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Effect of a Probiotic Intake on Oxidant and Antioxidant Parameters in Plasma of Athletes During Intense Exercise Training

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Abstract The aim of this study was to evaluate the effect of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® on oxidative stress in athletes during a four-week period of intense physical activity. Two groups of twelve subjects each were selected for this analysis. The first group consumed a daily dose of a mixture of the two probiotic strains (1:1 *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®; $\sim 10^9$ cells/day) for 4 weeks. The second group (control) did not consume any supplements during the 4 weeks. Blood samples collected immediately before and after the supplementation were analyzed, and plasma levels of reactive oxygen metabolites and biological antioxidant potential were determined. Faeces were also collected and analyzed before and at the end of the probiotic supplementation. Antioxidative activity and oxidative stress resistance of the two strains were determined in vitro. Results demonstrated that intense physical activity induced oxidative stress and that probiotic supplementation increased plasma antioxidant levels, thus neutralizing reactive oxygen species. The two strains, *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®, exert strong antioxidant activity. Athletes and all those exposed to oxidative stress may benefit from the ability of these probiotics to increase antioxidant levels and neutralize the effects of reactive oxygen species.

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Introduction

During the past decade, several studies have supported the potential health benefits of probiotics, such as the improvement of gastrointestinal microbiota ecosystems, stimulation of the immunological system, anticarcinogenic activities, and reduction of oxidative stress. The most widely studied probiotics are *Lactobacillus* and *Bifidobacterium*. Most *Lactobacillus* species are normal and non-pathogenic inhabitants of human and animal intestines, and their presence is important for the maintenance of the intestinal microbial ecosystem [23]. Lactobacilli have been shown to possess inhibitory activity towards the multiplication of enteropathogens, and they are highly competitive, largely due to their production of several antimicrobial compounds [3]. Few studies have explored the antioxidative properties of probiotics and those few investigated a limited number of strains [2, 16, 21, 26, 28, 34, 42, 45]. These studies indicated that some probiotic strains exert antioxidant activity and may be useful in reducing systemic oxidative stress through several mechanisms. The expression of high levels of antioxidant enzymes can neutralize oxidants directly in the intestinal tract. Moreover, the stimulation of the immune system reduces inflammation and prevents cytokine-induced oxidative stress. Also, the inhibition of intestinal pathogens reduces inflammation and its associated oxidative damage. Probiotics also seem to enhance the absorption of macro and micronutrients,

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including antioxidants. For example, they assemble lipoprotein particles with lower levels of oxidant products and higher concentrations of antioxidant enzymes. Finally, probiotics reduce postprandial lipids that are related to significant oxidative damage, which is responsible for several food-related pathologies [34]. Oxidative stress is considered an imbalance between oxidant and antioxidant levels. Since humans use oxygen to obtain energy from food, the production of reactive oxygen species (ROS) and their metabolites (reactive oxygen metabolites, ROMs) is a normal outcome of the biochemical pathways. To neutralize the oxidant molecules, the bodies possess enzymes that, together with the antioxidants contained in food, form the biological antioxidant barrier. Nevertheless, oxidative stress impacts a wide range of subjects and seems to be related to several conditions such as heart disease [15, 35], rheumatoid arthritis [18, 20], hypertension [27, 37], Alzheimer's disease [31, 53], Parkinson's disease [24], atherosclerosis [4], and aging [25]. Owing to their intense physical activity and augmented oxygen consumption, athletes are often exposed to an excess of circulating reactive oxygen species. A single session of intense physical exercise is sufficient to produce significant amounts of ROS. In exhaustive and prolonged exercise, ROS production is even higher and results in oxidative stress [13, 22]. The increase in oxygen consumption and the inflammation induced by tissue damage underlie the inevitable production of oxidants during physical activity. In this context, due to their wide range of effects, probiotics may represent an effective supplementation for athletes to furnish an appropriate antioxidant barrier essential for preventing dangerous levels of oxidative stress. Because the authors have recently published the probiotic characterization of two new *Lactobacillus* strains [40, 46] and in light of the above findings, in this study, the authors evaluated their effect on oxidative stress in athletes during a 4-week period of intense physical activity.

