Ibuprofen intake increases exercise time to exhaustion: A possible role for preventing exercise-induced fatigue

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Although the intake of nonsteroidal anti-inflammatory drugs (NSAIDs) intake by athletes prevents soreness, little is known concerning their role in exercise performance. This study assessed the effects of ibuprofen intake on an exhaustive protocol test after 6 weeks of swimming training in rats. Animals were divided into sedentary and training groups. After training, animals were subdivided into two subsets: saline or ibuprofen. Afterwards, three repeated swimming bouts were performed by the groups. Ibuprofen (15 mg/kg) was administered once a day. Pain measurements were performed and inflammatory and oxidative stress parameters were assayed in cerebral cortex and gastrocnemius muscle. Training, ibuprofen administration, or both combined (P<0.05; 211 ± 18s, 200 ± 31s, and 279 ± 23s) increased exercise time to exhaustion. Training decreased the acetylcholinesterase (AChE) activity (P<0.05; 149 ± 11) in cerebral cortex. Ibuprofen intake decreased the AChE activity after exhaustive protocol test in trained and sedentary rats (P<0.05; 270 ± 60; 171 ± 38; and 273 ± 29). It also prevented neuronal tumor necrosis factor-α (TNF-α) and interleukin (IL 1β) increase. Fatigue elicited by this exhaustive protocol may involve disturbances of the central nervous system. Additive anti-inflammatory effects of exercise and ibuprofen intake support the hypothesis that this combination may constitute a more effective approach. In addition, ergogenic aids may be a useful means to prevent exercise-induced fatigue.

Evidence accumulated over many decades illustrates the beneficial role of physical activity in maintaining and improving neural and muscular functions in humans and animals (Kramer et al., 1999). However, high-intensity exercise, more specifically a high-intensity training session or a high performance competition, is a known event that triggers several events in the skeletal muscle such as microtrauma through mechanical and metabolic stress. Consequently, it leads to inflammation processes, reactive oxygen species (ROS), nitrogen species generation, and tissue damage in an important process responsible for muscle repair and regeneration, in other words, training adaptation (McAnulty et al., 2007). Considering that skeletal muscle damage occurs after phagocytic cell infiltration (Petersen & Pedersen, 2005), it is not surprising that a variety of biochemical markers such as pro-inflammatory and ROS production may be related to impaired exercise performance and fatigue possibly in an attempt to prevent a larger damage (McHugh, 1999).

Fatigue is characterized by a decreased performance linked with an increase in real and/or perceived difficulty to overcome a task and/or exercise (MacIntosh et al., 2004), and it has been divided into central – metabolic, circulatory, neurotransmitter, thermodynamic changes, or other disturbances of central nervous system (CNS) and peripheral – linked to depletion of substrates, accumulation of metabolites, and changes at the neuromuscular junctions (NMJs; Gandevia, 2001). Based on this perspective, a considerable body of evidence suggests that inflammation and immunological regulation exerted by exercise may be involved in alterations on neuroendocrine and neurotransmitter activities (Tidball, 2005). Indeed, studies have demonstrated that aerobic exercise influences the production of pro-inflammatory cytokines (Morgado et al., 2012).

Furthermore, the ability exercise trainings provide increasing parasympathetic tone and heart rate variability as well as enhancing acetylcholine (ACh) levels in...
the brain may promote additional benefits in such chronic diseases (Das, 2001), as ACh has been proved to significantly attenuate the release of pro-inflammatory cytokines (Pavlov et al., 2009).

Although integrative physiology studies have shown a potential link between oxidative stress and immune system interplay toward behavioral modulation, little is known regarding the role of pro-inflammatory cytokines, ROS production, and neuronal cholinergic pathway on experimental fatigue-induce sets after exercise training (Morgado et al., 2012). In this context, several procedures have been undertaken in an attempt to investigate pathways related to exercise-induced fatigue. Ergogenic aids have also been tested in order to increase performance outcomes (for a review, see Tarnopolsky, 2010).

