of Neurovascular Dysfunctions

181. Ayatollahi H, Darabi Mahboub MR, Mohammadian N, Parizadeh MR, Kianoosh T, Khabbaz Khoob M, et al. Ratios of free to total prostate-specific antigen and total prostate specific antigen to protein concentrations in saliva and serum of healthy men. *Urol J.* (2007) 4:238–41.

182. Byrne ML, O’Brien-Simpson NM, Reynolds EC, Walsh KA, Laughton K, Waloszek JM, et al. Acute phase protein and cytokine levels in serum and saliva: a comparison of detectable levels and correlations in a depressed and healthy adolescent sample. *Brain Behav Immun.* (2013) 34:164–75. doi: 10.1016/j.bbi.2013.08.010.

183. La Fratta I, Tatangelo R, Campagna G, Rizzato A, Franceschelli S, Ferrone A, et al. The plasmatic and salivary levels of IL-1beta, IL-18 and IL-6 are associated to emotional difference during stress in young male. *Sci Rep.* (2018) 8:3031. doi: 10.1038/s41598-018-21474-y.

184. Peters SA. Evaluation of a generic physiologically based pharmacokinetic model for lineshape analysis. *Clin Pharmacokinet.* (2008) 47:261–75. doi: 10.2165/00003088-200807400-00004.

185. Wang J, Liang Y, Wang Y, Cui J, Liu M, Du W, et al. Computational prediction of human salivary proteins from blood circulation and application to diagnostic biomarker identification. *PLoS ONE.* (2013) 8:e80211. doi: 10.1371/journal.pone.0080211.

186. Sun Y, Du W, Zhou C, Zhou Y, Cao Z, Tian Y, et al. A computational method for prediction of salivary-secretory proteins and its application to identification of head and neck cancer biomarkers for salivary diagnosis. *IEEE Trans Nanobioscience.* (2015) 14:167–74. doi: 10.1109/TNB.2015.2395143.

187. Ship JA, DeCarli C, Friedland RP, Baum BJ. Diminished submandibular salivary flow in dementia of the Alzheimer type. *J Gerontol.* (1990) 45:M61–66. doi: 10.1093/geronj/45.2.M61.

188. Siqueira WL, Dawes C. The salivary proteome: challenges and perspectives. *Proteomics Clin Appl.* (2011) 5:575–9. doi: 10.1002/prca.201100046.

189. Bailey DM, Oxygen and brain death; back from the brink. *Exp Physiol.* (2019) 104:1769–79. doi: 10.1113/EP088005.

190. Northcutt RG. Understanding vertebrate brain evolution. *Comp Biol Chem.* (2002) 42:743–56. doi: 10.1016/j.cbbi.2002.07.011.

191. Calverley TA, Ogoh S, Marley CJ, Steggall M, Marchi N, Brassard P, et al. HITing the brain with exercise; mechanisms, consequences and practical recommendations. *J Physiol.* (2020) 11:25021.

192. Bailey DM, Marley CJ, Brugnaux JV, Hodson D, New KJ, Ogoh S, et al. Elevated aerobic fitness sustained throughout the adult lifespan is associated with improved cerebral hemodynamics. *Stroke.* (2013) 44:3233–8. doi: 10.1161/STROKEAHA.113.002589.

193. Ainslie PN, Cotter JD, George KP, Shave R, et al. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. *J Physiol.* (2008) 586:4005–10. doi: 10.1113/jphysiol.2008.158279.

194. Prestgaard E, Mariampillai J, Engeseth K, Erikssen J, Bodegård J, Liestøl et al. Physical activity and amyloid-beta plasma and brain levels: results from the Australian imaging, biomarkers and lifestyle study of ageing. *Exp Physiol.* (2019) 104:1769–79. doi: 10.1113/EP088005.

