Blood-brain barrier integrity may be threatened by exercise in a warm environment

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Watson, Phillip, Susan M. Shirreffs, and Ronald J. Maughan. Blood-brain barrier integrity may be threatened by exercise in a warm environment. Am J Physiol Regul Integr Comp Physiol 288: R1689–R1694, 2005. First published January 13, 2005; doi:10.1152/ajpregu.00676.2004.—Seven active men were recruited to examine changes in the serum concentration of S100β, a proposed peripheral marker of blood-brain barrier permeability, following prolonged exercise in temperate (T) and warm (W) conditions. Subjects were seated immersed to the neck in water at 35.0 (1.0)°C (T) or 39.0 (0.1)°C (W) for 30 min. Subjects then entered a room maintained at either 18.3 (1.8)°C (T) or 35.0 (0.3)°C (W) and completed 60 min of cycle exercise at 60% peak oxygen uptake. Serum S100β concentration was elevated after exercise in the W trial (+0.12 (0.10) µg/l; P = 0.02) but not after the T trial (P = 0.238). Water immersion and exercise elevated core temperature by 2.1 (0.5)°C to 39.5 (0.3)°C at the end of exercise in the W trial compared with a 0.9 (0.2)°C increase during the T trial (P < 0.001). Weighted mean skin temperature was higher throughout the W trial compared with the T trial (P < 0.001). Heart rate (P < 0.001) and blood glucose (P < 0.001) and lactate (P < 0.001) concentrations were elevated to a greater extent during exercise in the W trial than in the T trial. Ratings of perceived exertion (P < 0.001) and thermal comfort (P < 0.001) were markedly higher throughout the W trial than in the T trial. The results of this study demonstrate that serum S100β was elevated after water immersion and prolonged exercise in a warm environment, suggesting that blood-brain barrier permeability may be altered.

The blood-brain barrier (BBB) is a dynamic structure consisting of microvascular endothelial cells, characterized by the presence of tight junctions and restricted vesicular transport. The foot processes of surrounding astrocytes and the presence of pericytes reinforce these tight junctions. The BBB functions through these specialized structures to maintain a stable environment for the CNS by tightly regulating the exchange of molecules between the CNS and the peripheral circulation. When the BBB is intact, diffusion is largely restricted, with transport mediated through a series of selective molecular carrier systems specific to a wide range of substances, including glucose, amino acids, vitamins, electrolytes, and peptides (13).

The BBB is largely resistant to changes in permeability, but there are situations, including bacterial and viral infections, brain tumors, stroke, and traumatic brain damage, where the function of the BBB may become compromised (20). Changes to the permeability of this barrier may allow the entry or exit of species that can affect the metabolism and functioning of the brain and consequently influence a wide range of homeostatic mechanisms. Paradoxically, a transient opening of the BBB is desirable in the treatment of some conditions to allow the delivery of therapeutic agents into the CNS that would typically not cross the intact BBB (34).

An increase in the permeability of the BBB can result in a detectable leakage of substances from the blood into the CNS and from the CNS into the peripheral circulation. Kapural et al. (17) proposed that the appearance of CNS-specific proteins, in particular S100β, in the circulation might be used as a relatively noninvasive peripheral marker of BBB function. S100β is a low-molecular-mass (21 kDa) calcium-binding protein expressed predominantly within the CNS by astrocytes and Schwann cells. It is typically found in low concentrations in the serum of healthy individuals, with a cerebrospinal fluid-to-serum concentration ratio of ~18:1 (35). BBB opening would therefore be expected to significantly elevate serum S100β concentrations (17). Because brain trauma is known to result in a marked elevation in cerebrospinal fluid S100β concentrations and damage to the brain is typically accompanied by a breakdown in barrier function (20), serum S100β has been widely employed in a clinical setting as a peripheral index of CNS damage.

There is some evidence that prolonged exercise may lead to increased BBB permeability. Animal studies have established that the BBB can be widely disrupted after 30 min of forced swimming exercise (37, 40). These changes were found to be relatively acute, with normal BBB function restored 2 h after exercise. Additionally, there is some evidence that exercise leads to an increase in serum S100β concentrations (9, 15, 23, 30), but the possibility that this resulted from a change in BBB integrity has not been considered.