Previous surveys on the use of nonsteroidal anti-inflammatory drugs (NSAIDs) by athletes who participate in international sports events have demonstrated this sort of drugs to be the most frequently used by high-class athletes (Corrigan & Kazlauskas, 2003; Tscholl et al., 2009). NSAIDs are drugs commonly used to reduce acute pain and muscle injuries related to inflammation caused by stress, generated by both acute and chronic exercises. Ibuprofen is known to be among the most commonly NSAIDs used by athletes (Ziltener et al., 2010).

The anti-inflammatory action of this type of NSAIDs is based on the inhibition of prostaglandin endoperoxide synthase, which responds to the synthesis of endoperoxidase prostaglandins responsible for cytokine production. Consequently, the production of cytokines secreted by macrophages at the injury site is hampered, reducing the inflammatory response (Rainsford, 2009). Furthermore, researchers have shown that ibuprofen reduces the activity of inducible isoform of nitric oxide synthase, which produces changes in transmembrane ion flux (Liles & Flecknell, 1992) and is also involved in inflammatory processes (Di Girolamo et al., 2003). Interestingly, a study described by Da Silva et al. (2011) revealed that athletes who participated in the anti-doping control of the XV Pan-American Games reported a high intake of NSAIDs during competition when compared with out-of-competition athletes. The use of this drug suggests that it may be used as an ergogenic aid to enhance athletic performance on an indirect way, which may cause a delay on fatigue onset and consequently maintain this performance. However, the role of these medicines is not completely defined, mainly regarding the side effects of chronic use by athletes.

Thus, the purpose of this study was to investigate the effects of ibuprofen administration on time to exhaustion on a swimming test, pain, inflammatory markers, and ROS production in cerebral cortex, as well as gastrocnemius muscle of trained rats submitted to repeated exhaustive swimming bouts.

**Methods**

**Animals**

Male Wistar rats (180–250 g), between 60 and 90 days old, were kept in plastic boxes containing a maximum of five animals per cage (total of 48 animals), under controlled environment conditions (12:12 h light–dark cycle, with onset of light phase at 7:00 h, 25 ± 1 °C, 55% relative humidity) with food and water *ad libitum*. All experiments were carried out in accordance with national and international legislation and with the approval of the Ethics Committee for Animal Research of the university. Assay reagents were purchased from Sigma (St Louis, Missouri, USA).

**Study design**

In this study, animals were randomly divided into training (*n* = 24) and sedentary (*n* = 24) groups. The training group performed a 6-week swimming training with body weight overload. After 24 h of the last training session, both training and sedentary groups performed a lactate concentration (LC) test to assay training adaptations. Subsets of training and sedentary groups were sacrificed after LC test, in order to assess possible training effects upon the biomarkers herein assayed. After LC test, trained and sedentary groups were subdivided into saline or ibuprofen administration groups, and rats performed three exhaustive swimming bouts. Each bout was separated for a 72 h time period. Pain measurements were performed before each exhaustive swimming bout. Rats were then sacrificed, and cerebral cortex and gastrocnemius muscle were immediately removed for further biochemical assays. Figure 1 depicts the study design.

**Water adaptation**

Rats were adapted to the water before the beginning of the experiment. The adaptation consisted on keeping the animals in shallow water (5 cm) at 31 ± 1 °C between 9:00 and 11:00 h. The adaptation period was carried out during the week before the swimming training onset. The purpose of the water adaption was to reduce stress without promoting exercise training adaptation.

