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Exercise Pre-conditioning Reduces Brain Inflammation and Protects against Toxicity Induced by Traumatic Brain Injury: Behavioral and Neurochemical Approach

Bibiana Castagna Mota · Leticia Pereira · Mauren Assis Souza · Luiz Fernando Almeida Silva · Danieli Valnes Magni · Ana Paula Oliveira Ferreira · Mauro Schneider Oliveira · Ana Flávia Furian · Leidiane Mazzardo-Martins · Morgana Duarte da Silva · Adair Roberto Soares Santos · Juliano Ferreira · Michele Rechia Fighera · Luiz Fernando Freire Royes

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Abstract Although the favorable effects of physical exercise in neurorehabilitation after traumatic brain injury (TBI) are well known, detailed pathologic and functional alterations exerted by previous physical exercise on post-traumatic cerebral inflammation have been limited. In the present study, it is showed that fluid percussion brain injury (FPI) induced motor function impairment, followed by increased plasma fluorescein extravasation and cerebral—inflammation characterized by interleukin-1β, tumor necrosis factor-α (TNF-α) increase, and decreased IL-10. In addition, myeloperoxidase (MPO) increase and Na^+,K^+-ATPase activity inhibition after FPI suggest that the opening of blood–brain barrier (BBB) followed by neutrophils infiltration and cerebral inflammation may contribute to the failure of selected targets leading to secondary damage. In fact, Pearson's correlation analysis revealed strong correlation of MPO activity increase with Na^+,K^+-ATPase activity inhibition in sedentary rats. Statistical analysis also revealed that previous running exercise (4 weeks) protected against FPI-induced motor function impairment and fluorescein extravasation. Previous physical training also induced IL-10 increase per se and protected against cerebral IL-1β, and TNF-α increase and IL-10 decrease induced by FPI. This protocol of physical training was effective against MPO activity increase and Na^+,K^+-ATPase activity inhibition after FPI. The present protection correlated with MPO activity decrease suggests that the alteration of cerebral inflammatory status profile elicited by previous physical training reduces initial damage and limits long-term secondary degeneration after TBI. This prophylactic effect may facilitate functional recovery in patients suffering from brain injury induced by TBI.

Keywords Traumatic brain injury · Physical exercise · Inflammation · Na^+K^+-ATPase

Bibiana Castagna Mota and Letícia Pereira contributed equally to this study.

B. C. Mota · L. Pereira · M. A. Souza · L. F. A. Silva · A. P. O. Ferreira · L. F. F. Royes (✉)
Laboratório de Bioquímica do Exercício, Departamento de Métodos e Técnicas Desportivas, Centro de Educação Física e Desportos, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil
e-mail: nandoroyes@yahoo.com.br

M. A. Souza · L. F. A. Silva · D. V. Magni · A. P. O. Ferreira · M. S. Oliveira · J. Ferreira · M. R. Fighera · L. F. F. Royes
Programa de Pós-graduação em Ciências Biológicas: Bioquímica Toxicológica, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil

M. S. Oliveira · A. F. Furian
Universidade Federal do Pampa—Campus Itaqui, Itaqui, RS 97650-000, Brazil

M. R. Fighera
Instituto do Cérebro, Serviço de Neurologia, Hospital São Lucas, PUC-RS, Porto Alegre, RS, Brazil

L. Mazzardo-Martins · M. D. d. Silva · A. R. S. Santos
Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, Florianólpolis, SC, Brazil

Introduction

Traumatic brain injury (TBI) is a major cause of disability and death in young adults that could be present in diffuse axonal injury, neuronal cell loss, microglial activation, and

intraparenchymal hemorrhage in cortical regions (Dixon et al. 1991; Chen and Swanson 2003; Fujimoto et al. 2004). In this context, the early inflammatory response after tissue injury is believed to be triggered from several factors, such as extravasated blood products, intracellular compounds, and reactive species generation (Mathew et al. 1994; Juliet et al. 2008). Post-traumatic cerebral inflammation is characterized by glial activation, leukocyte recruitment, upregulation, and secretion of mediators, such as cytokines. In fact, alterations in systemic and cerebral spinal fluid (CSF) concentrations of cytokines have been reported in human patients and experimental models following severe head injury (Fan et al. 1995; Kossmann et al. 1995; Csuka et al. 1999). Leukocytes influx to the inflammatory site is orchestrated by a sequential upregulation of adhesion molecules on vascular endothelium leading to post-traumatic edema and blood–brain barrier (BBB) breakdown (Baskaya et al. 1997; Unterberg et al. 1997). In addition, it increased cerebral tissue water content and BBB dysfunction after TBI causing delayed neuronal dysfunction and death through secondary processes involving increased amino acids excitatory levels and loss of ionic equilibrium (Lenzlinger et al. 2004; Shlosberg et al. 2010).

The ion pump Na^+,K^+-ATPase is a ubiquitous plasma membrane protein which plays a key role in intracellular electrolyte homeostasis maintenance in virtually all tissues (Skou and Esmann 1992). In the Central Nervous System, decreased Na^+,K^+-ATPase activity directly affects neurotransmitter signaling, neural activity, as well as the whole animal behavior (Lees et al. 1990; Jamme et al, 1995; Li and Stys 2001). In this context, experimental findings have suggested that TBI-induced reactive oxygen species (ROS) generation decreases Na^+,K^+-ATPase activity by decreasing the total number of enzyme molecules while physical exercise protects against this effect (Lima et al. 2008).

Maintaining brain health and plasticity throughout life is an important public health goal and thus, beneficial effects of exercise on the brain are becoming increasingly evident (Ang and Gomez-Pinilla 2007). Cross-sectional studies also have suggested that regular exercise protects against diseases associated with chronic low-grade system inflammation (Petersen and Pedersen 2005) and that this effective protection may be partly mediated, by muscle-derived IL-6 (Steensberg 2003). In fact, physiological IL-6 concentrations after physical exercise stimulate the appearance of the anti-inflammatory cytokines IL-1 receptor antagonist (IL-ra) and IL-10 as well as inhibit the production of the pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) in the circulation (Steensberg et al. 2003). Moreover, altering the inflammatory status, profile performing exercise before brain trauma also produces prophylactic effects on attendant brain damage, such as limiting the infarct size following forebrain ischemia (Endres et al. 2003).