There is a growing body of evidence to suggest that the CNS is important in the development of fatigue during prolonged exercise. The capacity to perform prolonged exercise is significantly reduced when exercise is performed in a warm environment (12). In contrast to temperate conditions, fatigue during endurance exercise in high ambient temperatures does not appear to be adequately explained by the depletion of muscle glycogen or by cardiovascular and fluid balance factors (25).

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exercise in a warm environment, but the influences of combined exercise and heat stress on BBB function have yet to be explored. The aim of the present investigation was to determine whether a combination of water immersion and prolonged exercise in temperate (T trial) and warm (W trial) environmental conditions results in a change in serum S100β.

The appearance of S100β in the circulation has been proposed as a peripheral marker of BBB disruption. Because the development of whole-body hyperthermia was hypothesized to increase BBB permeability, preexercise water immersion was included to accelerate the change in core temperature during the W trial. Although the present study did not employ an exhausting exercise protocol, the aim was to examine a response that may be important to the development of fatigue during prolonged exercise.

METHODS

Subjects. Seven healthy men [age 25.7 (5.0) yr, height 1.76 (0.08) m, mass 77.1 (5.0) kg, peak oxygen uptake (VO2 peak) 4.1 (0.2) l/min] volunteered to participate in this study. At the time of the study, all subjects were taking part in regular endurance exercise, but were not accustomed to exercise in a warm environment. Because of the nature of this investigation, those with a history of metabolic disease or psychiatric illness were excluded. Before volunteering, all subjects received written details outlining the nature of the study. After any questions regarding the protocol were addressed, a written statement of consent was signed. The protocol received approval from the Loughborough University Ethical Advisory Committee.

Experimental protocol. All subjects completed a preliminary test to determine VO2 peak, a familiarization trial, and two experimental trials. The preliminary test consisted of a discontinuous, incremental cycle ride to exhaustion to determine VO2 peak and the power output required to elicit 60% VO2 peak. The familiarization trial was identical to the W trial and was undertaken to ensure that the subjects were accustomed to the protocol and environmental conditions employed throughout the investigation. Experimental trials were completed under either T or W conditions, administered in a randomized order separated by at least 7 days to minimize the development of heat acclimation. To help ensure that metabolic conditions were similar before the experimental trials, subjects were instructed to record all food and fluid intake, as well as any exercise performed, in a diary over the 2 days before the first trial. This pattern of dietary intake and physical activity was replicated as closely as possible during the 2 days before subsequent trials. Subjects were also asked not to perform any strenuous exercise or consume alcoholic beverages in the 24 h before all trials.

All experimental trials commenced in the morning after an overnight fast, other than the ingestion of ~500 ml of plain water at least 90 min before the start of the trial. Once subjects arrived at the laboratory, a rectal thermometer was inserted 10 cm beyond the anal sphincter. Thermistor probes were attached to the skin surface at four locations (chest, triceps, thigh, calf) to determine weighted mean skin temperature (33), and a heart rate (HR) telemetry band was positioned. Postvoid, nude body mass was determined after probes were positioned to allow ease of measurement after water immersion. Subjects were seated for 15 min in a comfortable environment (24–26°C) with one hand immersed in warm water (42°C) for 10 min to allow arterialized venous blood to be drawn at rest. A 21-g cannula was introduced into a superficial forearm vein to enable repeated blood sampling. To ensure the cannula remained patent between blood sampling, it was flushed with a small volume of heparinized saline after each collection. Two resting 7.5-ml blood samples were drawn (~5 and 0 min), and baseline measurements of temperature and HR were made at 5-min intervals.

Subjects then entered a water tank and were seated for 30 min immersed to the neck with the exception of the cannulated forearm. Water temperature was maintained at either 35.0 (0.1)°C or 39.0 (0.1)°C in the T and W trials, respectively. Core and skin temperatures and HR were recorded at 5-min intervals during immersion, and the subject’s perceived thermal stress was assessed every 10 min using a 21-point scale ranging from unbearable cold (~10) to unbearable heat (~10). During the final minute of water immersion, a blood sample was drawn, after which the subject left the water tank and toweled dry. Body mass was then remeasured to determine sweat losses occurring during the period of immersion. Subjects then dressed in shorts and shoes.