![Fig. 1. Timeline of the exercise training schedule and exhaustive protocol test data collection.](image-url)
Training protocol and lactate concentration test

Animals were weighed and randomly assigned to the following groups: (1) sedentary and (2) training. The exercise training protocol consisted of 6 weeks, five sessions per week of 60 min each. The training tank used in this study was 80 cm in length, 50 cm in width, and 90 cm in depth. The swimming was always performed in water temperature of 31 ± 1 °C between 10 and 12 h. Animals were subjected to swimming training with a 5% body weight overload attached to the back to improve endurance (Gobatto et al., 2001). Along with the training session, sedentary rats were placed in a separate but similar tank with shallow water (5 cm) at the same temperature for 30 min, 5 days a week without the back overload.

After 6 weeks of swimming training, a test protocol was used to determine the lactate concentration test (LC) in sedentary (n = 8) and trained rats (n = 8). The LC test was carried out according to the protocol described by Marquezi et al. (2003) with few modifications. The test consisted of three swimming bouts with progressive overload corresponding to 5%, 7%, and 9% of each animal body weight for a period of 3 min for each load, with a 1-min resting period between bouts. During the resting periods, 25 μL of blood was collected from the tail vein for lactate concentration assay, resulting in four blood samples, measured with a lactimeter (Accutrend® Plus, Roche Diagnostics GmbH, Penzberg, Germany). The LC for each animal was calculated based on the graph for lactate concentration with increasing workload. Twenty-four hours after the LC assay, sedentary and trained animal subsets were killed by decapitation. Cerebral cortex and gastrocnemius muscle were immediately removed and immediately frozen at −80 °C for further biochemical assays.

Exhaustive protocol test

Three days after the LC test, all four groups (sedentary and training groups subdivided into saline or ibuprofen) performed the exhaustive protocol test according to de Araujo et al. (2007) with few modifications. The protocol consisted of three repeated exhaustive swimming bouts: 72 h, 144 h, and 216 h after the LC test (Fig. 1). Animals swam individually in the tank with an overload of 13% of body weight until exhaustion in order to determine the time to exhaustion. Exhaustion was characterized by the moment at which animals were no longer able to maintain themselves in the water surface, reaching 10 s submerged. When exhaustion was reached, animals were taken out of the tank, dried, and after the third bout, sacrificed. Then, cerebral cortex and gastrocnemius muscle were immediately removed and immediately frozen at −80 °C for further biochemical assays.

Ibuprofen administration

In order to evaluate the effects of ibuprofen administration on time to exhaustion in sedentary (n = 8) and trained (n = 8) rats submitted to the exhaustive protocol test, a subset of animals was supplemented with ibuprofen or saline. Ibuprofen was dissolved in water and, as saline, injected via intragastric gavage, at a dose of 15 mg/kg daily (Liles & Flecknell, 1992), after LC test until 24 h before the last exhaustion bout. As all animals were sacrificed immediately after the last exhaustion bout, there was no drug administration on this day because there would be no time for ibuprofen action. The tests of exhaustion and ibuprofen administration were carried out blinded.

Hyperalgesia test

For measuring the thermal hyperalgesia, the Plantar Test (Ugo Basile, Varese, Italy) has been used according to Hargreaves et al. (1988). Briefly, 1 h before each exhaustive swimming bout, rats were accustomed to the place of observation and an infrared beam generated by a 60 W lamp was focused on the animal’s right hind leg. The time required for the animal to withdraw the paw of the incident ray was recorded automatically and used as an index of nociception. Significant decreases in paw withdrawal time compared with baseline were considered as hyperalgesia.

Nociception assessment

Mechanical allodynia is considered an indicator of nociception and was herein assessed as previously described by Chaplan et al. (1994). Rats were placed in Plexiglas boxes (9 cm × 7 cm × 11 cm) on elevated, wire-mesh platforms in order to access the ventral surface of the hind paws that were in contact with one of seven von Frey hairs (6–100 g). Von Frey hairs were applied perpendicularly to the paw’s surface in order to cause a slight buckling for approximately 2 s. The 50% withdraw threshold was determined using the up and down method of Dixon (1980). In this scenario, nociception assessment was initiated with the 15 g hair. Stimuli were continuously consecutive whether ascending or descending. Paw withdrawal thresholds were verified before each bout of the exhaustive protocol test.

