

Increase in Serum S100B Protein Level After a Swimming Race

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Abstract/Résumé

Physical activity has been shown to be a beneficial stimulus to the central and peripheral nervous systems. The S100B is a cytokine physiologically produced and released predominantly by astrocytes on the central nervous system (CNS). In order to study the possible influence of a nonimpact exercise on S100B serum levels, we measured this protein serum level after a 7,600-meter swimming race. We observed an increase in S100B levels in athletes post-race compared with their baseline values, pointing to a potential acute influence of physical exercise on serum S100B levels not related with CNS injury. We discuss this result and emphasize the possible central and peripheral origins of S100B serum levels.

Les bénéfices de l'activité physique sur les systèmes nerveux central et périphérique ne sont plus à prouver. Les S100B sont des cytokines produites physiologiquement et surtout libérées par les astrocytes du système nerveux central (CNS). Pour étudier l'effet potentiel d'une activité physique sans impact, nous avons mesuré les concentrations sériques de cette protéine après une compétition à la nage sur une distance de 7 600 m. Par comparaison au niveau de base, les concentrations de S100B étaient plus importantes après la compétition; l'activité physique aurait donc un effet potentiel immédiat sur les niveaux sériques de S100B qui ne

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sont pas reliés à des lésions du CNS. Cette observation est analysée en fonction des causes possibles, centrale et périphérique, des augmentations de niveau sérique de S100B.

Introduction

The practice of regular exercise and a physically active lifestyle have been correlated with many health and fitness benefits that include diminishment of stress and risk for cardiovascular diseases (American College of Sports Medicine, 2000). In addition, regular exercise exerts direct effects on the central nervous system (CNS) by improving cognitive function, stabilizing mood, and acting as a neuroprotector agent against chronic and acute brain diseases (Katula et al., 1999; Laurin et al., 2001; Meyer and Brooks, 2000). Brain imaging techniques and biochemical analysis have been used to study hemodynamic, metabolic, and immunological responses of human and animal brains to physical activity. Some researchers have suggested that the effects of exercise on the CNS could be associated with changes in neurotransmitter levels such as serotonin (5-HT) (Chaouloff, 1997; Dishman, 1997; Gomez-Merino et al., 2001), as well as in the expression of neurotrophic factors and cytokines such as brain-derived neurotrophic factor (Cotman et al., 2002), fibroblast growth factor (Gomez-Pinilla et al., 1997), and interleukins (Moldoveanu et al., 2001). Together, these studies point to short- and long-term adaptations of neuronal and glial cells to exercise.

The S100B protein is a cytokine that is physiologically produced and released predominantly by astrocytes in the CNS, where it exerts neurotrophic and gliotrophic actions. Accordingly, it appears to be involved in neurodevelopment processes (Portela et al., 2002b) such as proliferation, maturation, and maintenance of glial and neuronal cells (Azmitia et al., 1992; Donato, 2001). Moreover, it has been reported that the stimulation of serotonin receptor 5HT_{1A} increases the intracellular content and release of astrocytic S100B (Azmitia et al., 1992; 2001; Haring et al., 1993), pointing to a possible interaction between S100B and serotonin at physiological conditions. Furthermore, an increased serum level of this protein was observed in response to CNS injury (Berger et al., 2002; Portela et al., 2002a; Walz et al., 2000).

To our knowledge, only one study has evaluated the interaction among different modalities of exercise and serum S100B levels. Otto et al. (2000) found an increase in serum S100B levels after a competitive boxing match, which was associated with head injury due to punches. Furthermore, they also found an interesting increase in serum S100B levels after a 25-km running race, and proposed that vertical vibration of the brain at each step during running could be involved in the increase of serum S100B levels.

We postulated that increases in serum S100B levels after an exercise session could be related to the exercise, independent of CNS injury. To test this hypothesis, we measured serum S100B levels after a nonimpact exercise session in a 7,600-meter swimming race. Additionally, in order to determine whether the reported increase in CNS serotonin activity during exercise could be an underlying mechanism related to variations in serum S100B levels, we measured the serum prolactin of athletes, since some studies suggest that the level of this hormone reflects central serotonin activity (Eriksen et al., 2002).

Material and Methods

SUBJECTS AND STUDY DESIGN

Trained swimmers, 16 men, competed in the XIII Travessia do Pontal de Tapes (Tapes, RS, Brazil) during the VI Brazilian Championship of Open Swimming Marathon in March 2002. The swimmers read and signed an informed consent form. Blood samples were collected from them 24 hours before the competition (baseline value) and up to 15 minutes after the race (post-race value). The local ethics committee approved all experiments.