Central Nervous System inflammation induced by brain injury is commonly believed to cause sustained functional impairment and contribute to post-injury cell death. Indeed, studies indicated that treatment with drugs that antagonize endogenous inflammatory mediators reduces post-injury edema and improves motor function (Nimmo et al. 2004; Ivashkova et al. 2006). Although it is believed that physical exercise on general health and neurorehabilitation after TBI may be useful (Ang and Gomez-Pinilla 2007), little information is available regarding the prophylactic role of physical exercise on deleterious effects induced by TBI (Lima et al. 2008). Therefore, it was investigated whether events, such as acute inflammatory response (IL-1; IL-6; IL-10 TNF-α), myeloperoxidase (MPO, neutrophil infiltration indicator), and BBB breakdown are involved in fluid percussion brain injury (FPI)-induced motor function impairment and Na^+,K^+-ATPase inhibition. Furthermore, it was investigated whether previous aerobic physical training prevents against these deleterious effects elicited by FPI.

Materials and Methods

Animal and Reagents

All experiments involving animals were conducted in compliance with the policy statement of the American College of Sports Medicine and policy statement of the European Communities Council Directive (86/609/EEC), and adequate measures were taken to minimize pain and discomfort. In the present study, 90-day-old male Wistar rats, weighing 220–260 g at the beginning and 270–320 g at the end of the experimental period were used. During this period, animals were maintained in controlled environment (12:12 h light:dark cycle, 24 ± 1°C, 55% relative humidity) with free access to food (Guabi, Santa Maria, Brazil) and water. Animal utilization protocols followed the Official Government Ethics guidelines and were approved by the University Ethics Committee. All efforts were made to reduce the number of animals used, as well as minimizing their suffering. Reagents were purchased from Sigma (St. Louis, MO).

Physical Training Procedure

The physical training was carried out according to the protocol described by Arida and collaborators (2007). Before surgical procedure, animals were familiarized with the apparatus for 3 days by placing them on a treadmill (Insight Instruments) for 10 min/day at 10 m/min and 0% inclination degree. To provide a trainability measure, each animal's treadmill performance was rated on a scale of 1–5 according Dishman et al. (1988). After 4 weeks of training,

a test protocol was employed to determine the lactate threshold (LT) in sedentary ($n = 6$) and trained rats ($n = 6$). The LT test was carried out according to the protocol described by Marquezi and collaborators (2003).

Traumatic Brain Injury

After 4 weeks of training, sedentary and trained rats underwent FPI. Rats assigned to sham groups were anesthetized and connected to the injury device, but receiving no injury. The FPI was carried out as previously described (D'Ambrosio et al. 1999, 2004). In brief, animals were anesthetized with a single i.p. injection of Equithesin (6 ml/kg), a mixture containing sodium pentobarbital (58 mg/kg), chloral hydrate (60 mg/kg), magnesium sulfate (127.2 mg/kg), propylene glycol (42.8%), and absolute ethanol (11.6%) and placed in a rodent stereotaxic apparatus. A 3-mm-diameter burr hole was drilled on the right convexity, 2 mm posterior to the bregma and 3 mm lateral to the midline, taking care to keep the dura mater intact. A plastic injury cannula was placed over the craniotomy with dental cement. When dental cement has hardened, the cannula was filled with Chloramphenicol, closed with a proper plastic cap and the animal removed from the stereotaxic device and returned to its homecage. After 24 h, animals were anesthetized with Isoflurane and had the injury cannula attached to the fluid percussion device and placed in a heatpad maintained at 37 ± 0.2°C. The TBI was produced by a fluid-percussion device developed in our laboratory. A brief (10–15 ms) transient pressure fluid pulse (3.53 ± 0.17 atm) impact was applied against the exposed dura. Pressure pulses were measured extracranially by a transducer (Fluid Control Automação Hidráulica, Belo Horizonte, MG, Brazil) and recorded on a storage oscilloscope (Tektronix TDS 210). Sham-operated animals underwent an identical procedure, with the exception of FPI. Immediately after these procedures, the cannula was removed, and the orifice was covered with dental cement. Naive rats underwent randomization with no further intervention.

Motor Function Assessment

The neuroscore (NS) test was employed to assess motor function at 24 h post-injury or sham-injury. The NS test was performed based on McIntosh et al. (1989). In brief, animals were allowed to walk on an open wire grid for 3 min, during which a qualitative assessment of the number of foot-faults was performed to establish whether an observable motor deficit was present, defined as the inability of the animal to immediately retract its paw after falling through the grid. This evaluation (deficit/no deficit) has been shown to sensitively detect both forelimb and hindlimb deficits (Laurer et al. 2001). Subsequently, forelimb and hindlimb functions were evaluated by suspending the animals by the tail and observing how the animal grasp the top of the cage when they are lowered toward it (for the forelimbs) and the pattern of toespread and hindlimb extension during the suspension (for hindlimbs). Finally, the animals were tested for vestibulomotor function using a wire-grip test accordingly (Hall et al. 1988). Rats were placed on a metal wire 40 cm long, suspended 40 cm above a foam mat between 2 vertical bars, and introduced to the wire so that both front paws came in contact with the wire, and there was equal chance at grasping the wire. The latency that a rat remained on the wire within a 60-s interval was measured. Animals were scored from 4 (normal) to 0 (severely impaired) for each of the following indices, and the maximum score for each animal was 12.

Tissue Processing for Analysis of MPO, Na^+, K^+-ATPase Activity and Cytokine Levels

Considering that the early inflammatory activation after tissue injury exerts adverse effects on synaptic function and neuronal plasticity (Cederberg and Siesjo 2010), animals were killed by decapitation 24 h after TBI, and a coronal section (7 mm, Fig. 1) of the injured hemisphere corresponding to the injury impact site was dissected. Tissue was homogenized in solution containing bovine serum albumin (BSA 10 mg/ml), EGTA (2 mM), EDTA (2 mM) and PMSF (0.2 mM) in phosphate-buffered saline (PBS, pH 7.4). After homogenization, the sample was centrifuged (3,000×*g* for 10 min) and cytokine levels measured using a commercially available ELISA Kit from R&D Systems (Minneapolis, MN). The detection limit was 15 pg/ml. Assay of MPO and Na^+,K^+-ATPase activities were performed according to Suzuki et al. (1983) and Wyse et al. (2000), respectively. Protein content was measured colorimetrically by the method of Bradford (1976) using BSA (1 mg/ml) as standard.

