



Saccharomyces boulardii supplementation does not affect anaerobic power gain induced by short-term sprint interval training in physically active individuals

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Abstract

Sprint interval training (SIT), which consists of vigorous-intensity exercise interspersed with periods of rest or low-intensity exercise, can improve human anaerobic performance. Probiotic strains, including yeasts (e.g. *Saccharomyces boulardii*; Sb), have beneficial effects on human health; however, evidence regarding the effects of probiotics on anaerobic performance is unavailable. The current study investigated whether Sb supplementation influences the SIT-induced changes to the following performance variables: peak (PPO) and mean (MPO) power output. Fifteen healthy individuals (twelve men and three women) were randomly divided into two groups: placebo (PLA; n=8) and Sb (n=7). The individuals performed six SIT sessions on a cycle ergometer (four to seven 30-s all-out sprints thrice weekly). During the training period, participants ingested a capsule containing PLA or at least 1×10^9 Sb cells daily for 14 days. Performance-related variables were compared between the first and last training sessions. Sb supplementation did not influence the changes in PPO and MPO across the two weeks of training ($P > 0.05$); therefore, the data from both groups were analyzed collectively to assess performance changes induced by SIT. Training increased PPO, an index of anaerobic power, in the sixth session compared to the first session (by $8 \pm 11\%$ in the first sprint; $+1.0 \pm 1.2$ W/kg; $P=0.008$) but did not change MPO. In conclusion, short-term SIT improved the participants' anaerobic performance (power), as evidenced by increased PPO. Sb supplementation did not affect the improved anaerobic power caused by SIT.

Key words: Athletic performance; Exercise tests; Probiotics; Yeasts; Sprint interval training

Introduction

Several lines of evidence link gut microbiota to physical exercise. Cross-sectional studies demonstrate a relationship between trained states and healthy gut microbiota (1,2), whereas regular physical exercise increases the abundance of beneficial bacterial taxa (2,3). These earlier findings boosted research addressing the modulatory role of probiotic supplementation on the gut microbiome as a strategy to enhance physical performance. Probiotics positively impact human physiology by inducing beneficial changes in the gut and extra-intestinal tissues (2) and have been associated with improved performance (4,5).

Performance in high-intensity exercises lasting from seconds to a few minutes depends heavily on the energy released by cellular anaerobic processes (6). Previous

investigations involving treatment/supplementation with bacterial strains have highlighted some physiological mechanisms that may underlie an improved anaerobic performance caused by probiotics. Treatment with *Lactiplantibacillus plantarum* increased muscle glycogen stores and improved muscle mass and strength in mice (7). The improved performance was attributed to the crosstalk between skeletal muscles and the gut, likely due to the effects of probiotics/microbiota composition on muscle mass and function (8). Furthermore, *L. plantarum* supplementation increased the plasma concentration of branched-chain amino acids (BCAAs) and improved aerobic and anaerobic performance in athletes after a simulated triathlon (5). As previously shown, the regulation of BCAA catabolism in muscles is essential for

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precise control of muscle energy metabolism and, at least in part, for adaptation to physical training (9).

Most studies have focused on supplementation with bacterial strains, and there is no evidence of the effects of a yeast strain with probiotic properties on performance, particularly on anaerobic performance gains induced by physical training. *Saccharomyces boulardii* (Sb), the focus of the current study, is a non-pathogenic yeast widely marketed worldwide due to its probiotic properties, such as for the treatment of several types of diarrheas (10). None of the probiotic-induced muscle benefits previously mentioned has been investigated following Sb supplementation, and the only study to date addressing the effects of Sb on physical performance showed that a 10-day supplementation increased maximum oxygen consumption ($\dot{V}O_{2max}$) and maximum aerobic speed in rats subjected to an incremental exercise session (11). Interestingly, the authors of the latter study hypothesized that Sb could override the inhibitory signals arising from the central nervous system, thus favoring prolonged exertion (11), and available evidence indicates that chronic oral administration of Sb has central effects (12). Performing a 30-s Wingate test induces peripheral and central fatigue, as revealed by decreased maximum voluntary contraction and voluntary activation in a subsequent isometric knee extension test (13). However, the translation of findings obtained in rodents (e.g. the central or performance-enhancing effects of Sb) to human physiology is not direct since many interspecies differences, such as diet, microbiota composition, intestine size, and exposure to environmental stressors, may influence the effects of probiotic supplementation.