Acetylcholinesterase (AChE) activity

The AChE activity was determined by the method of Ellman et al. (1961). Cerebral cortex and gastrocnemius muscle were homogenized 1:20 with 10 mM Tris HCl, pH 7.4. The homogenates were centrifuged at 1000 × g for 30 min at 4 °C and the supernatant was used as enzymatic source. The mixture assay contained 1.04 mM DTNB (5,5′-dithiobis-2-nitrobenzoic acid), 24 mM phosphate buffered saline (PBS; pH 7.2) and 100 μL of enzymatic material. It was pre-incubated for 2 min at 28 °C and the reaction was started with the addition of 0.83 mM ACh. The product from the thiocholine reaction with DTNB was determined at 412 nm every 30 s for 2 min with an absorption coefficient of 0.0136 M/cm for the TNB anion. The specific activity was expressed as nmol ACh hydrolyzed/h/mg protein.

Interleukin 1beta (IL-1β) and tumor necrosis factor alpha (TNF-α) content

Cerebral cortex and gastrocnemius muscle were homogenized in a solution containing bovine serum albumin (10 mg/mL), 2 mM EGTA, 2 mM EDTA, and 0.2 mM PMSF in PBS (pH 7.4) for the IL-1β and TNF-α assays. Cytokine content was measured according to the protocol of the manufacturer, using a commercially available ELISA kit from R & D Systems (Minneapolis, Minnesota, USA). Results are expressed as pg/mg protein for tissue homogenate.

Estimation of ROS production

Production of ROS was estimated with the fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), as described by Ali et al. (1992). Briefly, tissues were homogenized in 2.5 mL of saline solution (0.9% NaCl). Aliquots of 2.5 mL were incubated in the presence of DCFH-DA (5 μM) at 37 °C for 60 min. The DCFH-DA was enzymatically hydrolyzed by intracellular esterases to form nonfluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. A calibration curve was established with standard DCF (0.1 nM to 1 μM) and ROS levels were expressed as percentages of control (sedentary or sedentary/saline groups).
Protein carbonyl levels

Protein oxidation in cerebral cortex and gastrocnemius muscle was measured as a concentration of protein carbonyls formed, and levels were determined using 2,4 dinitrophenylhydrazine (DNPH) assay (Levine et al. 1990). The cortex was divided into two portions containing 1 mg prot/mL each. To one portion, 1 mL of 2 N HCl was added and incubated at room temperature shaking intermittently for 1 h. The other portion was treated with 1 mL of 10 mM DNPH in 2 N HCl and incubated by shaking intermittently for 1 h at room temperature. After incubation, the mixture was precipitated with 10% TCA and centrifuged. The precipitate was washed thrice with 1 mL of ethanol : ethyl acetate (1:1). The final protein precipitate was dissolved in denaturation buffer (3% SDS and 150 mM NaH2PO4; pH 6.8) and the absorption at 370 nm (DNPH-treated sample minus sample blank) was determined. Carbonyl content was calculated using the molar extinction coefficient of 22 000 and expressed as nmol carbonyl/mg protein.

Protein determination

The protein content was measured colorimetrically by the method of Bradford (1976) using bovine serum albumin (1 mg/mL) as standard.

Statistical analysis

The Statistical Package for Social Sciences (SPSS, Inc, Chicago, Illinois, USA) version 17 was used for all analyses. Data were expressed as mean ± standard error of means (SEM). Significance was assessed by F-test and one- or two-way analysis of variance (ANOVA), followed by Newman–Keuls test for post-hoc comparison when appropriate. Statistical significance was set at P < 0.05. The F-test was used when the objective was to compare two independent groups. Two-way ANOVA was performed when we had two factors in study (ibuprofen and training) and the Student–Newman–Keuls test was only performed when two-way ANOVA presented significance. One-way ANOVA with repeated measure was chosen when the objective was to compare one factor in different levels, as lactate content.