BBB Permeability Assay Using Fluorescein

For evaluation of BBB permeability to small molecular mass compounds in rats, a subset of animals were injected with 10 mg sodium fluorescein in 0.1 ml sterile saline, i.p. administered (Olsen et al. 2007). In brief, animals were anaesthetized with ketamine HCl (100–200 mg/kg) i.p. 45 min after sodium fluorescein injection for blood sampling. Transcardial perfusion with PBS was performed, and then, the brain was removed, weighed, homogenized in 1-ml sterile PBS, and then stored at −70°C until processed further. Protein was precipitated from brain and serum samples with trichloroacetic acid (TCA) and diluted in sterile PBS 1:10 before adding 1:10 dilution in 20% TCA.

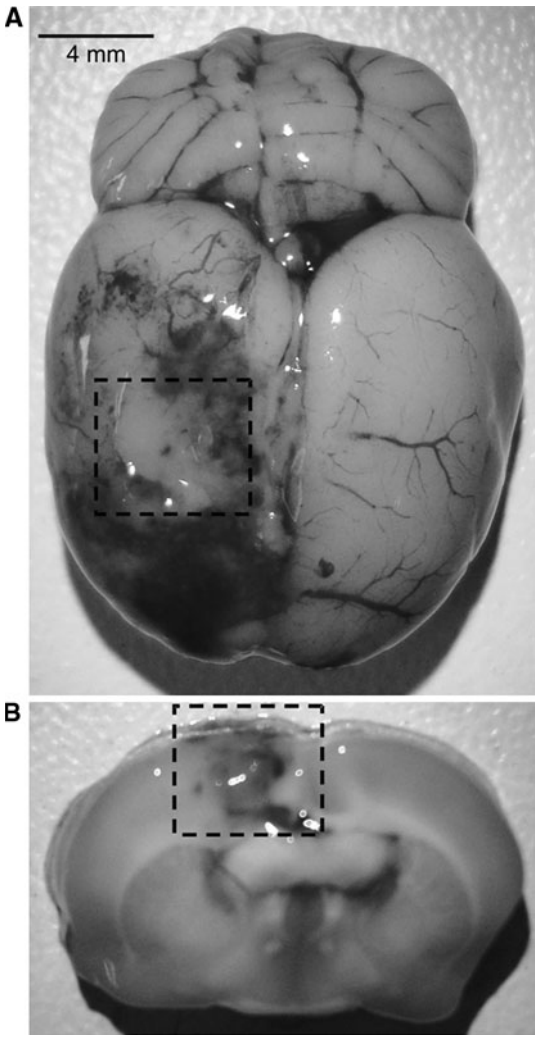

Fig. 1 Scheme of dorsal and coronal view of contusion site and pericontusional cortical tissue that was collectively sampled for the current study

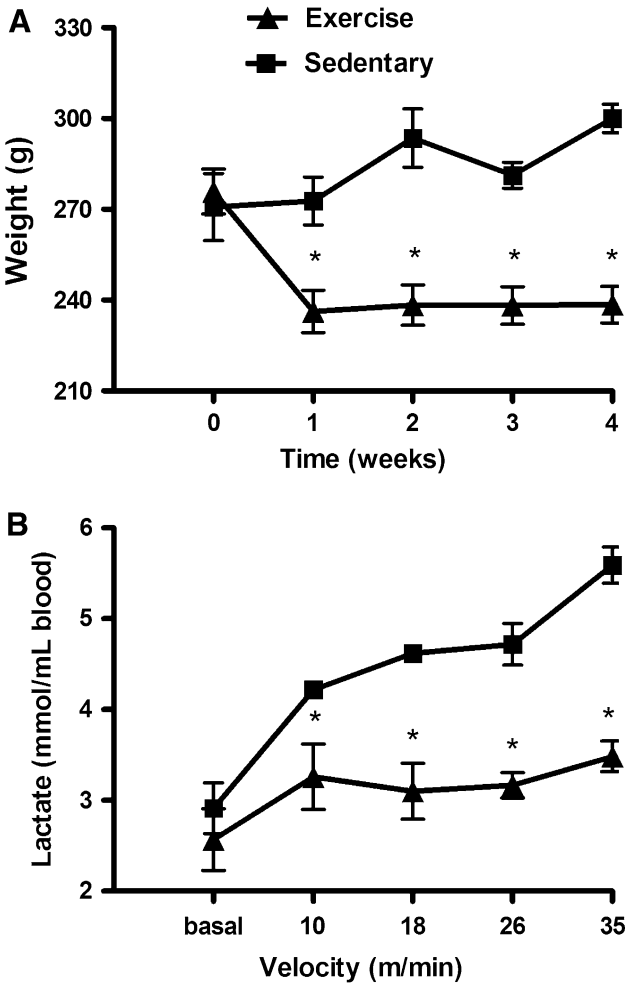

Fig. 2 Effect of 4-week treadmill exercise on body weight (**a**) and LT assay (**b**). Mean ± SEM for $n = 6$–8 in each group. *$P < 0.05$ compared with sedentary group (F test for simple effect) (two-way ANOVA)

Brain samples were first centrifuged at 1,250 g for 5 min, after which the resulting supernatant was diluted 1:10 in 20% TCA. All samples were incubated at 4°C for 24 h. Samples were centrifuged at 10,000 g for 15 min to remove precipitated protein. The supernatant was removed and diluted with equal volumes of borate buffer (0.05 M, pH 10), resulting in a 10% TCA final concentration and 0.025 M buffer borat. Samples were analyzed on fluorometer.BBB permeability degree was measured as the percentage (w/v) of sodium fluorescein per amount of sodium fluorescein in a milliliter of serum.

Statistical Analyses

Statistical analyses were carried out by one or two-way analyses of variance (ANOVA), Kruskal–Wallis test, and Pearson's correlation. Values of F and H are only presented if $P < 0.05$. Post-hoc analyses were carried out, while appropriately using the Student–Newman–Keuls test. Data were expressed as mean ± SEM or mean ± inter quartile.

Results

In the present study, significant increase in the total body weight (mean = 300 g) in sedentary versus trained rats (mean = 230 g) along the 4 weeks of physical training [$F(4,56) = 7.01$; $P < 0.05$; Fig. 2a] are shown. Statistical analyses also showed a clear stabilization of blood lactate concentration in the trained group compared with that of the sedentary [$F(4,40) = 4.39$; $P < 0.05$; Fig. 2b], indicating that the training program increased animal's aerobic resistance.