Materials and Methods

Study Product

The probiotics used in this study were supplied by Synbiotec S.r.l. in Camerino, Italy. The study product is a powder consisting of a mixture of two lyophilized probiotic strains (1:1, about 10^9 cells/g of product), *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®. The choice to use a combination of the two bacterial strains is justified by the results obtained by Verdenelli et al. [46], wherein the mixture expressed higher in vitro adherence than the two single *Lactobacillus* strains to the intestinal cell line. These two bacterial strains were isolated from faecal samples of Italian elderly subjects during an EU project named Crownalife, as previously described [40]. The probiotic properties of *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® were previously characterized, including gastro-intestinal transit (acid and bile tolerance), in vitro adhesion to cell lines, antimicrobial activity, antibiotic susceptibility, and in vivo colonization [46, 47]. The bacterial strains were phenotypically and genotypically characterized and deposited in the culture collection Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany) with number DSM 16104 and DSM 16105 and this was assigned Italian patent no. RM2004 A000166 [46] and International Publication Number WO 2005/095656.

Subjects and Sample Size Calculation

In order to determine the effects of probiotic supplementation on oxidant and antioxidant levels in plasma of athletes engaged in a prolonged intense exercise program, the authors performed a retrospective analysis of data collected by trainers and medical doctors of a local cycling team during the training season. The authors selected data from subjects which were in good health based on a medical examination. None of the subjects had taken medications or supplements that might alter the study outcome within the past 10 days. Furthermore, no subjects had a history of medical or surgical events that could affect the study outcome, including cardiovascular disease or metabolic, renal, hepatic or musculoskeletal disorders. The number of subjects required for the study was determined by power analysis and sample size estimation (see Statistical Analysis). Based on the results obtained from sample size calculation, data from twenty-four amateur male athletes aged 32.03 ± 6.12 years (±SD) were analyzed. Their mean height and weight were 177.84 ± 6.78 cm and 72.83 ± 5.90 kg, respectively. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

Experimental Procedure

Volunteers were randomly distributed into two groups: control group (12 subjects) and probiotic group (12 subjects). The study was performed in a parallel manner, with a 4-weeks run-in period followed by a 4-weeks intervention period. During the intervention period the probiotic group consumed a daily dose of a mixture of the two probiotic strains (1:1 *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®; $\sim 10^9$ cells/day). The second group (control) did not consume any supplements during the 4 weeks. Throughout the study, all the athletes followed the same intense

exercise training and an individual diet developed on the basis of their basal metabolism, body composition and energy expenditure. Diets were proportionally equivalent in macro and micronutrient quantity for all the subjects, containing 100% of the recommended daily allowance (RDA) for all nutrients. Blood samples were collected at 8.00 a.m. on an empty stomach. Two samples were analyzed for each subject immediately before and after the supplementation, and plasma levels of reactive oxygen metabolites (ROMs) and biological antioxidant potential (BAP) were determined. Faeces were also collected for microbiological analysis before and at the end of the probiotic supplementation. The athletes were strictly monitored and the compliance with the study was assessed weekly by individual interview. The antioxidative activity and oxidative stress resistance of *Lactobacillus* strains were also evaluated in vitro.