Subjects entered a climatic chamber maintained at either 18.3 (1.8)°C (T trial) or 35.0 (0.3)°C (W trial) and began a bout of cycle exercise (Gould Corvial 300, Groningen, Holland) corresponding to 60% VO2 peak for 60 min. Under both conditions, the relative humidity was 60 (5%). The time between leaving the water tank and commencing exercise in the climatic chamber was ~5 min. Core and skin temperatures and HR were recorded every 5 min throughout exercise. Ratings of perceived exertion (4) and thermal stress were obtained at 10-min intervals. Blood samples (7.5 ml) were drawn during exercise at 15-min intervals. Trials were stopped if a subject’s rectal temperature reached 40.0°C. After the cessation of exercise, subjects returned to a comfortable environment where recovery was monitored for 15 min and postexercise body mass was measured to determine sweat losses.

Blood handling and analysis. Blood samples collected throughout the experimental protocol were drawn into dry syringes with a 2.5-ml aliquot dispensed into tubes containing K2EDTA and the remaining 5 ml into plain tubes. Duplicate 100-μl aliquots of EDTA-treated whole blood were rapidly deproteinized in 1 ml of ice-cold 0.3 N perchloric acid. These were centrifuged, and the resulting supernatant was used for determination of blood glucose (God-PAP, Randox, Antrim, UK) and blood lactate concentrations (21). Hemoglobin (cyanmethemoglobin method) and hematocrit (microcentrifugation) values were used to estimate percent changes in blood, plasma, and red cell volumes relative to the second resting sample (10). The 5-ml aliquot added to a plain tube was kept on ice until the end of each trial before being centrifuged to yield serum; this was kept frozen at −20°C for the analyses of S100β using a commercially available enzyme-linked immunosorbant assay (Sangtec Medical, Bromma, Sweden) and free fatty acids (FFA; Roche Diagnostics, Mannheim, Germany). The intra-assay coefficient of variation for serum S100β measurements was 6.7%.

Statistical analyses. Data are presented as means (SD) unless otherwise stated. To identify differences in normally distributed results, two-way (time-by-trial) repeated-measures ANOVA was employed. Where a significant interaction was apparent, pair-wise differences were evaluated using Tukey’s post hoc procedure. For the purpose of hypothesis testing, the 95% level of confidence was predetermined as the minimum criterion to denote a statistical difference (P < 0.05).

RESULTS

One subject was stopped after 52 min of exercise during the W trial due to the attainment of a rectal temperature of 40.0°C. The final exercise blood sample was drawn at this point, and the recovery was monitored as usual. This subject’s data were included in all analyses.

Serum S100β data are presented in Fig. 1. Serum concentrations of S100β were not different at rest between trials, with values of 0.06 (0.04) and 0.07 (0.05) μg/l measured in the T and W trials, respectively (P = 0.372). A 2.6-fold increase in S100β was apparent after water immersion and prolonged exercise in the W trial (+0.12 (0.10) μg/l; P = 0.020), with no change apparent after the T trial (+0.06 (0.09) μg/l; P = 0.238). Two subjects who experienced no change in serum
S100β in either trial were primarily responsible for the large variation in S100β observed after exercise (see Fig. 3).

The blood glucose and lactate and plasma FFA responses to the experimental trials are presented in Table 1. Blood glucose concentrations were not different at rest between conditions ($P = 0.457$). Throughout exercise in the W trial, blood glucose was significantly elevated above corresponding values in the T trial. Blood lactate concentrations were elevated above rest during exercise in both trials and maintained at a significantly higher level during the W trial than in the T trial ($P < 0.001$). There was no difference between trials in serum FFA at rest ($P = 0.459$). Although FFA concentrations did not change significantly during exercise, there was a marked increase apparent after 15 min of recovery, with this response more pronounced in the W trial. Percent changes in plasma volume were not different between trials. The experimental protocol produced a fall of ~7–9% in plasma volume during exercise on both trials, with a marked reduction apparent from the first exercise blood sample (15 min; Table 1). There was no further change in plasma volume throughout exercise.