Results

In the present study, a significant increase in total body weight in sedentary versus trained rats along the 6 weeks of swimming training was found \([F(1, 12) = 20.58; P < 0.05; \text{Fig. 2a}]\). In addition, statistical analysis revealed a clear lower lactate concentration on blood in the trained group than in the sedentary group in the lactate concentration test \([F(1, 14) = 22.56; P < 0.05; \text{Fig. 2b}]\).

Figure 3 shows the effect of training, ibuprofen intake or both combined on time to exhaustion. Swimming training increased time to exhaustion, as expected, on the first \([F(3, 28) = 24.31; P < 0.01; \text{Fig. 3a}]\) and second \([F(3, 28) = 7.03; P < 0.05; \text{Fig. 3b}]\) bouts of exhaustive exercise protocol when compared with the sedentary-saline group. At the same time, experimental findings also showed that ibuprofen administration in sedentary rats as well as the combination of training and ibuprofen administration presented higher increase on time to exhaustion on the third bout \([F(3, 28) = 10.28; P < 0.05; \text{Fig. 3c}]\) of exhaustive protocol test when compared with the other groups. This finding showed that this combination may have a synergistic effect on fatigue-related exercise.

**Fig. 2.** Effect of 6 weeks of exercise training on body weight (a), and lactate concentration (b). *P < 0.05 when compared with the sedentary group (F-test for simple effect). Data mean + SEM for \(n = 8\) in each group.

**Fig. 3.** Effect of 6 weeks of exercise training and/or ibuprofen intake on exhaustion time on first (a), second (b), and third (c) bouts of the exhaustive protocol test. *P < 0.05 when compared with the sedentary group (F-test for simple effect); #P < 0.05 when compared with the training group (F-test for simple effect). Data mean + SEM for \(n = 8\) in each group.
Results presented in this report revealed that this fatiguing protocol and/or ibuprofen administration had no effect on total body weight in sedentary or trained rats after the exhaustive protocol test \( F(3, 28) = 21.79; P < 0.05 \) (Fig. 4).

Statistical analysis revealed that repeated swimming bouts preceded by training and/or ibuprofen intake had no effect on hyperalgesia in Hargreaves (Fig. 5a) or mechanical allodynia (Fig. 5b) tests.

In the present study, 6 weeks of swimming training decreased the AChE activity on cortex \( F(1, 14) = 18.40; P < 0.05 \) (Fig. 6a). At the same time, after three bouts of exhaustive exercise, both aerobic training and ibuprofen prevented the increase of AChE activity \( F(3, 28) = 10.68; P < 0.05 \) (Fig. 6b) when compared with the sedentary/saline group. There was no statistical difference of AChE activity in muscle (data not shown).

Figure 7 shows that the effect of 6 weeks of swimming training did not elicit detectable alterations in pro-inflammatory cytokine TNF-\( \alpha \) (Fig. 7a); however, after three bouts of exhaustive exercise, just ibuprofen administration decreased the TNF-\( \alpha \) levels in cerebral cortex \( F(3, 28) = 11.03; P < 0.05 \) (Fig. 7b). Unlikely, aerobic training decreased the content of TNF-\( \alpha \) \( F(1, 14) = 30.78; P < 0.05 \) (Fig. 7c) in gastrocnemius muscle, but three bouts of exhaustive exercise elicated no changes in these cytokine levels (Fig. 7d).

Likewise, there were no statistical differences in pro-inflammatory cytokines IL-1\( \beta \) levels (Fig. 8a) in cortex after aerobic training. After the last bout of exhaustive exercise, statistical analysis presented a decrease in IL-1\( \beta \) levels with ibuprofen administration and a higher decrease with the combination with exercise \( F(3, 28) = 22.57; P < 0.05 \) (Fig. 8b) after the exhaustive protocol test. In the meantime, swimming training decreased the IL-1\( \beta \) content in muscle \( F(1, 14) = 19.99; P < 0.05 \) (Fig. 8c), but the exhaustive test did not change the IL-1\( \beta \) content (Fig. 8d).