Microbial Analysis

In order to verify the presence of the two strains before and after the 4 weeks, faecal samples were suspended (1:10 w/v) in saline solution and 10-fold serially diluted and 100 µl of appropriate dilutions were plated on Rogosa agar (Oxoid, Basingstoke, Umpshire, UK) with or without 12 µg/ml of vancomycin and gentamicin (Sigma-Aldrich, Missouri, USA). Vancomycin and gentamicin-resistant lactobacilli were enumerated on Rogosa-vancomycin and gentamicin agar. Plates were anaerobically incubated for 3 days at 37°C. Ten to 20% of the total colonies, randomly selected from countable Rogosa vancomycin and gentamicin agar plates, were isolated and checked for purity. DNA was extracted using the Qiagen Dneasy Tissue kit and analyzed using the Randomly Amplified Polymorphic DNA (RAPD) technique as previously reported [46].

Antioxidative Activity of Probiotic Bacterial Strains

The antioxidative activity of the two probiotic strains was determined by the thiobarbituric acid (TBA) method in vitro [19]. This method was based on the monitoring of inhibition of linoleic acid peroxidation by intact cells and by intracellular cell-free extracts. Linoleic acid was chosen as the source for unsaturated fatty acid [48] and a Fe/H_2O_2 system was used for the catalysis of oxidation [14]. In brief, the linoleic acid emulsion was made up of 1 ml of linoleic acid, 0.2 ml of Tween 20 and 19.7 ml of deionised water. Phosphate buffer solution (0.5 ml, 0.02 M, pH 7.4), 1 ml of linoleic acid emulsion, 0.2 ml of $FeSO_4$ (0.01%), 0.2 ml of H_2O_2 (0.56 mM), and 0.4 ml of intact cells or intracellular cell-free extract were mixed and incubated at 37°C. Distilled water was substituted for intracellular cell-free extract in the blank sample. After 3 h of incubation, 2 ml of the reaction solution was mixed with 0.2 ml of trichloroacetic acid (TCA; 4%), 2 ml of TBA (0.8%) and 0.2 ml of butylated hydroxytoluene (BHT; 0.4%). This mixture was incubated at 100°C for 30 min and allowed to cool. The absorbance was measured at 532 nm. The percentage of inhibition of linoleic acid peroxidation was defined as follows: $[1-A_{532}(\text{sample})/A_{532}(\text{blank})] \times 100$.

Determination of the Oxidative Stress Resistance of Probiotic Bacterial Strains

The oxidative stress resistance of *Lactobacillus* strains was determined in vitro [43]. The organisms were grown in MRS (de Mann, Rogosa and Sharpe) broth (Oxoid) until the culture reached a density of 4 McFarland. A volume of 2.5 ml of the broth was introduced into 50 ml of 0.4% MRS agar and mixed well. A volume of 4 ml of inoculated 0.4% MRS agar was poured onto pre-prepared MRS agar plates and allowed to solidify. A sterile paper disk (6 mm; Wathman International Ltd, Maidstone, UK) was placed on each Petri dish and a volume of 10 µl of 3% H_2O_2 was dispensed onto the paper disk. The plates were anaerobically incubated at 37°C for 24 h and the diameters of the inhibition zones around the paper disks were measured.

Determination of Reactive Oxygen Metabolites

Oxidative stress was measured by performing the dROMs test, which determines the level of reactive oxygen metabolites (ROMs) [11]. ROMs are produced by the interaction of ROS with organic substrates such as glucids, lipids, amino acids, proteins and nucleotides [38]. Of the known ROMs, hydrogen peroxides are considered markers and amplifiers of oxidative stress [1]. The dROMs test is based on the concept that plasma hydrogen peroxides react with the transition metal ions liberated from proteins in the acidic medium and are converted to alkoxy- and peroxy-radicals. These newly formed radicals are able to oxidize *N*,*N*-diethyl-para-phenylenediamine to the corresponding radical cation, and the concentration of this cation can be determined by spectrophotometry (absorption at 505 nm wavelength). The dROMs test is expressed in U CARR (Carratelli units) where 1 U CARR = 0.08 mg H_2O_2/dl. Values higher than 300 U CARR are indicative of ongoing oxidative stress.