Core temperature at rest before water immersion was not different between trials ($P = 0.253$; Fig. 2, top). Warm water immersion caused an increase in core temperature, but no change was observed during water immersion in the T trial. Exercise resulted in a marked elevation in core temperature in both trials, with a plateau apparent after 30 min in the T trial compared with a near linear increase in the W trial. There was a greater increase in core temperature in the W trial (2.1 (0.5)°C) than in the T trial (0.9 (0.2)°C; $P < 0.001$). Core temperature at the end of exercise in the W trial was 39.5 (0.3)°C. There was no apparent association between the change in S100β and the change in core temperature, with correlation coefficients calculated as $-0.318 (P = 0.486)$ for the T trial and 0.021 ($P = 0.965$) for the W trial (Fig. 3). Weighted mean skin temperature was different between trials throughout the period of water immersion and during exercise and recovery ($P < 0.001$; Fig. 2, bottom). Body mass loss during the experimental protocol was greater in the W trial (1.79 (0.35) kg) than in the T trial (0.70 (0.14) kg) ($P < 0.001$).

Warm water immersion resulted in a marked elevation in HR above corresponding values in the T trial, with this difference maintained throughout exercise and recovery ($P < 0.001$). HR at the end of exercise in the T trial was 138 (8) beats/min compared with 174 (11) beats/min during the W trial ($P < 0.001$). Throughout the W trial, ratings of perceived exertion were elevated above those in the T trial ($P < 0.001$; Fig. 4). Perceived exertion increased with time during both trials, reaching values of 12 (0) and 16 (2) in the T and W trials, respectively. Perceived thermal stress was relatively stable during the T trial, with little change apparent during water immersion or exercise (Fig. 5). In the W trial, the subjects’ thermal stress progressively increased and was persistently higher than values obtained in the T trial ($P < 0.001$).

![Fig. 1. Individual serum S100β concentrations at rest (0) and at the end of exercise (60) in the temperate (T) and warm (W) trials. Bars and whiskers represent means ± SD. *Significant difference from the 0 time point in the W trial, $P < 0.05$. †Significant difference between the T trial and the corresponding time point in the W trial, $P < 0.05$.](image)

### Table 1. Concentrations of glucose and lactate in blood and FFA in plasma and the percent change in plasma volume

<table>
<thead>
<tr>
<th></th>
<th>Preimmersion</th>
<th>Postimmersion</th>
<th>15</th>
<th>30</th>
<th>45</th>
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<th>15 min postexercise</th>
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<tr>
<td><strong>Glucose, mmol/l</strong></td>
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<td>T</td>
<td>5.3 (0.4)</td>
<td>4.9 (0.3)</td>
<td>4.9 (0.2)</td>
<td>4.9 (0.4)</td>
<td>4.9 (0.4)</td>
<td>4.8 (0.3)</td>
<td>5.2 (0.4)</td>
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<tr>
<td>W</td>
<td>5.4 (0.5)</td>
<td>5.0 (0.4)</td>
<td>5.4 (0.3)</td>
<td>5.5 (0.4)</td>
<td>5.6 (0.4)</td>
<td>5.5 (0.5)</td>
<td>5.8 (0.5)</td>
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<td><strong>Lactate, mmol/l</strong></td>
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<tr>
<td>T</td>
<td>0.70 (0.17)</td>
<td>0.73 (0.16)</td>
<td>2.54 (0.78)*</td>
<td>2.12 (0.68)*</td>
<td>1.79 (0.58)*</td>
<td>1.42 (0.25)</td>
<td>0.80 (0.20)</td>
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<tr>
<td>W</td>
<td>0.80 (0.31)</td>
<td>0.89 (0.24)</td>
<td>3.37 (0.90)**</td>
<td>2.91 (0.88)**</td>
<td>2.85 (0.68)**</td>
<td>2.55 (0.41)**</td>
<td>1.51 (0.37)**</td>
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<td><strong>FFA, mmol/l</strong></td>
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<td>T</td>
<td>0.31 (0.11)</td>
<td>0.29 (0.12)</td>
<td>0.20 (0.08)</td>
<td>0.24 (0.10)</td>
<td>0.31 (0.14)</td>
<td>0.41 (0.23)</td>
<td>0.72 (0.28)</td>
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<tr>
<td>W</td>
<td>0.33 (0.14)</td>
<td>0.35 (0.20)</td>
<td>0.25 (0.15)</td>
<td>0.32 (0.19)</td>
<td>0.40 (0.22)</td>
<td>0.56 (0.36)</td>
<td>1.13 (0.40)**</td>
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<tr>
<td><strong>% ΔPV</strong></td>
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<tr>
<td>T</td>
<td>1.8 (1.3)</td>
<td>9.1 (3.2)*</td>
<td>−7.2 (1.3)*</td>
<td>−7.6 (1.2)*</td>
<td>−8.1 (0.8)*</td>
<td>−8.5 (1.2)*</td>
<td>−2.0 (1.9)</td>
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<tr>
<td>W</td>
<td>0.6 (1.8)</td>
<td>4.0 (4.5)</td>
<td>−7.8 (2.3)**</td>
<td>−8.3 (1.0)**</td>
<td>−8.9 (2.0)**</td>
<td>−8.9 (1.2)**</td>
<td>−5.3 (2.4)**</td>
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Values are means (with SD in parentheses). ΔPV, change in plasma volume; T and W, temperate and warm trial, respectively. Significant difference from the 5-min time point in the *T and †W trials respectively, $P < 0.05$. ‡$P < 0.05$, significantly different between the T trial and the corresponding time point in the W trial.
Exercise-induced hyperthermia has been suggested to result in a reduction in motivation and drive to continue exercise, and this may to some extent explain the decline in capacity to perform prolonged exercise apparent when ambient temperatures are high (25). Recent work has demonstrated that hyperthermia results in a reduction in sustained maximal muscle activation (26), altered brain activity (24), and elevated perceived exertion (27), but the underlying neurobiological mechanisms responsible for these changes are not clear at present.