The period of 6 weeks of aerobic training demonstrated the capacity to reduce DCFH-DA oxidation both in cortex \( F(1, 14) = 1.502; P < 0.01 \) (Fig. 9b) and in muscle \( F(1, 14) = 1.189; P < 0.01 \) (Fig. 9b). Similarly, the decreased DCFH-DA oxidation remained lower after three bouts of exhaustive exercise and/or ibuprofen administration in cortex \( F(3, 24) = 10.99; P < 0.01 \) (Fig. 9b) and in muscle \( F(3, 28) = 14.74; P < 0.01 \) (Fig. 9d).

Figure 10 shows that the effect of 6 weeks of swimming training did not elicit any detectable alterations in carbonyl protein in cerebral cortex (Fig. 10a) or in gastrocnemius muscle (Fig. 10c). Similarly, three bouts of exhaustive exercise elicited no changes in the carbonyl protein in cerebral cortex (Fig. 10b) or in gastrocnemius muscle (Fig. 10d).

**Discussion**

Results presented in this report show for the first time that training or its combination with a worldwide type of NSAID (ibuprofen) increased time to exhaustion, decreased neural AChE activity, and protected against neuronal inflammation as well as ROS production.
after an exhaustive protocol test in rats. In this study, there was an increased time to exhaustion induced by swimming training and/or ibuprofen intake but it was not due to the reduction of pain as speculated in the literature. Ibuprofen was administered for 9 days (15 mg/kg daily) and removed 24 h before the last exhaustive bout because there is no evidence about side effect and this period would not be enough to stop a possible therapeutic effect (McElwee et al., 1990).

NSAIDs are routinely used during and after competition on injury treatments to suppress minor soreness symptoms in muscle and joint stiffness associated with overexertion. At the same line, it is generally agreed that delayed onset muscle soreness after eccentric exercise peaks roughly at 2–3 days post-exercise (Clarkson & Newham, 1995). In line of this view, our experimental findings revealed that trained groups showed a higher time to exhaustion than sedentary groups on first and second bouts of exhaustive exercise. The combination of training and ibuprofen intake significantly increased the time to exhaustion on the third day of the exhaustive protocol test when compared with the trained group, indicating that this combination has additional effects on exercise-related fatigue onset.

In fact, the result could reflect a more trained/adapted state rather than reduce fatigue if the time to fatigue were statistically the same as all groups performed the exhaustive bout. However, on the referred chart, sedentary/saline group did not show an increase on time to fatigue, unlike sedentary/ibuprofen group. Under these circumstances, we may speculate that there could be another cause for the increased time to fatigue on the referred group.

It is important to emphasize that the trained group approached here performed a high-intensity aerobic training protocol to mimic athletes’ routine, promoting body weight reduction in humans because of intense lipid metabolism activation (Yoshioka et al., 2001). In this way, muscle glycogen resynthesis is of high metabolic priority resulting in preferential use of intramuscular triacylglycerol and circulating lipids by the recovering muscle, being such mechanisms also reported in rats (Botezelli et al., 2011). In line with this, blood lactate concentration was lower and time to exhaustion was higher in the trained group, corroborating previous findings (Lima et al., 2009).

