

# Letters

## RESEARCH LETTER

### Imaging Blood-Brain Barrier Dysfunction in Football Players

There has been an increasing awareness of the long-term neuropsychiatric pathologies associated with repeated mild traumatic brain injury (mTBI) and specifically sports-related concussive and subconcussive head impacts.<sup>1</sup> While mTBI had been associated with diffusion tensor imaging evidence of diffusivity changes in soccer,<sup>2</sup> American football, and hockey players,<sup>3</sup> the mechanisms underlying the development of post-mTBI neurodegenerative complications are poorly understood.

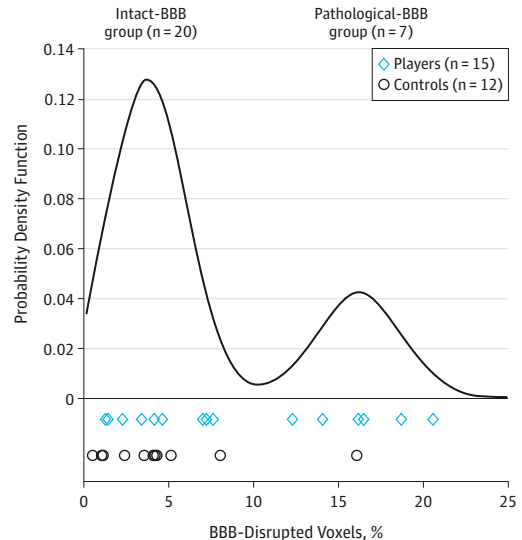
Accumulating evidence points to vascular pathology and dysfunction of the blood-brain barrier (BBB) as a potential link between severe TBI and neurodegeneration.<sup>4</sup> Moreover, participation in American football has been associated with changes in blood proteins reflecting BBB leakage.<sup>5</sup> Thus, here we set out to visualize the extent and location of BBB dysfunction in football players using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).

**Methods** | Sixteen male amateur football players (mean [SD] age, 26.53 [3.3] years) and 13 male track and field athlete control participants (mean [SD] age, 28.54 [2.2] years) were recruited during the 2013/2014 season of American football in Israel. Exclusion criteria included previous psychiatric/neurological disorders. The study was approved by the Soroka University Medical Center Helsinki institutional review board, and all participants gave written informed consent.

After at least 2 months of training and competing, all participants underwent DCE-MRI (3.0T Philips Ingenia), and BBB permeability maps were created for each individual.<sup>6</sup> In brief, a linear fit was used to calculate the slope of contrast agent concentration in each voxel over time. As positive slopes reflect contrast-agent accumulation due to BBB dysfunction, a threshold for high permeability was defined as the 95th percentile of all slopes in the control group. The percentage of brain volume with suprathreshold voxels was used as a measure of overall BBB pathology in each individual. A gaussian mixture model was applied to automatically cluster all participants into 2 BBB integrity-based groups.

History, symptoms, and cognitive function were evaluated using the National Football League Sideline Concussion Assessment Tool and the Standardized Assessment of Concussion (<http://www.nflevolution.com>). Comparisons between groups were performed using the Mann-Whitney *U* test. Data analysis was performed in Matlab 2013 and results are shown as mean (SD).