Determination of Biological Antioxidant Potential

Antioxidant defence status was assessed by performing the biological antioxidant potential (BAP) test, which measures

plasma levels of antioxidants [1]. The BAP test is based on the ability of a coloured solution, containing a source of ferric (Fe^{3+}) ions bound to a special chromogenic substrate, to decolourize when the Fe^{3+} ions are reduced to ferrous ions (Fe^{2+}). This reduction occurs only when the solution is added to a reducing/antioxidant system. First, 50 µl of ferric chloride reagent is transferred into the cuvette containing the thiocyanate derivative reagent. The resulting coloured solution is gently mixed by inversion and then subjected to a 550 nm photometric reading. Then, 10 µl of plasma is added to the same cuvette, and the solution is gently mixed and incubated in a thermostatic block for 5 min at 37°C. After incubation, the sample is tested for absorbance at 550 nm. BAP test results are expressed in µmol Fe^{2+}/litre of sample. Samples with values greater than 2200 µmol Fe^{2+}/litre are considered to have normal biological antioxidant potential. The dROMS and BAP tests were performed using apposite kits and the appropriate FRAS4 instrumentation (Free Radical Analytical System 4, Health & Diagnostics Limited Co., Parma, Italy).

Statistical Analysis

For sample size calculation, the standard deviation (SD) of the population and the minimal significant variation of parameters was set a priori, based on previously pilot studies [32, 33]. For ROMs levels, SD was set to 27. As previously done by other researchers, a change of at least 15% from the baseline (~270 U CARR) value was taken as the cut-off value for a significant variation in oxidative stress [12]. Therefore, the minimal significant variation of parameters was set to 40. For BAP levels, SD was set to 60. For BAP levels, generally a cut-off value of 200 is used to distinguish between different degrees of deficiency [36]. Since the authors were interested in even smaller changes, the minimal significant variation was set to 100. Both, for ROMs and BAP levels, power goals were set to 90%. Distribution of variables was assessed by the Kolmogorov–Smirnov test. Results were normally distributed. The combined effect of the two factors (exercise and supplementation) on ROMs and BAP was analyzed by repeated measures two-way ANOVA followed by the Student Newman–Keuls test (*post-hoc* comparisons). A P-value <0.05 indicated statistical significance. Statistics were compiled using the Statistica 7 software.

Results

Microbial Analysis

Faecal specimens collected at various time points, before and after probiotic administration, were analysed and the *Lactobacillus* flora was assessed by RAPD. Prior to probiotic treatment it was found that the subjects harboured lactobacilli at a mean count of 4.8 ± 0.9 Log_{10} CFU/g of faeces in probiotic group and 5.1 ± 0.7 Log_{10} CFU/g of faeces in control group. No strains were recovered which had RAPD fingerprints corresponding to *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®. At the end of probiotic treatment the *Lactobacillus* count remained almost stable in the control group (5.2 ± 0.5 Log_{10} CFU/g of faeces) while in the probiotic group it significantly increased ($P < 0.05$) at a value of 6.6 ± 0.8 Log_{10} CFU/g of faeces. *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® were detected in the faecal samples of all of the subjects (Table 1), even if in different proportions for each subject.

Table 1 RAPD PCR detection of *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® in faecal samples of subjects before (day 0) and after cessation (day 28) of oral administration of a combination of these strains

	No. of *L. rhamnosus* IMC501®		No. of *L. paracasei* IMC502®	
	Colonies/no. analyzed		Colonies/no. analyzed	
Subject no.	Day 0	Day 28	Day 0	Day 28
1	0/10	9/14	0/10	3/14
2	0/10	2/10	0/10	8/10
3	0/20	0/18	0/20	11/18
4	0/20	6/16	0/20	7/16
5	0/15	8/18	0/15	5/18
6	0/10	0/10	0/10	7/10
7	0/18	11/20	0/18	5/20
8	0/10	6/14	0/10	6/14
9	0/20	6/18	0/20	6/18
10	0/16	2/16	0/16	10/10
11	0/14	12/20	0/14	4/20
12	0/10	4/14	0/10	8/14