In athletes, most pain reports present a mechanical origin, which may be related to muscle strains (Alaranta et al., 2006). For this reason, a high prevalence of NSAID intake by athletes was observed in the XV Pan American Games (Da Silva et al., 2011). A possible reason for this high intake is the fact that NSAIDs help to

**Fig. 6.** Effect of 6 weeks of exercise training on acetylcholinesterase activity in cortex and muscle (a and c, respectively) and after exhaustive protocol test with or without ibuprofen intake (b and d, respectively). *P < 0.05 when compared with the sedentary-saline group [F-test for simple effect (a) and Student-Newman-Keuls test (b)]. Data mean + SEM for n = 8 in each group.
carry on training or even competitions without the need of a recovery window when minor injuries take place (Corrigan & Kazlauskas, 2003). Although analgesic drugs are commonly consumed to reduce or prevent pain and soreness after exhaustive exercise (Ziltener et al., 2010), statistical analysis revealed that repeated swimming bouts preceded by training and/or ibuprofen intake had no effect on hyperalgesia in Hargreaves or mechanical allodynia tests. These results indicated that the effect of training and/or ibuprofen intake on time to exhaustion was not due to an antinociceptive effect of this drug. It is interesting to consider that the exhaustive protocol test was designed in order to mimic as close as possible individual sports competitions (swimming, for example) during important worldwide events, such as the Olympics. In this kind of championship, athletes are asked to perform all-out trials during consecutive days without proper rest, for what metabolic byproducts (i.e., lactate, hydrogen ions, adenosine, K⁺ ion, and arachidonic acid) may accumulate to the last day and thus influence on performance outcomes. In this scenario, our experimental results suggest that improvement in the performance elicited by ibuprofen intake (15 mg/kg, daily) in trained rats is not due to the antinociceptive response.

The discovery that cytokines are related to a range of disease conditions has opened a completely new investigation field on the physiological mechanisms to maintain and control health by restraining or counter-regulating cytokine release (Nathan, 2002). More recently, this scenario has evolved to suggest that exercise may also control inflammatory responses within the CNS (Parachikova et al., 2008). CNS is able to inhibit cytokine release through an inflammatory reflex of the vagus nerve, thereby preventing tissue injury and death. Furthermore, it has been demonstrated that ACh, a major parasympathetic neurotransmitter, inhibits pro-inflammatory cytokines such as IL-1β and TNF-α from macrophages (Pavlov et al., 2009). Accordingly, our experimental findings showed that 6 weeks of swimming training decreased the neural AChE activity, although it presented no effect on IL-1β and TNF-α levels in cerebral cortex. However, after the repeated exhaustive bouts, the neural AChE activity in the trained groups remained lower, as the effect of aerobic training, and
ibuprofen administration alone showed the same effect in the sedentary group, being also capable of decreasing IL-1β and TNF-α levels. On the other hand, although aerobic training did not change the AChE activity in muscle, there was a decrease in IL-1β and TNF-α levels. After repeated exhaustive bouts, no difference was observed in the AChE activity or in IL-1β and TNF-α levels. Considering that motor functions can be modulated by signals originated in the cerebral cortex and that blockade of cholinergic pathway accelerates the exercise-induced fatigue (Pavlov et al., 2009), it is plausible to propose that the neuronal cholinergic pathway maintenance induced by exercise training protects against IL-1β and TNF-α increase after repeated exhaustive exercise bouts. In addition, it is possible to state that a lower content of pro-inflammatory cytokines induced by cholinergic pathway activation could be responsible for a continuous communication between cerebral cortex and NMJs maintaining muscle contraction.

Although exercise training has long been known to regulate immune responses, recent studies suggest that exercise may also modulate inflammatory and ROS production responses within the CNS (Radak et al., 2007). Several studies have pointed out that exercise-induced modulation of the redox status is an important means by which exercise may benefit the brain function, increasing the resistance against oxidative stress, and facilitating recovery from oxidative stress (Lima et al., 2009).