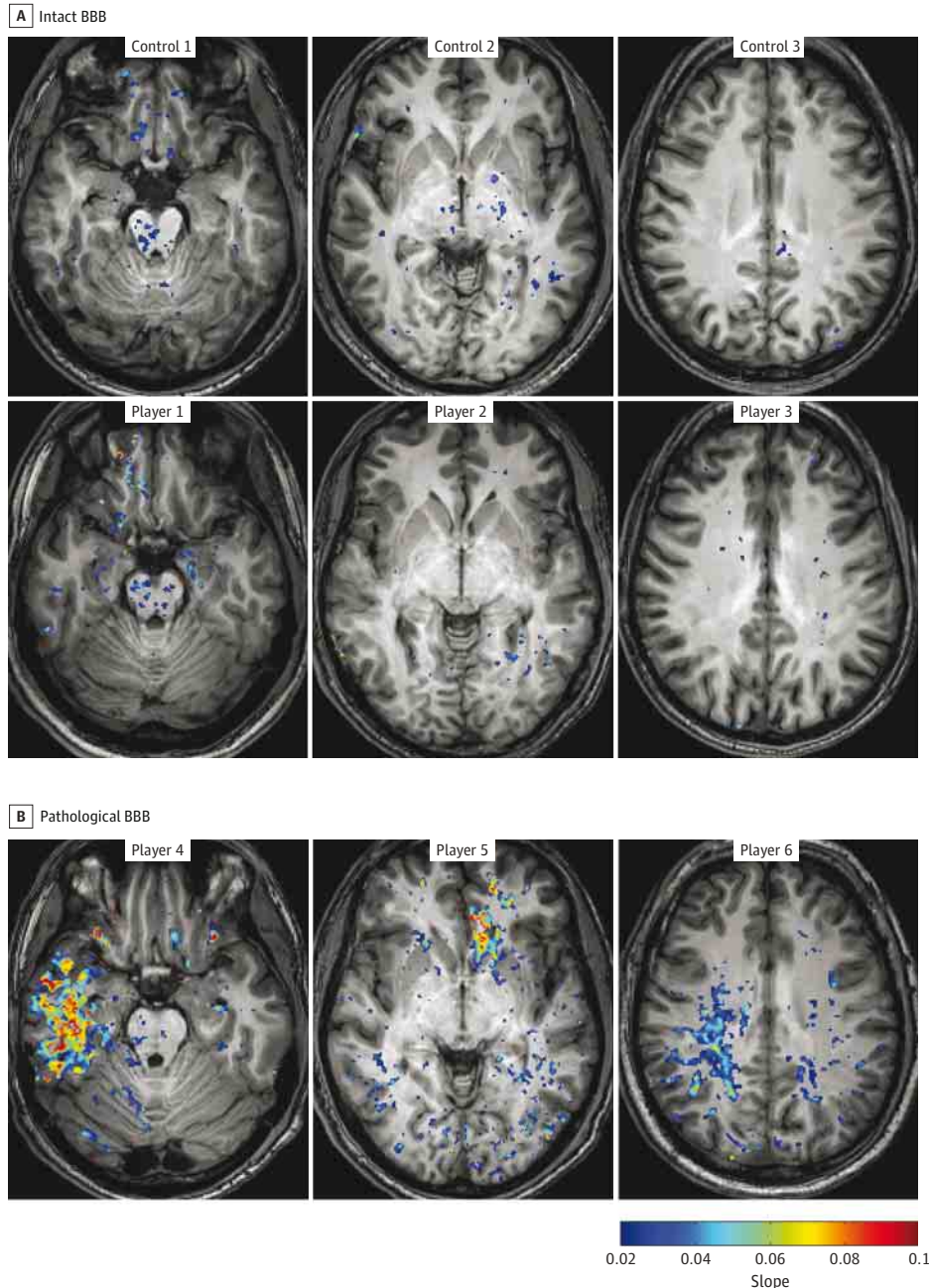
**Figure 1. Clustering of Participants Based on Percentage of Brain Volume With High Blood-Brain Barrier (BBB) Permeability**



The slope of contrast-agent intensity in each voxel was calculated, with negative slopes reflecting contrast-agent washout from blood vessels, near zero slopes corresponding to BBB-protected brain tissue, and positive slopes reflecting contrast-agent accumulation due to BBB breakdown. The 95th percentile of all slopes in the control group (0.02) was defined as the threshold corresponding to high permeability. Automatic gaussian clustering of the percentage of suprathreshold voxels in each individual revealed 2 significantly different subpopulations (Mann-Whitney *U* test,  $P < .001$ ): a group with low percentages, consisting of 11 control athletes and 9 players, and a group with high percentages, consisting of 6 football players and 1 control athlete.

**Results** | Following the exclusion of 1 player and 1 control participant owing to motion artifacts, gaussian mixture model clustering divided participants into 2 groups ( $P < .001$ ): an intact-BBB group (mean [SD] suprathreshold voxels, 3.86% [2.2%];  $n = 20$ ) with 9 football players and 11 control participants and a pathological-BBB group (mean [SD] suprathreshold voxels, 16.29% [2.74%];  $n = 7$ ), of whom 6 were players (Figure 1). In the pathological group, high-BBB permeability was found in both gray and white matter of the cerebral cortex, with focal BBB lesions located in the base of temporal ( $n = 4$ ), frontal ( $n = 5$ ), parietal ( $n = 6$ ), and occipital ( $n = 3$ ) lobes (Figure 2). No significant differences in self-reported concussions were found between players (mean [SD], 1 [1.75]) and control participants (mean [SD], 1 [1.88]) nor between the intact-BBB (mean [SD], 0.93 [2.4]) and pathological-BBB (mean [SD], 1.16 [2.4]) groups. Similarly, comparisons of Sideline Concussion Assessment Tool and the Standardized Assessment of Concussion scores revealed no significant differences.

Figure 2. Blood-Brain Barrier (BBB) Permeability in Football Players and Sex- and Age-Matched Control Individuals



Representative maps of suprathreshold voxels (slope  $>0.02$ , see color bar). The players in the pathological-BBB group presented focal BBB lesions in different cortical regions including the temporal (player 4), frontal (player 5), and parietal (player 6) lobes. Both gray and white matter were involved.