Table 2 Antioxidative activity and oxidative stress resistance of probiotic bacterial strains

	Antioxidative activity (inhibition of linoleic acid peroxidation)		Oxidative stress resistance* (diameter of the inhibition zone)
	Intact cells	Intracellular extracts	
L. rhamnosus IMC 501®	34%	42%	16.2 ± 0.28
L. paracasei IMC 502®	42%	46%	14.5 ± 0.56

* The values (mm) are shown as mean ± standard deviation (SD) of three replicate experiments

Antioxidative Activity and Oxidative Stress Resistance of Probiotic Bacterial Strains

As shown on Table 2, both bacterial strains either as intact cells or as intracellular extracts, demonstrated an antioxidative effect on the inhibition of linoleic acid peroxidation. However, the intracellular extracts, in comparison with the intact cells, exhibited a greater inhibitory effect. In addition both strains showed good resistance to the oxidative stress (Table 2).

Changes in Plasma Reactive Oxygen Metabolites

Figure 1 shows ROMs levels before and after the physical activity in both the supplemented and control groups. ANOVA analysis revealed a significant effect of the exercise on ROMs level ($P < 0.01$). *Post-hoc* comparison confirmed that the ROMs level of the control group at the end of the training program was significantly higher than that measured before the physical activity ($P < 0.05$). This result indicates that the exercise program was intense and induced oxidative stress. Although after exercise ROMs levels were higher in the control group compared to the probiotic group, the overall ANOVA and *post-hoc* revealed that ROMs levels did not significantly differ between the two groups ($P > 0.05$). Also, ANOVA indicated that the interaction between treatment and exercise on ROMs levels was not significant ($P > 0.05$). However, as opposed to the control group, the probiotic group *post-hoc* comparison showed no differences on ROMs levels before exercise compared to those measured after exercise ($P > 0.05$). Overall, these results indirectly indicate that probiotics neutralized ROMs and are confirmed by the BAP test results.

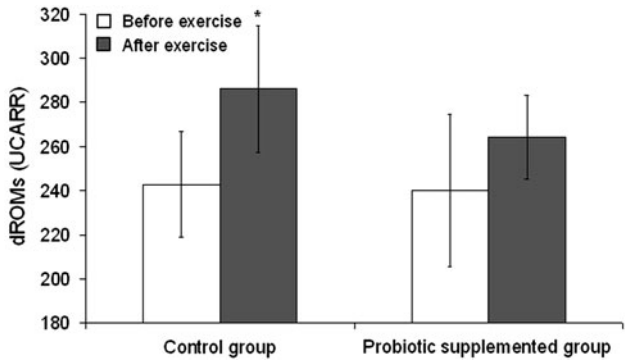

Fig. 1 Effects of probiotics supplementation on ROMs levels before and after the physical activity in both the probiotic supplemented and control groups. ANOVA: F-treatment $(1.46) = 2.75$; $P > 0.05$—F-interaction $(1.46) = 1.30$; $P > 0.05$—F-exercise $(1.46) = 16.35$; $P < 0.01$. *Post-hoc* comparisons: *: Control group, after exercise versus control group before exercise and versus probiotic supplemented group before exercise; *post-hoc* comparisons $P < 0.05$

Plasma Biological Antioxidant Potential

Figure 2 shows BAP levels before and after the physical activity in both the supplemented and control groups. ANOVA revealed a significant effect of probiotic supplementation on BAP levels ($P < 0.01$). In the probiotic supplemented group, *post-hoc* comparison confirmed that BAP levels significantly increased after supplementation, despite the physical activity ($P < 0.05$). Moreover, BAP levels after exercise were significantly higher in the probiotic supplemented group compared to the control group ($P < 0.01$). The interaction between treatment and exercise on BAP levels was also significant ($P < 0.01$), thus confirming that supplementation significantly increased plasma antioxidant levels.