195. Brown BM, Evans KA, McEneny J, Young IS, Hullin DA, James PE, et al. Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. *Exp Physiol.* (2011) 96:1196–207. doi: 10.1113/expphysiol.2011.060178.
203. Ernsting J. The effect of brief profound hypoxia upon the arterial and venous oxygen tensions in man. J. Physiol. (1963) 169:292–311. doi: 10.1113/jphysiol.1963.sp007257

204. Grocott MP, Martin DS, Levett DZ, McMorrow R, Windsor J, Montgomery HE, et al. Arterial blood gases and oxygen content in climbers on Mount Everest. N Engl J Med. (2009) 360:140–9. doi: 10.1056/NEJMoa0801581

205. Bailey DM, Roukens R, Knauth M, Kallenberg K, Christ S, Mohr A, et al. Free radical-mediated damage to barrier function is not associated with altered brain morphology in high-altitude headache. J Cereb Blood Flow Metab. (2006) 26:99–111. doi: 10.1088/jcbfm.9600169

206. Kallenberg K, Bailey DM, Christ S, Mohr A, Roukens R, Menold E, et al. Magnetic resonance imaging evidence of cytoxic cerebral edema in acute mountain sickness. J Cereb Blood Flow Metab. (2007) 27:1064–71. doi: 10.1038/jcbfm.9600404

207. Bailey DM, Taudorf S, Berg RMG, Lundby C, McEneny J, Young IS, et al. Increased cerebral output of free radicals during hypoxia: implications for acute mountain sickness? Am J Physiol. (2009) 297:R1283–1292. doi: 10.1152/ajpregu.00365.2009

208. Bailey DM, Rasmussen P, Evans KA, Bohm AM, Zaar M, Nielsen HB, et al. Hypoxia compounds exercise-induced free radical formation in humans; partitioning contributions from the cerebral and femoral circulation. Free Rad Biol Med. (2018) 124:104–13. doi: 10.1016/j.freeradbiomed.2018.05.090

209. Bailey DM, Rasmussen P, Overgaard M, Evans KA, Bohm AM, Seifert T, et al. Nitrite and S-Nitroshomoglobin exchange across the human cerebral and femoral circulation: relationship to basal and exercise blood flow responses to hypoxia. Circulation. (2017) 135:166–76. doi: 10.1161/CIRCULATIONAHA.116.024226

210. Bailey DM, Evans KA, James PE, McEneny J, Young IS, Fall L, et al. Altered free radical metabolism in acute mountain sickness: implications for dynamic cerebral autoregulation and blood-brain barrier function. J Physiol. (2009) 587:73–85. doi: 10.1113/jphysiol.2008.159855

211. Bailey DM, Bartsch P, Knauth M, Baumgartner RW. Emerging concepts in acute mountain sickness and high-altitude cerebral edema: from the molecular to the morphological. Cellular and Molecular Life Sciences. (2009) 66:583–94. doi: 10.1007/s00018-009-0145-9

212. Bailey DM, Willis CK, Hooland RL, Bain AR, MacLeod DB, Santoro MA, et al. Surviving without oxygen: how low can the human brain go? High Altitude Med Biol. (2017) 18:73–9. doi: 10.1089/ham.2016.0081

213. Willis CK, Ainslie PN, Drvis I, MacLeod DB, Bain AR, Madden D, et al. Regulation of brain blood flow and oxygen delivery in elite breath-hold divers. J Cerebral Blood Flow Metab. (2015) 35:66–73. doi: 10.1038/jcbfm.2014.170

214. Bain AR, Ainslie PN, Hoiland RL, Barak OF, Drvis I, Stembridge M, et al. Competitive apnea and its effect on the human brain: focus on the redox integrity. J Cerebral Blood Flow Metab. (2015) 35:2989–97. doi: 10.1007/s10067-016-3339-1

215. Falcone T, Carlson SI, Bertilacci MT, Ballabio E, Maier JA. Endothelial stress by gravitational unloading: effects on cell growth and cytoskeletal organization. Biochim Biophy Acta. (2013) 1834:1642–50. doi: 10.1016/j.bbamcr.2013.08.003

216. Porte Y, Morel JL. Learning on Jupiter, learning on the Moon: the dark side of the G-force. Effects of gravity changes on neurovascular unit modulation and learning and memory. Front Behav Neurosci. (2012) 6:64. doi: 10.3389/fnbeh.2012.00064