S100B ASSAY

Blood samples were collected without anticoagulant at the antecubital vein and drawn into 7-cc vacuum tubes. Serum was obtained by centrifugation at $3,000 \times g$ for 7 minutes, and immediately frozen and stored at -70°C until analyses. Serum S100B levels were measured using a monoclonal immuno-luminometric assay (LIA-mat[®] BYK-Sangtec[®]100, Dietzembach, Germany) in a Lumat LB9507 luminometer (EG&G Berthold) as previously described (Portela et al., 2002b). Values below the detection limit of 20 pg/mL, as specified by the manufacturer, were considered as 10 pg/mL. All determinations were carried out in duplicate in the same experiment, and the coefficient of variation was within 5%.

PROLACTIN ASSAY

Prolactin was measured using an automated chemiluminescent assay (Prolactin ACS: 180 Bayer[®], U.S.), by a two-site sandwich immunoassay using an antibody labeled with acridinium ester as a tracer. All determinations were carried out in the same experiment in duplicate, and the coefficient of variation was within 5%.

STATISTICAL ANALYSIS

The data are shown as mean \pm standard error of mean (*SEM*) and were analysed using a paired-samples *t*-test. A *p* value < 0.05 was considered statistically significant. A Pearson's correlation was performed between baseline and post-race measures of S100B and prolactin.

Results

Table 1 presents the epidemiological data and serum S100B levels of the athletes. Their mean age was 25.4 ± 2.2 years, and the race time was 107.6 ± 3.4 minutes. Serum S100B levels of the athletes at baseline were statistically different from their post-race condition ($p < 0.001$, $t = -3.892$). To further characterize this increase, we conducted a linear correlation analysis between baseline and post-race S100B levels. Figure 1 illustrates the high positive correlation obtained (Pearson's coefficient = 0.89, $r^2 = 0.79$, $p < 0.0001$), indicating that the increase was not chaotic (i.e., the rank of S100B values obtained at baseline was related to that observed in the post-race condition).

Athletes after a 7,600-m swimming race exhibited an increase in prolactin serum levels (mean = 16.0 ± 2.2 ng/dL) compared with their baseline value (mean = 10.2 ± 0.9 ng/dL; $p = 0.035$, $t = -2.32$). However, we did not observe a correlation between serum levels of S100B and prolactin (data not shown).

Table 1 Subjects' Age, Race Time, and Serum S100B Levels (mean \pm SEM)

Subj. no.	Age (yrs)	Race time (min)	Serum S100B levels (pg/mL)		Diff. between post race & baseline
			Baseline	Post-race	
1	26	85	10.0	49.0	39.0
2	26	84	10.0	69.0	59.0
3	17	96	175.0	211.0	36.0
4	19	96	121.0	131.0	10.0
5	28	104	116.0	127.0	11.0
6	17	106	247.0	297.0	50.0
7	21	110	81.0	122.0	41.0
8	30	104	29.0	92.0	63.0
9	41	134	143.0	126.0	-17.0
10	25	109	27.0	10.0	-17.0
11	18	106	10.0	10.0	0.0
12	19	111	41.0	149.0	108.0
13	29	125	81.0	130.0	49.0
14	48	118	10.0	39.0	29.0
15	26	123	10.0	10.0	0.0
16	17	111	86.0	158.0	72.0
Mean			70.66	108.13	33.31
\pm SEM			\pm 17.66	\pm 19.49*	\pm 8.55

Note: All subjects were male. Serum S100B levels at post-race are different from baseline condition, * $p < 0.001$.

Discussion

Despite the experience of our group in dealing with serum S100B levels as a biochemical marker of brain injuries (Portela et al., 2002a; Walz et al., 2000), we previously demonstrated that these levels vary physiologically with age in humans (Portela et al., 2002b), decreasing markedly from birth to around 18–20 years of age. This indicates that increases in serum S100B levels not only reflect brain injury but could also reflect variations in physiological brain activity. As it has been shown that physical activity affects brain functional parameters, such as increasing trophic factors, it is worthwhile to examine whether exercise in athletes could affect peripheral levels of S100B protein apart from the occurrence of injury.