Using the composite NS, there were no pre-injury, baseline deficits among any of the groups (data not shown). On

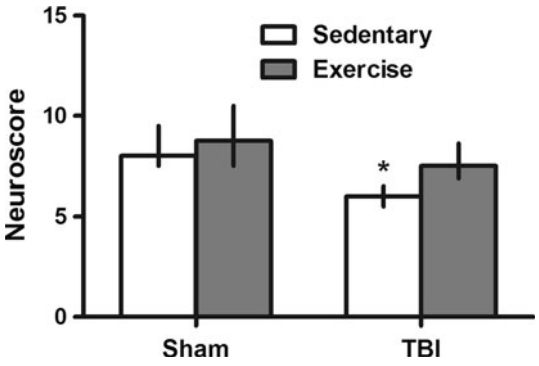

Fig. 3 Effects of physical exercise and TBI on motor function. Mean ± inter quartile for $n = 8$–9 in each group $^*P < 0.001$ compared to TBI + sed and sham groups (Kruskal–Wallis test)

the other hand, 24 h after FPI, brain-injured sedentary rats showed significant neurological deficits compared with sham-injured controls [$H(3) = 22.73$; $P < 0.001$; Fig. 3]. In addition, previous physical training protected against FPI-induced motor impairment. This study also showed that FPI induced the increase of IL-1β [$F(2,39) = 20.35$; $P < 0.002$; Fig. 4a] and TNF-α [$F(2,39) = 9.05$; $P < 0.05$; Fig. 4b] levels, despite not having increased IL-6 [$F(2,39) = 2.22$; $P > 0.05$; Fig. 4c], and FPI having decreased IL-10 levels [$F(2,39) = 16.14$; $P < 0.05$; Fig. 4d]. Statistical analysis also revealed that the chronic physical training induced IL-10 increase per se [$F(2,39) = 36.41$; $P < 0.001$; Fig. 4d] and protected against FPI-induced IL-10 decrease [$F(2,39) = 2.41$; $P < 0.05$; Fig. 4d]. Previous physical training also protected against the FPI-induced IL-1β [$F(2,39) = 5.49$; $P < 0.05$; Fig. 4a] and TNF-α [$F(2,39) = 11.00$; $P < 0.002$; Fig. 4b] accumulation.

Furthermore, the statistical analysis showed that physical exercise significantly attenuated FPI-induced increase of plasma fluorescein extravasation (BBB breakdown indicator) [$F(1,15) = 5.95$; $P < 0.05$; Fig. 5a] and protected against MPO activity increase [$F(2,36) = 8.24$; $P < 0.001$; Fig. 5b] as well Na^+,K^+-ATPase activity inhibition [$F(2,36) = 4.74$; $P < 0.05$; Fig. 5c]. Correlation analysis (Pearson's correlation) showed that MPO activity increases in correlation with Na^+,K^+-ATPase activity inhibition in sedentary rats ($r = -0.979$; $P < 0.002$; Fig. 6). On the other hand, the negative correlation between MPO and Na^+,K^+-ATPase activities in animals

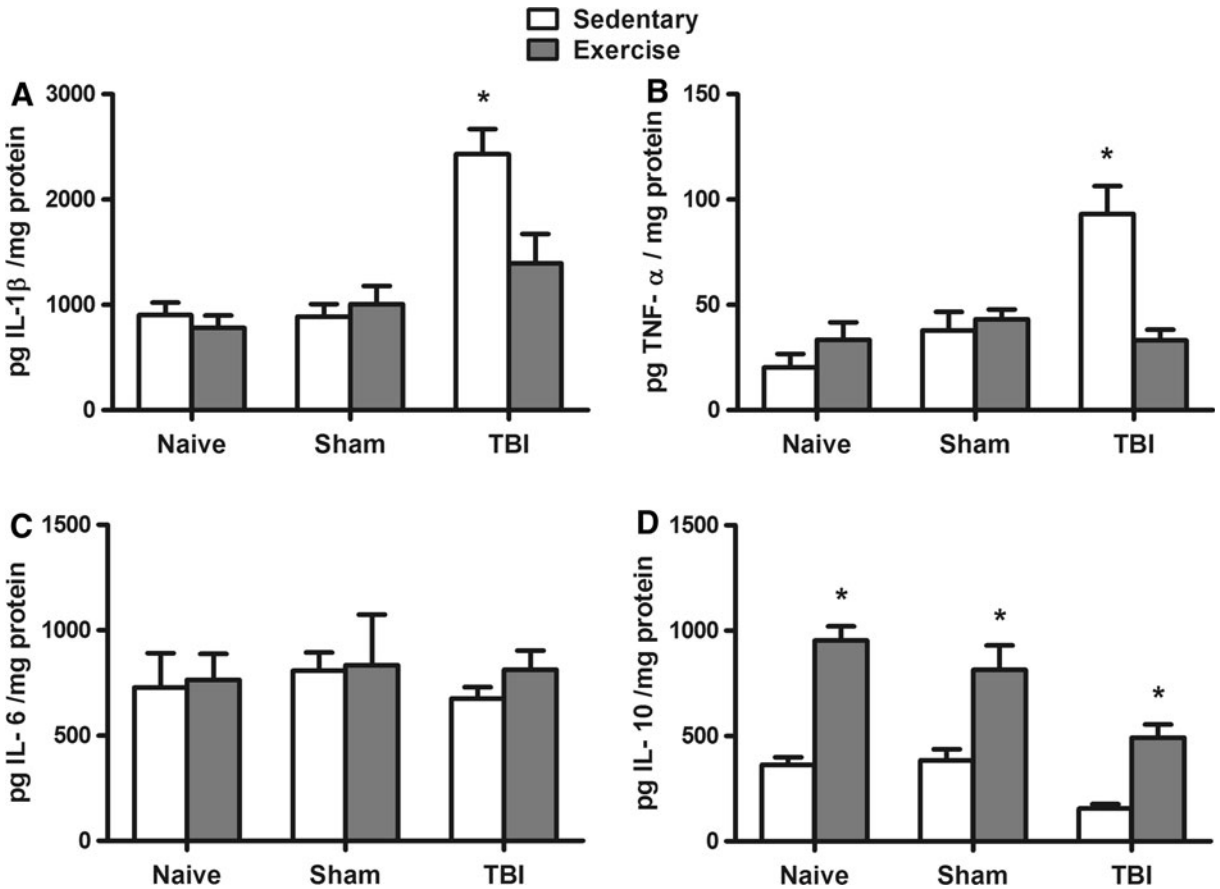

Fig. 4 Effects of physical exercise and TBI on the IL-1β content (**a**), TNF-α content (**b**), IL-6 content (**c**), and IL-10 content (**d**). $^*P < 0.05$ compared to sham and naive group. Mean ± SEM for $n = 8$ in each group (Student–Newman–Keuls test)