Lack of time is one of the primary reasons people do not adhere to regular physical exercise programs (14). In this sense, interval training (i.e., high-intensity stimuli interspersed with low-intensity recovery or rest) is an attractive, time-effective strategy for improving physical performance of elite and recreational athletes (15). Interval training can be divided into high-intensity interval training and sprint interval training (SIT) (16). While high-intensity interval training comprises “near maximal” efforts generally performed at an intensity that elicits $\geq 80\%$ of the maximal heart rate (16), SIT is characterized by efforts performed at intensities equal to or greater than the pace that would elicit $\dot{V}O_{2max}$, including “all-out” or “supramaximal” efforts (16). The most studied SIT protocol consists of four to six 30-s all-out cycle ergometer efforts (i.e., Wingate tests), interspersed by 4-min recovery intervals (16,17). Despite having a lower exercise volume and reduced time commitment, SIT induces similar aerobic adaptations (e.g. augmented mitochondrial content) as moderate-intensity constant training (18).

In addition to improving aerobic performance, SIT has been reported to enhance anaerobic performance, particularly when SIT protocols last at least four weeks

(17,19–21). These studies, which investigated anaerobic performance during Wingate tests (22), reported improved peak power output (PPO), an index of anaerobic power (23), but had controversial findings regarding mean power output (MPO), an index of anaerobic capacity (23). Therefore, the present study aimed to analyze the effects of Sb supplementation on the changes in anaerobic performance induced by short-term SIT in physically active individuals. We hypothesized that short-term SIT improves anaerobic performance-related variables, especially PPO, and that Sb supplementation enhances these SIT-induced improvements.

As a secondary objective, we investigated whether the initial (baseline) performance level influences the magnitude of change in anaerobic performance resulting from SIT. It is generally assumed that individuals with lower initial performance (i.e., less trained) exhibit greater trainability (i.e., more significant gains). This assumption is supported by studies addressing $\dot{V}O_{2max}$ trainability (24), where initial performance is negatively correlated with performance change. Thus, we hypothesized that individuals with lower baseline anaerobic conditioning exhibit greater anaerobic performance gains induced by SIT. Notably, the current study will improve our understanding of the interactions among probiotics, interval training, initial performance, and performance gains. This knowledge is essential for better understanding training prescription, athlete nutrition, and expected adaptations during short-term training protocols.

Material and methods

Ethical aspects

All procedures were approved by the Ethics Committee of the Universidade Federal de Minas Gerais (protocol number - CAAE: 65916517.0.0000.5149) and conformed to the Declaration of Helsinki. The research objectives, experimental procedures, and risks and discomforts associated with study participation were explained to all potential volunteers. The individuals who voluntarily agreed to participate in the study signed an informed consent form.

Individuals

The sample size calculation was based on the findings of Burgomaster et al. (17), who reported increased PPO measured during a Wingate test after six sessions of SIT (effect size=0.76; estimated from data reported in the 2005 article). The following parameters were used for *a priori* sample size calculation: *t*-tests, differences between two dependent means (matched pairs), two-tailed test, $\alpha=0.05$, and power=0.80. This calculation was conducted in G*Power software (v 3.1.9.7; Germany) and indicated that 16 participants were required to observe increased anaerobic power after six training sessions.

Several strategies were used to recruit volunteers, including advertising on social media and through fliers posted in different locations on the university campus.

As an inclusion criterion, the individuals should be recreationally active (i.e., perform physical activity at least twice a week). Moreover, they should not have used non-steroidal anti-inflammatory drugs, antibiotics, probiotics, or food supplements in the month preceding the experiment trials and should not present any gastrointestinal disease verified by self-report. The women volunteers should have been using monophasic oral contraceptives for at least 3 months. The three participating women were taking oral contraceptive as follows: 1 pill daily for 21 days followed by 7-day no-pill break.

Experimental design

This study was part of a broader project to assess the combined effects of short-term SIT and Sb supplementation on performance and physiological and clinical parameters of physically active individuals. In the current study, the 16 participants were allocated into two experimental groups: placebo (PLA) or *S. boulardii* supplementation (Sb). One female participant who was allocated to the *S. boulardii* supplementation group withdrew from the study for personal reasons. Therefore, only 7 individuals allocated to the Sb group completed the experiments.

Block randomization was conducted according to peak aerobic power output reached by the participants during a cardiorespiratory incremental test on a cycle ergometer. Based on their performance, the participants were divided into two strata (superior and inferior performance), and then a draw was made within each stratum to randomly allocate participants to the experimental groups.

On the first day of the experiment, the volunteers attended the laboratory for measurements of body mass, height, and skinfolds. They also performed an incremental test on a cycle ergometer to determine their maximum aerobic power (P_{max} ; Figure 1). The female volunteers were in the first week of contraceptive use when this first day of experiments took place. The participants started ingesting the Sb or PLA capsules on the sixth day. The capsules were ingested preferably before breakfast for

14 consecutive days (i.e., from the 6th to the 19th day of the timeline). On the eighth day, the 15 volunteers started training, comprising 6 sessions distributed across two weeks. Both interventions (SIT and supplementation) were terminated on the 19th day of the timeline. The experiments were conducted in a double-blinded manner.