**Discussion** | In this study, DCE-MRI was able to reveal BBB pathology in 40% of the examined football players and 8.3% of the control athletes, with football players comprising 85.7% of the pathological-BBB group. While indirect evidence of BBB permeability in football players was previously inferred by relative changes in serum protein levels,<sup>5</sup> DCE-MRI enabled direct mapping of BBB lesions and quantitative assessment of overall BBB dysfunction. Although no

correlation was found between BBB pathology and concussion history, possibly owing to BBB damage associated with repeated subconcussive impacts or unreported concussions, our results do associate football with an increased risk for BBB pathology. Limitations of this study include a relatively small sample size and lack of long-term follow-up.

Further research is warranted toward understanding the natural course of BBB dysfunction in mTBI, establishing

BBB imaging as a reliable diagnostic tool, and potentially targeting the BBB for the prevention of post-mTBI complications.

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- DeKosky ST, Blennow K, Ikonovic MD, Gandy S. Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. *Nat Rev Neurol*. 2013;9(4):192-200.
- Koerte IK, Ertl-Wagner B, Reiser M, Zafonte R, Shenton ME. White matter integrity in the brains of professional soccer players without a symptomatic concussion. *JAMA*. 2012;308(18):1859-1861.
- McAllister TW, Ford JC, Flashman LA, et al. Effect of head impacts on diffusivity measures in a cohort of collegiate contact sport athletes. *Neurology*. 2014;82(1):63-69.
- Shlosberg D, Benifla M, Kaufner D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat Rev Neurol*. 2010;6(7):393-403.
- Marchi N, Bazarian JJ, Puvanna V, et al. Consequences of repeated blood-brain barrier disruption in football players. *PLoS One*. 2013;8(3):e56805.
- Chassidim Y, Veksler R, Lublinsky S, Pell GS, Friedman A, Shelef I. Quantitative imaging assessment of blood-brain barrier permeability in humans. *Fluids Barriers CNS*. 2013;10(1):9.

## COMMENT & RESPONSE

### The Central Clock in Patients With Parkinson Disease

**To the Editor** The regulation of sleep-wakefulness behavior involves 2 physiological processes. A circadian process, based in the suprachiasmatic nucleus, is responsible for the timing of sleep and wakefulness, and a homeostatic process that monitors and responds to the quality and quantity of prior sleep and wakefulness.<sup>1</sup> In patients with Parkinson disease (PD), sleep disturbances are among the most debilitating nonmotor symptoms.<sup>2</sup> The underlying neuropathology is multifactorial and involves complex disease-medication interactions.<sup>2</sup> Given this complex pathophysiology, the contribution of a dysfunctional suprachiasmatic nucleus clock has remained elusive.

In a study published in *JAMA Neurology*, Breen et al<sup>3</sup> assessed sleep architecture and the circadian profile of cortisol, melatonin, and peripheral clock gene expression in 30 patients diagnosed as having PD. In addition to confirming the well-established alterations of sleep in PD,<sup>2</sup> a significant reduction in the amplitude of melatonin secretion, hypercortisolemia, and altered peripheral clock gene expression were found in patients with PD. Videnovic et al<sup>4</sup> also reported a 4-fold reduction in the amplitude of melatonin secretion in 20 patients with PD housed in a constant-routine protocol. Videnovic et al<sup>4</sup> went further by showing that patients with PD with excessive daytime sleepiness had a significant 2.5-fold reduction in the melatonin rhythm amplitude compared with patients with PD without excessive daytime sleepiness.

However, in both the Breen et al<sup>3</sup> and Videnovic et al<sup>4</sup> studies, no alterations in the markers of the circadian phase were reported in patients with PD. This is surprising given that in both studies, patients with PD were receiving dopaminergic therapy. Previous studies that investigated the phase of the melatonin rhythm in medicated and unmedicated patients with PD found a phase-advanced melatonin rhythm in patients receiving dopamine therapy.<sup>5</sup> Indeed, Bolitho et al<sup>6</sup> confirmed the alteration of the phase angle of entrainment of the melatonin rhythm in 16 treated compared with untreated de novo patients with PD and healthy control participants. Additionally, Bolitho et al<sup>6</sup> reported a 3-fold increase in melatonin secretion, contrasting the decrease reported by Breen et al<sup>3</sup> and Videnovic et al.<sup>4</sup> The reasons behind these discrepancies are not clear. As stated by Videnovic et al,<sup>4</sup> the experimental protocols of the earlier studies did not control for environmental conditions. Consequently, the melatonin rhythm phase and amplitude may have been influenced by external factors such as light exposure. However, this may not account for the results of Bolitho et al<sup>6</sup> given that melatonin samples were collected under controlled conditions. A more plausible explanation is that these differences reflect an intrinsic neuropathophysiological variability in the PD cohorts investigated. This conclusion is supported by significant differences in multiple features of the sleep/wake cycle between patients studied by Breen et al<sup>3</sup> and Bolitho et al.<sup>6</sup> Furthermore, the patients in both studies did not show an increase in total sleep duration, which departs from the hypersomnia characterizing sleep in PD.<sup>2</sup>