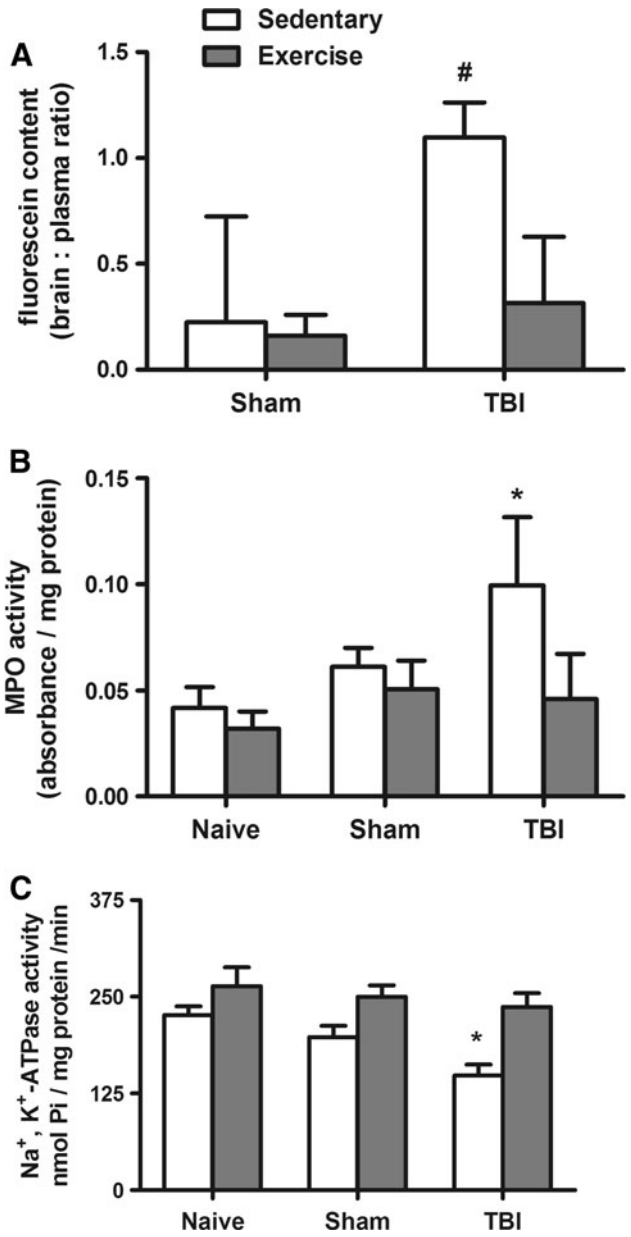

Fig. 5 Effects of physical training and TBI on the BBB breakdown (**a**), on the MPO activity (**b**), and on Na^+,K^+-ATPase activity (**c**). Mean ± SEM for $n = 4$–8 in each group. $^{\#}P < 0.001$ compared to TBI + sed and sham group (two-way ANOVA) and $^{*}P < 0.05$ compared to sham (Student–Newman–Keuls test)

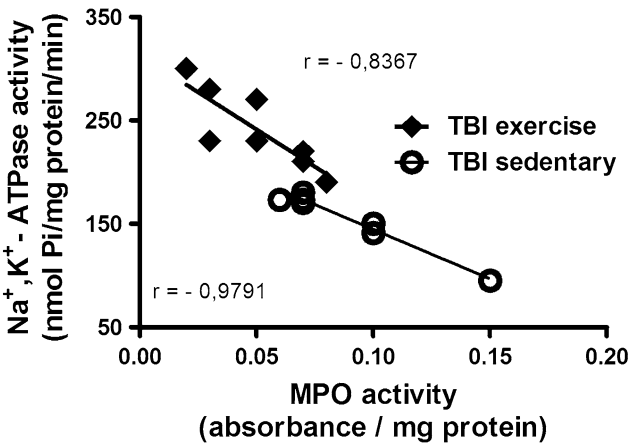

Fig. 6 Pearson's correlation coefficient between Na^+,K^+-ATPase, and MPO activities after FPI in sedentary and exercise groups. Data are individual values for $n = 8$ in each group (Pearson's correlation)

trained after FPI ($r = -0.838$; $P < 0.007$; Fig. 6) suggests FPI-induced neutrophil infiltration leads to Na^+,K^+-ATPase activity inhibition and that previous physical exercise protects against such effect.

Discussion

In the present study, we showed that FPI induced severe motor impairment followed by the increase in both cerebral interleukin-1β and TNF-α levels and decrease in IL-10 without altering IL-6 levels. These findings are consistent with previous studies that show TBI causes severe motor impairment (Marklund et al. 2009; Chow et al. 2010; Costa et al. 2010) BBB opening and brain function disturbance characterized by TNF-α, IL-1β, and ROS generation (Abbott et al. 2006). On the other hand, this experimental protocol revealed that FPI did not alter IL-6 levels when analyzed 24 h after neural injury. Although IL-6 has been widely used as severity marker of inflammatory response in many clinical trials (Lenz et al. 2007), the role of IL-6 in TBI pathophysiology is not completely defined. While some authors suggest that this cytokine plays regulatory effect upon the inflammatory response (Hammacher et al. 1994) by releasing soluble IL-1ra (Tilg et al. 1994), others reveal that IL-6 increase in CSF is associated with increased acute phase proteins (C-reactive protein, fibrinogen, and α1-antitrypsin), with severe BBB dysfunction (Kossmann et al. 1995). The determining factor for such a discrepancy is not known, but one might argue that methodological differences may account for it or the possible dual role exerted by this cytokine. However, further in-depth studies are necessary to establish the mechanism involved.

Since TBI is one of the most common neurologic disorders causing disability (Fujimoto et al. 2004), detailed information about motor recovery is essential for brain rehabilitation because it enables us to establish scientific rehabilitative strategies and predict motor outcome (Jang 2009). In this context, acute damage to the Central Nervous System has been recently associated with immunodeficiency and impaired cell-mediated immunity which may predispose patients to infection (Meisel et al. 2005). Furthermore, the severe motor impairment and disturbance of brain function after TBI in the present study agree with previous studies that

have demonstrated that superimposition of inflammatory process results in worsening of post-injury mortality and weight loss, significant exacerbation of post-injury motor deficit, and cognitive impairments (Venturi et al. 2009).