This study examined the possibility that hyperthermia induced by warm water immersion and exercise in the heat might cause an alteration in BBB permeability.

The main observation to arise from the present study was the marked elevation in the serum concentration of the CNS-specific protein S100β following exercise in a warm environment. This response was not apparent after exercise in temperate conditions. Increased serum concentrations of S100β have been reported following osmotic opening of the BBB (17), with the kinetics of change closely related to the extent and time course of BBB disruption determined using gadolinium-enhanced MRI (16). The appearance of this CNS-specific protein in the peripheral circulation up to serum concentrations of 0.34 μg/l has been proposed as a peripheral marker of BBB permeability (20), with higher concentrations associated with neuronal damage and poor patient outcome.

An increase in serum S100β concentrations has been reported following boxing competition (30), soccer heading drills (23), and prolonged running (15) and swimming exercise (9), but this work did not consider the possibility that these changes may have resulted from changes in BBB function. S100β has been employed as a peripheral marker of brain trauma in a clinical setting for a number of years, and more recently it has been adopted in sports medicine; however, a change in serum S100β does not occur unless there is a change in BBB integrity (20). At present, the functional consequences of changes in BBB permeability during exercise are not clear.

Previous reports of changes in BBB permeability with exercise have employed an animal model involving forced swimming (37, 40). This exercise model places an animal under severe stress and has been associated with the appearance of hemorrhagic spots on the wall of the stomach and the excretion of fecal pellets (40). It is important to note that the breakdown in BBB permeability reported in the series of studies by Sharma and colleagues was particularly severe and widespread, with the extravasation of an albumin-tracer complex (Evans blue and 131I-labeled sodium) into the brain. Any change in BBB permeability in humans occurring during exercise is unlikely to be as dramatic, although there have been numerous reports of fatalities associated with extreme physical exertion in hot climates.

Although it is accepted that the BBB integrity can be altered by a wide range of physiological disturbances, the molecular and cellular mechanisms behind these changes in vivo are not clear at present. Potential contributing factors that may be important regarding changes to BBB permeability during exercise in a warm environment include the development of hyperthermia (39, 41), changes to central serotonergic neurotransmission (37, 40), hyperammonemia (19), increased circulating epinephrine concentrations (1), and an upregulation in proinflammatory cytokine production (8). Prolonged exposure to physiological and psychological stress has also been demonstrated to result in widespread BBB disruption in humans.
The data collected during the present study (rating of perceived exertion, thermal stress, HR) suggest that subjects found the W trial significantly more difficult to complete. The lower levels of stress encountered during the T trial did not appear to be sufficient to produce a marked change in BBB function.