Anthropometric assessment

Body mass, height, and skinfold thickness were measured. Body mass (kg) was measured on a digital scale (Filizola, Brazil) with an accuracy of 0.02 kg, with the volunteers protected by a screen and wearing only underwear and socks. Height (cm) was measured with a stadiometer (Filizola) with an accuracy of 0.5 cm. The subscapular, triceps, pectoral, subaxillary, suprailiac, abdominal, and thigh skinfolds were measured with a caliper (Lange, NutriActiva LLC, USA), graduated in millimeters.

Body mass index (BMI, expressed in kg/m^2) was calculated by dividing body mass by the square of the height. Body density was calculated according to the participants' sex using the equations proposed by Jackson and Pollock (25): body density in men = $[1.112 - (0.00043499 \times \Sigma 7 \text{ skinfolds in cm})] + [0.00000055 \times (\Sigma 7 \text{ skinfolds in cm})^2] - (0.00028826 \times \text{age in years})$; body density in women = $[1.097 - (0.00046971 \times \Sigma 7 \text{ skinfolds in cm})] + [0.00000056 \times (\Sigma 7 \text{ skinfolds in cm})^2] - (0.00012828 \times \text{age in years})$. Finally, body fat percentage (%BF) was calculated using the following equation: $\%BF = [(4.95 / \text{body density}) - 4.5] \times 100$. Lean body mass (reported in kg) was calculated by subtracting fat mass from body mass using the following equation: $\text{body mass} - (\%BF \times \text{body mass} / 100)$.

Incremental test

The configuration of the incremental test depended on the participant's sex. Women started the test cycling at 50 W (26), while men started at 100 W (27). Intensity was increased by 25 W every 3 min until the participants were voluntarily fatigued. The test was interrupted when the participants scored 20 on the Borg's rating of perceived exertion (RPE) scale, were unable to maintain the predetermined cadence of 50 revolutions per minute (rpm), or voluntarily asked to stop cycling.

P_{max} (measured in W) and $\dot{V}O_{2max}$ ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) were determined using the equations proposed by Kuipers et al. (28) and the American College of Sports Medicine (29): $P_{max} = \text{power in the last completed stage in W} + [(\text{time spent in the uncompleted stage in seconds} / 180 \text{ s}) \times 25 \text{ W}]$ and $\dot{V}O_{2max} = (12 \times P_{max} + 300) / \text{body mass in kg}$.

The incremental test was performed on a Monark standard bicycle with a friction resistance system (model MAXX, Hidrofit, Brazil). The bicycle was positioned inside an environmental chamber (WMD 1150-5, Russels Technical Products, USA), programmed to maintain the

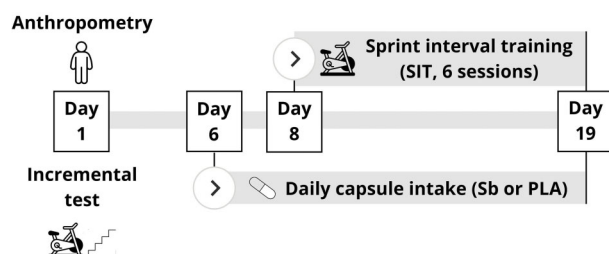


Figure 1. Schematic representation of the experimental design. SIT: sprint interval training; Sb: *Saccharomyces boulardii*; PLA: placebo.

dry-bulb ambient temperature and relative humidity at 25°C and 55%, respectively.

Supplementation

Participants ingested a capsule of Sb (at least 1×10^9 Sb cells per capsule; Floratil[®], Merck S.A., Germany) or placebo (100 mg of magnesium stearate and 100 mg of lactose - substances present in the probiotic capsule) daily for 14 days. They were instructed not to consume fermented products (e.g. yogurt) or additional probiotics during the experimental period. The participants reported not using medications during the study.

Sprint interval training

Six training sessions were carried out across two weeks, with three sessions per week. The SIT consisted of 30-s maximal sprints against a resistance of 0.075 kg/kg of body mass (i.e., Wingate test) with 4 min of recovery, during which the volunteers cycled at low intensity (<30 W) in the first 2 min and then rested for an additional 2 min. In the first session, four sprints were performed. The number of sprints was increased to five in the second session and six in the third and fourth sessions. In the last two training sessions, the participants performed seven sprints. This training protocol was similar to the one used by Burgomaster et al. (17), except for the number of sprints in the sixth session: our participants performed seven instead of four sprints.

Like the incremental test, all training sessions were carried out on the same equipment and conditions. During these sessions, the male participants wore a T-shirt, shorts, socks, and sneakers; the female participants wore a top under the T-shirt, shorts, socks, and sneakers. In addition, all participants with long hair were encouraged to use a hair clip. The participants were instructed to eat between 1 and 2 h before the SIT session to reduce the possibility of gastrointestinal discomfort or hunger.