In the present study, we also confirmed and extended our previous findings that a single FPI decreases Na^+,K^+-ATPase activity (Lima et al. 2009) and showed the involvement of the neutrophil infiltration (here characterized by MPO activity increase) and generation of inflammatory processes in the collapses of ion gradient homeostasis for the first time. In addition, Pearson's correlation analysis revealed strong correlation of MPO activity increase with Na^+,K^+-ATPase activity inhibition in sedentary rats. This experimental body of evidence suggests that secondary injury mechanisms including inflammation are believed to participate in the TBI pathophysiology here characterized by severe motor impairment. In fact, published data have demonstrated that TBI initiates a complex pattern of acute inflammatory events that may either aggravate outcome or reparative processes (Lucas et al. 2006).

Furthermore, it is important to point out that FPI-induced MPO activity increases in cortical tissues surrounding the injured tissue agreeing with the view that the hallmarks of early brain inflammation injured after TBI include activated microglia and presence of neutrophils (Potts et al. 2006). Thus, results presented in this report suggest that neutrophils infiltration induced by FPI may contribute to the failure of select targets leading to secondary damage. The correlation of MPO activity increase with Na^+,K^+-ATPase activity inhibition in sedentary rats also strongly reinforces the assumption that Na^+,K^+-ATPase may represent one of these targets since it is the main factor responsible for maintaining ion gradients across plasma membranes (Ang and Gomez-Pinilla 2007). In agreement with this view, we have demonstrated that a single FPI episode in rat parietal cortex decreases Na^+,K^+-ATPase activity with concomitant increase in the levels of oxidative stress markers (Lima et al. 2008). Accordingly, Na^+,K^+-ATPase inhibition is elicited by prostaglandin E_2 (Oliveira et al. 2009) suggesting that the major prostaglandin lipid mediators of inflammation may increase brain excitability and thus, contribute to a variety of inflammatory responses including TBI.

Recently, a considerable set of evidence support the notion that inflammation action may differ in the acute and delayed phase after TBI, and maintaining limited inflam2-mation is essential for repair (Ziebell and Morganti-Kossmann 2010). In this context, favorable changes in the profile of cerebral anti-inflammatory status (IL-10 increase) elicited by previous physical training may exert prophylactic effect on FPI-induced inflammatory response characterized by increased IL-1β, TNF-α levels, MPO activity, and BBB breakdown. Moreover, the significant protection exerted by the physical exercise against FPI-induced Na^+,K^+-ATPase activity inhibition suggests that the adaptive responses to regular and moderate endurance exercise protects against the failure of some selected targets, such as Na^+,K^+-ATPase enzyme in this TBI model. Results presented in this report demonstrated that previous physical training reduced the trauma-induced motor disability and release of pro-inflammatory mediators. Considering that motor function is mediated by a complex system of neural networks originating in the cortex and terminating in skeletal muscle (Hamm 1990), it is plausible to propose that any interference with secondary injury development induced by previous physical training can attenuate the disruption of this complex motor pathway in this TBI model. The negative correlation between the activities of MPO and Na^+,K^+-ATPase enzymes in animals trained after FPI reinforces this idea and suggest that previous physical exercise protects against FPI-induced neutrophil infiltration and subsequent Na^+,K^+-ATPase activity inhibition. This effective profile alteration of cerebral inflammatory status elicited by previous aerobic physical training (Woods 2005) may reduce initial damage and limit long-term secondary degeneration after TBI (Lenzlinger et al. 2004; Shlosberg et al. 2010).

Although a pre-injury regimen for humans may not be the most effective treatment since the injury time cannot be predicted, the effective protection exerted by physical activity in this TBI model is particularly interesting since it supports the assumption that physical activity alters neuronal functions and thus, delays or prevents secondary cascades that lead to long-term cell damage and neurobehavioral disability after TBI (Stahel et al. 2000). Another emerging finding is that, contrary to the transient proinflammatory effect found after a single exercise bout, regularly performed exercise or physical activity seems to have anti-inflammatory effect (Petersen and Pedersen 2005; Woods 2005). In this context, Funk et al. (2011) have demonstrated that voluntary exercise elevates hippocampal IL-6 offering protection against chemical-induced hippocampal injury associated with TNF receptor activation (Funk et al. 2011). Clinical studies suggest that a reduction in brain inflammation underlies positive effects of exercise on cognitive functioning in patients suffering from neurodegenerative disease or acute brain injury (Erickson et al. 2007). In addition, the down-regulation of TNF signaling is associated with the exercise amelioration of cognitive declines in the aged, and (van Praag et al. 2005; Nichol et al. 2008; Parachikova et al. 2008) in a model of ischemia/reperfusion, exercise preconditioning protects against damage in the brain via TNFα signal transduction pathway and TNF receptor (TNFR) down-regulation (Ding et al. 2006). Given the evidence that inflammation underlies the pathophysiology in many disease states (Black 2003),

results presented in this report highlight a new mechanism whereby physical exercise may be exerting its beneficial effects on disease outcome.

It is also important to point out that a significant increase in total body weight in sedentary versus trained rats was observed along 4 weeks of treadmill training. The difference in body weight between sedentary and trained rats may be explained by changes in body composition. For instance, decrease in subcutaneous adipose tissue of trained rats may explain why body mass was lower in this group. Since we have not determined body composition in the present study, this explanation remains speculative in nature and further studies are necessary to determine the mechanisms involved. In addition, the clear stabilization of blood lactate concentration in the trained group compared with the sedentary group for LT assay suggests that the training program increased aerobic resistance of animals (Gobatto et al. 2001).

In conclusion, results presented in this report revealed that alterations in the profile of cerebral inflammatory status after FPI are involved in the Na^+,K^+-ATPase inhibition. In addition, aerobic training exerts prophylactic effect in this TBI model by the enhancement of endogenous anti-inflammatory (1L-10), inhibition of neutrophil (MPO) infiltration, attenuating BBB breakdown, pro-inflammatory (IL-1β, TNF-α) accumulation, and neuromotor impairment. Considering that inflammatory events together with cytotoxic effects of immune mediators lead to injured brain, physical training may be a new therapeutic approach to control acute inflammation that lead to long-term cell damage and neurobehavioral disability after TBI.