Discussion

In the last several years, prebiotics, probiotics and synbiotics have become popular food supplements because of their beneficial effects on human health. These products improve the microbial environment of the gastro-intestinal tract and exert different positive effects, ranging from competitive exclusion of pathogens to immune system stimulation. The impact of probiotics on host metabolism is also significant, as they produce vitamins and participate in the processing of nutrients. In the case of inadequate diets, metabolic pathology and antibiotic therapies, the use of probiotics may represent an effective and sometimes necessary strategy to prevent deficiencies of vitamins, antioxidants and other valuable nutrients. Among these effects, several studies have established that some strains of probiotics positively prevent and correct oxidative stress in humans, through their direct antioxidative activity, improvement of food digestion and absorption, vitamin

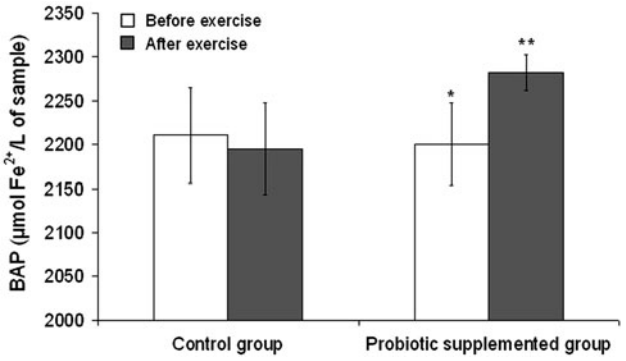

Fig. 2 Effects of probiotics supplementation on BAP levels before and after the physical activity in both the probiotic supplemented and control groups. ANOVA: F-treatment $(1.46) = 9.8$; $P < 0.01$—F-interaction $(1.46) = 12$; $P < 0.01$—F-exercise $(1.46) = 5.7$; $P < 0.05$ *Post-hoc* comparisons: * Probiotic supplemented group before supplementation versus probiotic supplemented group after supplementation. $P < 0.05$ ** probiotic supplemented group versus control group after supplementation. $P < 0.01$