Results presented in this report demonstrated parameters related to the antioxidant status and ROS production in cortical homogenates that were affected after repeated exhaustive swimming bouts. The DCFH-DA oxidation increase suggests that fatigue elicited by the exhaustive protocol test was accompanied by overproduction of ROS and nitrogen species. Such results are in agreement with several studies that have presented total antioxidant status upregulation of the main enzymes (superoxide dismutase, catalase, and glutathione peroxidase) after 14 days of swimming both in serum and hypothalamus. Intensive stress has shown to bring about changes in the antioxidant defense system in rats (Klotz & Sies, 2003). Moreover, increased production of ROS in the tissues causes lipid peroxidation (especially in membranes) and plays an important role in tissue injury (Lima et al.,

Fig. 8. Effect of 6 weeks of exercise training on IL-1β levels in cortex and muscle (a and c, respectively) and after exhaustive protocol test with or without ibuprofen intake (b and d, respectively). *P < 0.05 when compared with the sedentary-saline group; #P < 0.05 when compared with the sedentary-ibuprofen group [F-test for simple effect (c); Student–Newman–Keuls test (b)]. Data mean + SEM for n = 8 in each group. IL-1β, interleukin-1 beta.
Oxidative stress initiated by imbalance in oxidants and antioxidants in the hypothalamus may mediate cell damage and may be responsible for the neuronal disorders during stress (Halliwell & Gutteridge, 1985). On the other hand, the adaptive response to exercise training characterized here by DCFH-DA decrease led to the development of compensatory responses to oxidative stress and pro-inflammatory cytokine generation (Peake et al., 2007) after the exhaustive protocol test. In addition, the effective protection exerted by ibuprofen intake on the fatiguing protocol in trained and sedentary rats reinforces the assumption that fatigue and impaired recovery from exercise share a common link with inflammation and ROS overproduction. Considering that ibuprofen reduces ROS production after a repeated exhaustive exercise protocol, a mild indirect ergogenic effect may be suggested. However, the role of such drugs in sports is not completely understood. While some authors have revealed that prophylactic ibuprofen significantly attenuated the decline in quadriceps isometric contraction and eccentric torque at 24 h post-exercise (Lecomte et al., 1998), others have not found oral ibuprofen intake to be better than placebo in this setting (Bourgeois et al., 1999). In addition, its use has shown no beneficial effect on the relief of muscle damage and pain perception after an ultramarathon (Nieman et al., 2006). The key factor for such a discrepancy is not known, but one might argue that methodological differences may account for it. An interesting possibility is that ibuprofen intake effect on exercise-induced fatigue may vary with the model of training performed.

**Perspectives**

NSAIDs are not on the prohibited list of Worldwide Anti-Doping Agency, thus they are used by athletes to prevent pain and inflammation, two of the main causes of performance decrease. Despite the lack of consensus about the real effect of this kind of drug in sports, there is a great number of subjects using it. For this reason, we have assessed whether the combination of aerobic training and ibuprofen in sets of exhaustive exercise could lead to a decrease on muscle soreness and to an increase on performance. The present study reports that swimming training, ibuprofen intake, or both combined increase time to exhaustion induced by an exhaustive protocol test. Our experimental findings have also revealed that the neuronal cholinergic pathway maintenance elicited exercise training and its combination with...
ibuprofen administration protects against overproduction of pro-inflammatory cytokines and DCFH-DA oxidation increase after an exhaustive protocol test. These specific molecular systems modulated by ibuprofen in trained and sedentary rats provide a framework to guide further studies to examine the mechanisms by which NSAIDs may alter neuronal functions related to exercise-induced fatigue. However, there is some adverse reactions from NSAIDs, some more prevalent and some rare like stomach/intestinal ulcers and bleeding, abnormal kidney and liver functions, asthma and respiratory reactions, immune reactions, joint destruction (in osteoarthritis), kidney failure, liver injury and failure, and severe skin reaction.

Additive anti-inflammatory effects of exercise and ibuprofen intake support the hypothesis that this combination may constitute a more effective approach, as ergogenic aids may be a useful means to prevent exercise-induced fatigue.

Key words: Exercise training, NSAIDs intake, exhaustive exercise, inflammation, AChE activity.

References