References

Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7:41–53

Ang ET, Gomez-Pinilla F (2007) Potential therapeutic effects of exercise to the brain. Curr Med Chem 14:2564–2571

Arida RM, Scorza FA, de Lacerda AF, Gomes da Silva S, Cavalheiro EA (2007) Physical training in developing rats does not influence the kindling development in the adult life. Physiol Behav 90:629–633

Baskaya MK, Rao AM, Dogan A, Donaldson D, Dempsey RJ (1997) The biphasic opening of the blood-brain barrier in the cortex and hippocampus after traumatic brain injury in rats. Neurosci Lett 226:33–36

Black PH (2003) The inflammatory response is an integral part of the stress response: implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. Brain Behav Immun 17:350–364

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cederberg D, Siesjo P (2010) What has inflammation to do with traumatic brain injury? Childs Nerv Syst 26:221–226

Chen Y, Swanson RA (2003) Astrocytes and brain injury. J Cereb Blood Flow Metab 23:137–149

Chow JW, Yablon SA, Horn TS, Stokic DS (2010) Temporospatial characteristics of gait in patients with lower limb muscle hypertonia after traumatic brain injury. Brain Inj 24:1575–1584

Costa T, Constantino LC, Mendonca BP, Pereira JG, Herculano B, Tasca CI, Boeck CR (2010) *N*-methyl-D-aspartate preconditioning improves short-term motor deficits outcome after mild traumatic brain injury in mice. J Neurosci Res 88:1329–1337

Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T (1999) IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. J Neuroimmunol 101:211–221

D'Ambrosio R, Maris DO, Grady MS, Winn HR, Janigro D (1999) Impaired K(+) homeostasis and altered electrophysiological properties of post-traumatic hippocampal glia. J Neurosci 19:8152–8162

D'Ambrosio R, Fairbanks JP, Fender JS, Born DE, Doyle DL, Miller JW (2004) Post-traumatic epilepsy following fluid percussion injury in the rat. Brain 127:304–314

Ding YH, Mrizek M, Lai Q, Wu Y, Reyes R Jr, Li J, Davis WW, Ding Y (2006) Exercise preconditioning reduces brain damage and inhibits TNF-alpha receptor expression after hypoxia/reoxygenation: an in vivo and in vitro study. Curr Neurovasc Res 3:263–271

Dishman RK, Armstrong RB, Delp MD, Graham RE, Dunn AL (1988) Open-field behavior is not related to treadmill performance in exercising rats. Physiol Behav 43:541–546

Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL (1991) A controlled cortical impact model of traumatic brain injury in the rat. J Neurosci Methods 39:253–262

Endres M, Gertz K, Lindauer U, Katchanov J, Schultze J, Schrock H, Nickenig G, Kuschinsky W, Dirnagl U, Laufs U (2003) Mechanisms of stroke protection by physical activity. Ann Neurol 54:582–590

Erickson KI, Colcombe SJ, Elavsky S, McAuley E, Korol DL, Scalf PE, Kramer AF (2007) Interactive effects of fitness and hormone treatment on brain health in postmenopausal women. Neurobiol Aging 28:179–185

Fan L, Young PR, Barone FC, Feuerstein GZ, Smith DH, McIntosh TK (1995) Experimental brain injury induces expression of interleukin-1 beta mRNA in the rat brain. Brain Res Mol Brain Res 30:125–130

Fujimoto ST, Longhi L, Saatman KE, Conte V, Stocchetti N, McIntosh TK (2004) Motor and cognitive function evaluation following experimental traumatic brain injury. Neurosci Biobehav Rev 28:365–378

Funk JA, Gohlke J, Kraft AD, McPherson CA, Collins JB, Jean Harry G (2011) Voluntary exercise protects hippocampal neurons from trimethyltin injury: possible role of interleukin-6 to modulate tumor necrosis factor receptor-mediated neurotoxicity. Brain Behav Immun (in press). doi:10.1016/j.bbi.2011.03.012

Gobatto CA, de Mello MA, Sibuya CY, de Azevedo JR, dos Santos LA, Kokubun E (2001) Maximal lactate steady state in rats submitted to swimming exercise. Comp Biochem Physiol A Mol Integr Physiol 130:21–27

Hall ED, Yonkers PA, McCall JM, Braughler JM (1988) Effects of the 21-aminosteroid U74006F on experimental head injury in mice. J Neurosurg 68:456–461

Hamm TM (1990) Recurrent inhibition to and from motoneurons innervating the flexor digitorum and flexor hallucis longus muscles of the cat. J Neurophysiol 63:395–403

Hammacher A, Ward LD, Weinstock J, Treutlein H, Yasukawa K, Simpson RJ (1994) Structure-function analysis of human IL-6: identification of two distinct regions that are important for receptor binding. Protein Sci 3:2280–2293

Ivashkova Y, Svetnitsky A, Mayzler O, Pruneau D, Benifla M, Fuxman Y, Cohen A, Artru AA, Shapira Y (2006) Bradykinin B2 receptor antagonism with LF 18-1505T reduces brain edema and improves neurological outcome after closed head trauma in rats. J Trauma 61:879–885

Jamme I, Petit E, Divoux D, Gerbi A, Maixent JM, Nouvelot A (1995) Modulation of mouse cerebral Na+, K(+)-ATPase activity by oxygen free radicals. Neuroreport 7:333–337

Jang SH (2009) Review of motor recovery in patients with traumatic brain injury. Neurorehabilitation 24:349–353

Juliet PA, Mao X, Del Bigio MR (2008) Proinflammatory cytokine production by cultured neonatal rat microglia after exposure to blood products. Brain Res 1210:230–239

Kossmann T, Hans VH, Imhof HG, Stocker R, Grob P, Trentz O, Morganti-Kossmann C (1995) Intrathecal and serum interleukin-6 and the acute-phase response in patients with severe traumatic brain injuries. Shock 4:311–317

Laurer HL, Bareyre FM, Lee VM, Trojanowski JQ, Longhi L, Hoover R, Saatman KE, Raghupathi R, Hoshino S, Grady MS, McIntosh TK (2001) Mild head injury increasing the brain's vulnerability to a second concussive impact. J Neurosurg 95:859–870

Lees GJ, Lehmann A, Sandberg M, Hamberger A (1990) The neurotoxicity of ouabain, a sodium-potassium ATPase inhibitor, in the rat hippocampus. Neurosci Lett 120:159–162