Whole-body hyperthermia induced by prolonged exposure to passive heat stress has been demonstrated to result in impaired BBB integrity in rats (38, 42). In the present study, the W trial resulted in a marked and sustained increase in core (rectal) temperature, with levels elevated above 39.0°C during the final 25 min of exercise and throughout the recovery period, compared with a mean core temperature of 38.3°C at the end of exercise in the T trial (Fig. 2, top). Additionally, brain temperature, a more representative measure of the thermal load placed on the brain capillary network, is thought to be persistently maintained at least 0.2°C above core (esophageal) temperature during exercise in a warm environment (29). No significant relationship between changes in S100β and core temperature was found in the present study. In light of the previous work that examined changes in BBB integrity with exposure to whole-body hyperthermia, it is not clear why an association was not apparent, although it is possible that a number of the other factors mentioned above may have also played an important role.

It has been suggested that the activity of central serotonergic neurons may be important in mediating changes in BBB integrity during both exercise and exposure to hyperthermia (39), with the administration of p-chlorophenylalanine (a tryptophan hydroxylase inhibitor) reported to prevent exercise-induced BBB breakdown (40). Serotonin is a potent vasoactive agent, known to act on both central and peripheral blood vessels (7). Prolonged exercise has been demonstrated to result in a marked increase in serotonin neurotransmission (22). Although there is some limited evidence that central serotonergic activity is increased by a greater extent during exercise in a warm environment compared with exercise in temperate conditions (32), this is not supported by data in which cerebral tryptophan uptake was examined using arterial-venous difference across the brain (28). This does not discount a role for serotonin in mediating exercise-induced changes in BBB function, but it is likely that a complex interaction of various neuromodulators is involved in permeability control during exercise.

Although serum S100β is now widely employed in a clinical setting as a diagnostic tool for the assessment of brain trauma and, more recently, BBB dysfunction, a number of factors must be first considered when interpreting these data. Although the β-subunit of this protein is highly specific to the CNS (2), it is expressed, albeit in minute quantities, in peripheral tissues, including bone, muscle, heart, and adipose tissue (11, 20). Recent work suggests that S100β is liberated into the circulation from these tissues only when subjected to significant trauma, such as surgery (3, 11, 31). Exercise-induced muscle damage has been proposed to be a possible cause of changes in serum S100β (15), but the exercise task used in this study is unlikely to have resulted in significant loss of membrane integrity in the working muscles. Muscle damage is much more likely to occur in running exercise, as in the study of Hasselblatt et al. (15), than in the cycle exercise employed in this investigation (18). In addition, all subjects were habituated to cycling exercise, and it is well recognized that prior habituation greatly decreases the release of muscle-specific proteins into the circulation (5). As S100 proteins are cleared from the peripheral circulation by the kidneys, with a serum half-life of −2 h, the change observed after exercise may have resulted from reduced renal clearance. Prolonged exercise, in particular under conditions of heat stress, is associated with a marked reduction in renal blood flow (36), but there is evidence that extraction of protein remains high during strenuous exercise (6), suggesting that the clearance of S100β should not be altered.

The present study has demonstrated that serum S100β is elevated after prolonged exercise in a warm environment, suggesting that BBB permeability may be altered. Previous animal studies have also observed a marked increase in BBB permeability after forced swimming exercise. The development of hyperthermia, an increase in central serotonergic function across the brain (28). This does not discount a role for serotonin in mediating exercise-induced changes in BBB function, but it is likely that a complex interaction of various neuromodulators is involved in permeability control during exercise.

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activity, elevated circulating ammonia and epinephrine concentrations, and increased production of proinflammatory cytokines may contribute to this response. These findings may be important for two reasons: 1) a marked change in BBB integrity during exercise may disturb normal brain function and contribute to the development of central fatigue; and 2) serum S100β is now being employed as an index of brain trauma in individuals who suffer head injuries during sports (23, 30). Changes in the permeability of the BBB to this protein may give misleading results in exercising individuals, particularly under conditions that lead to significant heat stress.

REFERENCES