Accordingly, our results showed that a single bout of endurance exercise in athletes promoted an increase in serum S100B levels compared with baseline values, demonstrating an influence of exercise on S100B levels (see Table 1 and Figure 1). Considering that swimming has a low mechanical impact on the CNS, our results point out that physical activity produced an increase in serum S100B levels independent of any traumas caused by axial vibration of the brain, as discussed by Otto et al. (2000) in other exercises. Since Otto et al. found slight (but not signifi-

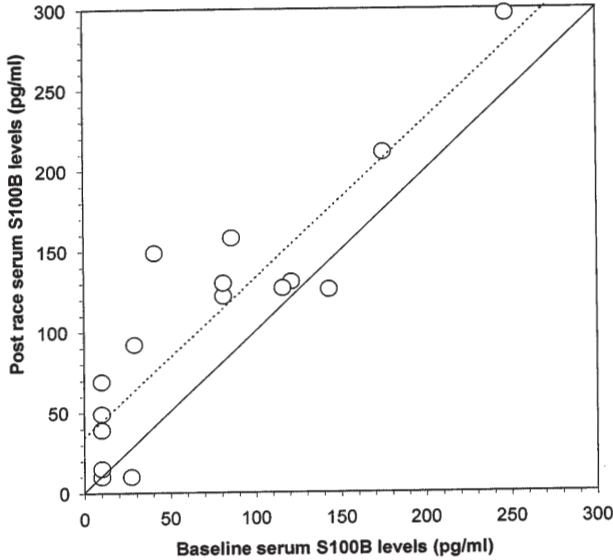


Figure 1. Correlation between athletes' baseline and post-race serum S100B levels. Pearson correlation coefficient (r) = 0.89, with r^2 of 0.79, $p < 0.0001$. Circles above the continuous line represent the athletes whose S100B levels increased after exercise. Dotted line shows the linear regression.

cant) changes in serum S100B levels after exercise of shorter duration, it is reasonable to postulate that exercise of medium to long duration may cause significant increases in serum S100B levels in athletes, as observed in 25-km running (Otto et al., 2000) and 7,600-m swimming (Table 1).

Regarding possible mechanisms involved in this increase, we postulated that it could be due to S100B release from the CNS, triggered by astrocytic responses to serotonergic stimulation. Accordingly, it has been reported that a single bout of endurance exercise promotes an increase in serotonin levels in the brain (Chaouloff et al., 1997; Gomez-Merino et al., 2001). Azmitia et al. (1992, 2001) showed that the modulation of astrocytic 5-HT_{1A} receptors by serotonin promotes S100B release from astrocytes. Additionally, an increase in S100B expression was obtained after treatment with an inhibitor of serotonin uptake, fluoxetine (Haring et al., 1993), whereas a treatment with tryptophan hydroxylase inhibitor decreases S100B expression (Eriksen et al., 2002).

To study the interaction between S100B and serotonin, we measured prolactin serum levels, which is an indirect parameter of central serotonergic activity (Van de Kar et al., 1996; for review, see Strüder et al., 2001). Interestingly, our results showed that the serum concentration markers of both prolactin and S100B protein were elevated immediately after physical activity in athletes. As prolactin is released mainly by the stimulatory action of serotonergic inputs in hypothalamic 5-HT_{1A} and 5-HT_{2A/C} receptors (Clemens et al., 1978; Van de Kar et al., 1996), we postulated that the increase in serum prolactin levels after the swim-

ming race could represent an enhancement in serotonin activity. In turn, with more serotonin acting in 5-HT_{1A} receptors, more S100B could be released. This is a speculative model based on previous reports, although other mechanisms involved in S100B release cannot be excluded.

Thus, we consider that the metabolic responses to endurance exercise increase CNS 5-HT synthesis, which consequently could be implicated in the release of S100B by astrocytes. However, as the increases in serum prolactin and S100B levels were not correlated, at this point we cannot entirely associate central serotonergic activity with peripheral S100B levels.

Importantly, despite the fact that S100B protein is predominantly present in the CNS (about 95%), other peripheral sources such as fat and cartilaginous tissues and melanocytes (Donato, 2001) cannot be excluded. Given that during exercise there is an increase in lipolysis, the contribution of this metabolic process to serum S100B levels during exercise cannot be ruled out. Accordingly, a previous study has demonstrated that the release of S100 (possibly S100B) from adipocytes of rats was enhanced by epinephrine *in vitro* (Suzuki et al., 1984). However, in physiological conditions, the relevance of peripheral sources on serum S100B levels is still unknown.

Conclusion

The present work showed that endurance exercise performed by athletes promoted an increase in serum S100B levels independent of CNS injury. Several mechanisms related to central and/or peripheral S100B sources may be involved. However, more studies are needed to clarify the influence of serum S100B protein in exercise physiology, as well as the mechanism involved in its secretion during physical activity.