217. Matsuzawa Y, Kobayashi T, Fujimoto K, Shinozaki SY. Cerebral edema in acute mountain sickness and high-altitude cerebral edema: from the molecular to the morphological. Biochim Biophy Acta. (2016) 1861:94–101. doi: 10.1016/j.bbacli.2015.09.016

218. Sanchez del Rio M, and Moskowitz MA. High altitude headache. Lessons from space travel. Neurology. (2020) 96:1–10. doi: 10.1212/WNL.0000000000009847

219. Monbailliu T, Goossens J, Hachimi-Idrissi S. Blood protein biomarkers as diagnostic tools for acute mountain sickness. J Cerebral Blood Flow Metab. (2017) 37:828–37. doi: 10.1038/jcbfm.2016.98

220. Wilson MH, Davagnanam I, Holland G, Dattani RS, Tamm A, Hirani SP, et al. Cerebral venous system and anatomical predisposition to high-altitude headache. Ann Neurol. (2013) 73:381–9. doi: 10.1002/ana.23796

221. Andersson JP, Liner MH, Jonsson H. Increased serum levels of the brain damage marker S100B after apnea in trained breath-hold divers: a study including respiratory and cardiovascular observations. J Appl Physiol. (2009) 107:809–15. doi: 10.1152/japplphysiol.91434.2008

222. Kjeld T, Jutti T, Nielsen HB, Goetze JP, Secher NH, Olsen NV. Release of erythropoietin and neuron-specific enolase after breath holding in competing free divers. Scand J Med Sci. (2015) 25:c253–7. doi: 10.1111/smss.12309

223. Hansen AJ. Effect of anoxia on ion distribution in the brain. Physiol Rev. (1985) 65:101–48. doi: 10.1152/physrev.1985.65.1.101

224. Lee AG, Mader TH, Gibson CR, Tarver W, Rahibe P, Riascos RF, et al. Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. NPJ Microgravity. (2020) 6:7. doi: 10.1038/s41526-020-00114-8

225. Janigro et al. Peripheral Biomarkers of Neurovascular Dysfunctions

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243. Montagne A, Huuskonen MT, Rajagopal G, Sweeney MD, Nation DA, Sephrhband F, et al. Undetectable gadolinium brain retention in individuals with an age-dependent blood-brain barrier breakdown in the hippocampus and mild cognitive impairment. Alzheimers Dement. (2019) 15:1568–75. doi: 10.1016/j.jalz.2019.07.012

244. Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. Nature. (2020) 581:71–6. doi: 10.1038/s41586-020-2247-3

245. Sweeney MD, Sagare AP, Pachicano M, Harrington MG, Joe E, Chui HC, et al. A novel sensitive assay for detection of a biomarker of pericyte injury in cerebrospinal fluid. Alzheimers Dement. (2020) 16:821–30. doi: 10.1002/alz.12061

246. Leuzy A, Chiotis K, Lemoine L, Gillberg PG, Almkvist O, Rodriguez-Vieitez E, et al. Tau PET imaging in neurodegenerative tauopathies—still a challenge. Mol Psychiatry. (2019) doi: 10.1038/s41380-018-0342-8

247. Cohen AD, Landa SM, Snitz BE, Klunk WE, Blennow K, Zetterberg H. Fluid and PET biomarkers for amyloid pathology in Alzheimer’s disease. Mol Cell Neurosci. (2018) doi: 10.1016/j.mcn.2018.12.004

248. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol. (2013) 9:201–10. doi: 10.1038/nrneurol.2013.9

249. Mayeux R. Biomarkers: potential uses and limitations. NeuroRx. (2004) 1:182–8. doi: 10.1602/neurorx.1.2.182

250. Marchi N, Johnson Al, Puvenna V, Johnson HL, Tierney W, Ghosh C, et al. Modulation of peripheral cytotoxic cells and ictogenesis in a model of seizures. Epilepsia. (2011) 52:1627–34. doi: 10.1111/j.1528-1167.2011.03080.x

Conflict of Interest: DJ was affiliated to the company FloTBI Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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