Lenz A, Franklin GA, Cheadle WG (2007) Systemic inflammation after trauma. Injury 38:1336–1345

Lenzlinger PM, Saatman KE, Hoover RC, Cheney JA, Bareyre FM, Raghupathi R, Arnold LD, McIntosh TK (2004) Inhibition of vascular endothelial growth factor receptor (VEGFR) signaling by BSF476921 attenuates regional cerebral edema following traumatic brain injury in rats. Restor Neurol Neurosci 22:73–79

Li S, Stys PK (2001) Na(+)-K(+)-ATPase inhibition and depolarization induce glutamate release via reverse Na(+)-dependent transport in spinal cord white matter. Neuroscience 107:675–683

Lima FD, Souza MA, Furian AF, Rambo LM, Ribeiro LR, Martignoni FV, Hoffmann MS, Fighera MR, Royes LF, Oliveira MS, de Mello CF (2008) Na^+,K^+-ATPase activity impairment after experimental traumatic brain injury: relationship to spatial learning deficits and oxidative stress. Behav Brain Res 193:306–310

Lima FD, Oliveira MS, Furian AF, Souza MA, Rambo LM, Ribeiro LR, Silva LF, Retamoso LT, Hoffmann MS, Magni DV, Pereira L, Fighera MR, Mello CF, Royes LF (2009) Adaptation to oxidative challenge induced by chronic physical exercise prevents Na^+,K^+-ATPase activity inhibition after traumatic brain injury. Brain Res 1279:147–155

Lucas SM, Rothwell NJ, Gibson RM (2006) The role of inflammation in CNS injury and disease. Br J Pharmacol 147(Suppl 1):S232–S240

Marklund N, Morales D, Clausen F, Hanell A, Kiwanuka O, Pitkanen A, Gimbel DA, Philipson O, Lannfelt L, Hillered L, Strittmatter SM, McIntosh TK (2009) Functional outcome is impaired following traumatic brain injury in aging Nogo-A/B-deficient mice. Neuroscience 163:540–551

Marquezi ML, Roschel HA, dos Santa Costa A, Sawada LA, Lancha AH Jr (2003) Effect of aspartate and asparagine supplementation on fatigue determinants in intense exercise. Int J Sport Nutr Exerc Metab 13:65–75

Mathew P, Graham DI, Bullock R, Maxwell W, McCulloch J, Teasdale G (1994) Focal brain injury: histological evidence of delayed inflammatory response in a new rodent model of focal cortical injury. Acta Neurochir Suppl (Wien) 60:428–430

McIntosh TK, Vink R, Noble L, Yamakami I, Fernyak S, Soares H, Faden AL (1989) Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. Neuroscience 28:233–244

Meisel C, Schwab JM, Prass K, Meisel A, Dirnagl U (2005) Central nervous system injury-induced immune deficiency syndrome. Nat Rev Neurosci 6:775–786

Nichol KE, Poon WW, Parachikova AI, Cribbs DH, Glabe CG, Cotman CW (2008) Exercise alters the immune profile in Tg2576 Alzheimer mice toward a response coincident with improved cognitive performance and decreased amyloid. J Neuroinflamm 5:13

Nimmo AJ, Cernak I, Heath DL, Hu X, Bennett CJ, Vink R (2004) Neurogenic inflammation is associated with development of edema and functional deficits following traumatic brain injury in rats. Neuropeptides 38:40–47

Oliveira MS, Furian AF, Rambo LM, Ribeiro LR, Royes LF, Ferreira J, Calixto JB, Otalora LF, Garrido-Sanabria ER, Mello CF (2009) Prostaglandin E2 modulates Na^+,K^+-ATPase activity in rat hippocampus: implications for neurological diseases. J Neurochem 109:416–426

Olsen AL, Morrey JD, Smee DF, Sidwell RW (2007) Correlation between breakdown of the blood-brain barrier and disease outcome of viral encephalitis in mice. Antiviral Res 75:104–112

Parachikova A, Nichol KE, Cotman CW (2008) Short-term exercise in aged Tg2576 mice alters neuroinflammation and improves cognition. Neurobiol Dis 30:121–129

Petersen AM, Pedersen BK (2005) The anti-inflammatory effect of exercise. J Appl Physiol 98:1154–1162

Potts MB, Koh SE, Whetstone WD, Walker BA, Yoneyama T, Claus CP, Manvelyan HM, Noble-Haeusslein LJ (2006) Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. NeuroRx 3:143–153

Shlosberg D, Benifla M, Kaufer D, Friedman A (2010) Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. Nat Rev Neurol 6:393–403

Skou JC, Esmann M (1992) The Na, K-ATPase. J Bioenerg Biomembr 24:249–261

Stahel PF, Kariya K, Shohami E, Barnum SR, Eugster H, Trentz O, Kossmann T, Morganti-Kossmann MC (2000) Intracerebral complement C5a receptor (CD88) expression is regulated by TNF and lymphotoxin-alpha following closed head injury in mice. J Neuroimmunol 109:164–172

Steensberg A (2003) The role of IL-6 in exercise-induced immune changes and metabolism. Exerc Immunol Rev 9:40–47

Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK (2003) IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab 285:E433–E437

Suzuki K, Ota H, Sasagawa S, Sakatani T, Fujikura T (1983) Assay method for myeloperoxidase in human polymorphonuclear leukocytes. Anal Biochem 132:345–352

Tilg H, Trehu E, Atkins MB, Dinarello CA, Mier JW (1994) Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood 83:113–118

Unterberg AW, Stroop R, Thomale UW, Kiening KL, Pauser S, Vollmann W (1997) Characterisation of brain edema following "controlled cortical impact injury" in rats. Acta Neurochir Suppl 70:106–108

van Praag H, Shubert T, Zhao C, Gage FH (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 25:8680–8685

Venturi L, Miranda M, Selmi V, Vitali L, Tani A, Margheri M, De Gaudio AR, Adembri C (2009) Systemic sepsis exacerbates mild post-traumatic brain injury in the rat. J Neurotrauma 26:1547–1556

Woods JA (2005) Physical activity, exercise, and immune function. Brain Behav Immun 19:369–370

Wyse AT, Streck EL, Barros SV, Brusque AM, Zugno AI, Wajner M (2000) Methylmalonate administration decreases Na^+,K^+-ATPase activity in cerebral cortex of rats. Neuroreport 11:2331–2334

Ziebell JM, Morganti-Kossmann MC (2010) Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. Neurotherapeutics 7:22–30