production and immune system modulation [2, 16, 21, 26, 28, 34, 42, 45]. Oxidative stress plays an important role in several diseases. It can be the cause or a consequence of pathologies such as hyperglycaemia and hyperlipidemia, where the associated oxidative stress seems to be responsible for cardiovascular diseases and atherosclerosis [5, 8–10, 44]. New probiotic strains with antioxidative activity are thus needed, and in this study, the authors analyzed the effects of *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® supplementation on exercise-induced oxidative stress. The authors analyzed data from athletes supplemented with *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® during 4 weeks of intense physical activity, which is a source of intense ROS production and potential oxidative stress, as confirmed by the results obtained. The recovery of *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® from human faeces after probiotic administration showed that both strains were recovered from the faecal samples of the athletes though in different proportions for each subject. This result indicates that the colonization of these probiotics is likely host-specific. The antioxidative activity of the two probiotics strains was first determined by measurement in vitro of the inhibition of linoleic acid peroxidation. Both intact cells and intracellular cell-free extracts of *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® showed an antioxidative effect on inhibiting lipid peroxidation. The fact that intracellular cell-free extracts possessed increased inhibitory effect suggests that the level of antioxidant factor in the intracellular extracts was greater than that released in the medium by the intact cells. Lactobacilli are anaerobic and, under aerobic conditions, highly toxic reactive oxygen intermediates such as superoxides, hydroxyl radicals and peroxides are formed within the cells [43]. The oxidative stress resistance is a measure of the ability of bacteria to survive in oxidative conditions because of their ability to neutralize ROS. *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® showed high-oxidative stress resistance, thus confirming their antioxidative activity. Beyond these in vitro studies and because the intestinal environment is complex, in vivo data are essential for a proper evaluation of the effects of probiotics. The data revealed that they decreased plasma levels of ROMs, which is consistent with an increase of antioxidants levels. In the control group, the ROMs levels after 4 weeks of intense exercise were higher compared to those measured before exercise (Fig. 1), thus demonstrating the proper utility of this physical activity as an oxidative stress paradigm. ANOVA analysis indicated that the interaction between treatment and exercise was not significant, *post-hoc* comparisons in the probiotics group provided evidence that there was not a significant difference of ROMs levels before and after exercise, and this indirectly indicates that probiotics neutralized the exercise-induced ROMs. However, oxidative stress is a complex condition and both ROMs and BAP levels must be considered simultaneously for a correct diagnosis. Actually, the antioxidative effects of the probiotics can be better evidenced analysing the BAP levels. In the control group, the BAP levels after exercise were lower than before exercise. BAP levels decreased because antioxidants were consumed for ROS neutralization. In contrast, in the probiotics group, the BAP levels after exercise were significantly higher than those measured before exercise, despite the elevated oxidant levels produced by the physical activity (Fig. 2). Finally, after exercise, the BAP levels in the probiotics group were significantly higher than those measured in the control group. Taken together, these data demonstrated that intense physical activity induced oxidative stress and that probiotic supplementation increased plasma antioxidant levels, thus neutralizing ROS and reducing oxidative stress. Antioxidants were also present in the food consumed as prescribed in the diet program of athletes enrolled in the study. Diets were proportionally equivalent in macro and micronutrient quantity for all the subjects, and contained 100% of the RDA for all nutrients, antioxidants included. Since the intestinal microflora regulates nutrient assimilation, one mechanism responsible for the augmented BAP levels could be the improvement of antioxidant absorption exerted by the two probiotic strains. However, due to their vast range of effects, other options must be considered, and the higher BAP levels determined after the probiotics supplementation may be the sum of several mechanisms. For example, both the strains analyzed in this study are a subspecies of *L. casei*, which is known to be a producer of vitamins of the B group. Among these, B1, B5 and B6 vitamins are known to be antioxidants [17, 29, 30, 41, 52]. Moreover, improved glucose and lipid metabolism may

contribute to the reduction of oxidative stress. A number of studies suggest that postprandial hyperglycaemia produces oxidative stress, leading to complications associated with diabetes [8–10]. Oxidative stress is also induced by hyperlipidemia after consumption of a meal rich in lipids [5, 44]. Since the intestinal microflora has the potential to reduce the adverse effects associated with high-fat diets and to restore normal glucose metabolism, it may modulate the redox balance of the organism leading to a reduction of oxidative stress [6, 7, 39]. Finally, intense exercise induces inflammation, which increases free radical activity by activating the neutrophil NADPH oxidase and/or uncoupling a variety of redox systems, including endothelial cell xanthine dehydrogenase [51]. Since probiotics modulate perturbations in immune function and inflammation after exercise [50], they may counter the exercise-induced oxidative stress. It was also believe that intense exercise has strongly influenced the results of this study. To the knowledge, this is the first work which explored the effect of probiotics on exercise-induced oxidative stress, and the authors do not have other comparative data. Probably, different effects of probiotic supplementation would be expected without intense exercise, which itself is sufficient to alter the plasma oxidant and antioxidant levels. For example, the suspension of exercise decreases oxidant production, so antioxidant defence increases. Moreover, during exercise, antioxidants are upregulated and also mobilized from tissues to blood [49]. Conversely, exercise represents an ideal situation for levelling parameters among subjects, which might be too heterogeneous for a statistical comparison of different experimental groups. In conclusion, it was demonstrated that the two strains, *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®, exert strong antioxidant activity in situations of elevated physical stress. Athletes and all those exposed to oxidative stress may benefit from the ability of these probiotics to increase antioxidant levels and neutralize the effects of reactive oxygen species.

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