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References

- American College of Sports Medicine. (2000). Benefits and risks associated with exercise. In: B.A. Franklin, M.H. Whaley, and E.T. Howley (Eds.), **ACSM's Guidelines for Exercise Testing and Prescription**, pp. 1-21. Philadelphia: Lippincott Wms & Wilkins.
- Azmitia, E.C. (2001). Modern views on an ancient chemical: Serotonin effects on cell proliferation, maturation, and apoptosis. **Brain Res. Bull.** 56: 413-424.
- Azmitia, E.C., Griffin, W.S., Marshak, D.R., Van Eldik, L.J., and Whitaker-Azmitia, P.M. (1992). S100 beta and serotonin: A possible astrocytic-neuronal link to neuropathology of Alzheimer's disease. **Prog. Brain Res.** 94: 459-473.
- Berger, R.P., Pierce, M.C., Wisniewski, S.R., Adelson, P.D., Clark, R.S., Ruppel, R.A., et al. (2002). Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. **Pediatrics** 109(2): E31.
- Chaouloff, F. (1997). Effects of acute physical exercise on central serotonergic systems. **Med. Sci. Sports Exerc.** 29: 58-62.

- Clemens, J.A., Roush, M.E., and Fuller, R.W. (1978). Evidence that serotonin neurons stimulate secretion of prolactin releasing factor. **Life Sci.** 22: 2209-2213.
- Cotman, C.W., and Engesser-Cesar, C. (2002). Exercise enhances and protects brain function. **Exerc. Sport Sci. Rev.** 30: 75-79.
- Dishman, R.K. (1997). Brain monoamines, exercise, and behavioral stress: Animal models. **Med. Sci. Sports Exerc.** 29: 63-74.
- Donato, R. (2001). S100: A multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. **Int. J. Biochem. Cell Biol.** 33: 637-668.
- Eriksen, J.L., Gillespie, R., and Druse, M.J. (2002). Effects of ethanol and 5-HT(1A) agonists on astroglial S100B. **Brain Res. Dev. Brain Res.** 139(2): 97-105
- Gomez-Merino, D., Bequet, F., Berthelot, M., Chennaoui, M., and Guezennec, C.Y. (2001). Site-dependent effects of an acute intensive exercise on extracellular 5-HT and 5-HIAA levels in rat brain. **Neurosci. Lett.** 301: 143-146.
- Gomez-Pinilla, F., Dao, L., and So, V. (1997). Physical exercise induces FGF-2 and its mRNA in the hippocampus. **Brain Res.** 764: 1-8.
- Haring, J.H., Hagan, A., Olson, J., and Rodgers, B. (1993). Hippocampal serotonin levels influence the expression of S100 beta detected by immunocytochemistry. **Brain Res.** 631: 119-123.
- Katula, J.A., Blissmer, B.J., and McAuley, E. (1999). Exercise intensity and self-efficacy effects on anxiety reduction in healthy, older adults. **J. Behav. Med.** 22: 233-247.
- Laurin, D., Verreault, R., Lindsay, J., MacPherson, K., and Rockwood, K. (2001). Physical activity and risk of cognitive impairment and dementia in elderly persons. **Arch. Neurol.** 58: 498-504.
- Meyer, T., and Brooks, A. (2000). Therapeutic impact of exercise on psychiatric diseases: Guidelines for exercise testing and prescription. **Sports Med.** 30: 269-279.
- Moldoveanu, A.I., Shephard, R.J., and Shek, P.N. (2001). The cytokine response to physical activity and training. **Sports Med.** 31: 115-144.
- Otto, M., Holthusen, S., Bahn, E., Sohnchen, N., Wiltfang, J., Geese, R., et al. (2000). Boxing and running lead to a rise in serum levels of S-100B protein. **Int. J. Sports Med.** 21: 551-555.
- Portela, L.V., Brenol, J.C., Walz, R., Bianchin, M., Tort, A.B., Canabarro, U.P., et al. (2002a). Serum S100B levels in patients with lupus erythematosus: Preliminary observation. **Clin. Diagn. Lab. Immunol.** 9: 164-166.
- Portela, L.V., Tort, A.B., Schaf, D.V., Ribeiro, L., Nora, D.B., Walz, R., et al. (2002b). The serum S100B concentration is age dependent. **Clin. Chem.** 48(6 Pt 1): 950-952.
- Strüder, H.K., and Weicker, H. (2001). Physiology and pathophysiology of the serotonergic system and its implications on mental and physical performance. Part I. **Int. J. Sports Med.** 22: 467-481.
- Suzuki, F., Kato, K., and Nakajima, T. (1984). Hormonal regulation of adipose S-100 protein release. **J. Neurochem.** 43: 1336-1341.
- Van de Kar, L.D., Rittenhous, P.A., Qjan, L., and Levy, A.D. (1996). Serotonergic regulation of renin and prolactin secretion. **Behav. Brain Res.** 73: 203-208.
- Walz, R., Portela, L.V., Tort, A.B., Neto, E.C., Fernandes, L.N., Gonçalves, C.A., et al. (2000). Serum S100B levels in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. **Neurology** 54: 2021-2022.