Each sprint was initiated from a stationary position, and the volunteers were required to remain seated throughout the 30-s all-out effort. The seat and handlebars were adjusted according to each participant's body size and comfort, with the same settings used for the incremental test applied to the Wingate exercise bouts. The same experimenter provided standardized verbal encouragement during the sprints.

Measured variables

The training-induced changes in physical performance were assessed by data collected during the first and last training sessions. The following variables were measured in each sprint: peak power output (PPO), mean power output (MPO), and fatigue index (FI). The cycle ergometer was connected to a software (Multi Cycle Ergometer, version 5.1, Warsaw Sports Institute, Poland) to record the power produced by the volunteers during the sprints and calculate the variables mentioned earlier. As described by

Lacerda et al. (30), an electronic sensor was placed on the pedal to measure the occurrence of a complete pedal cycle. Sensor status was sampled at 1,000 Hz, and the MCE software analyzed the data collected. Since the pedal is coupled to the wheel (i.e., a pedal-wheel system), one complete pedal revolution corresponds to 3.71-wheel revolutions, which, in turn, corresponds to $2\pi \times 25 \text{ cm} \times 3.71 = 6 \text{ m}$ (Monark standard cycle ergometer).

We recorded performance-related variables in the first sprint of each session and in the sprint in which the participants attained the highest value for the variables during the first and last training session. Notably, the highest value was not necessarily reached in the first sprint.

MPO, which is an index of anaerobic capacity (23), corresponded to the average power output sustained for the 30 s of each sprint. Power output, measured in $\text{kg} \cdot \text{m} \cdot \text{min}^{-1}$, was calculated using the following equation: $\text{Power output} = F \times D / T$, where “F” is the resistance placed on the cycle ergometer in kg; “D” is the distance, calculated by multiplying the distance covered at each revolution of the bicycle pedal (6 m) by the number of revolutions; and “T” is the time duration for each sprint in minutes. Data were converted into watts (i.e., data in $\text{kg} \cdot \text{m} \cdot \text{min}^{-1}$ were multiplied by 0.16344).

PPO in watts was the highest mechanical power achieved during each sprint. PPO is an index of anaerobic power and corresponds to the ability to produce the greatest amount of work in a given time (23). Both PPO and MPO data were normalized by the participants' lean body mass to allow more precise inter-group comparisons, mainly because the number of female volunteers differed between the PLA and Sb groups.

FI (or the rate of power decrease) represents the power reduction during the test and was calculated as the percent difference between PPO and the lowest power recorded during each sprint. The following equation was used: $\text{FI} (\%) = (\text{PPO} - P_{\text{Low}}) \times 100 / \text{PPO}$, where “ P_{Low} ” represents the lowest power recorded during the 30-s physical exertion.

Finally, total external work was computed by summing the external work done in all sprints during a training session.

Statistical analysis

The Shapiro-Wilk and Levene tests confirmed the data normality and homoscedasticity, respectively. All data are reported as means $\pm$ SD. Anthropometric variables and Pmax were compared between the Sb and PLA groups using Student's *t*-tests. The performance variables were initially compared between experimental groups (Sb vs PLA) and time points (sixth vs first training session) using mixed-model two-way ANOVAs. In these ANOVAs, repeated measures were only considered for the time point factor.

Next, the data from both groups were merged and then analyzed as a single group. Merging data was possible because Sb supplementation did not affect any

performance variable measured in the current study. Paired Student's *t*-tests were used to compare the performance between training sessions (i.e., second vs first, sixth vs first). The first and second sessions were compared to verify the reliability of performance measures. The reliability of our method was established by the intraclass correlation coefficient (ICC 3.1). The following benchmark values were used to classify ICC: less than 0.5 = poor, ≥ 0.5 and < 0.75 = moderate, ≥ 0.75 and ≤ 0.90 = good, and > 0.90 = excellent. The standard error of measurement (SEM) and minimum detectable difference (MDD) were calculated as follows: $SEM = SD \times \sqrt{(1 - ICC)}$ and $MDD = SEM \times 1.96 \times \sqrt{2}$.

Pearson's correlation coefficients were used to assess the association between performance variables obtained in the first training session or the accumulated external work and the performance changes. Performance changes (deltas) corresponded to the differences between the sixth and the first training sessions.

The statistical analyses were performed using software (Sigmaplot version 11.0 and SPSS version 21, USA), and the significance level adopted was 5%. Cohen's *d* effect size was used to assess the magnitude of differences between data and was calculated by subtracting a mean value of the sixth (or second) session from a mean value of the first session; the result was then divided by a combined standard deviation of the data. The effect size values were classified as trivial ($d < 0.2$), small ($0.2 \leq d < 0.6$), medium ($0.6 \leq d < 1.2$), or large ($d \geq 1.2$).

Results

Fifteen participants (12 men and 3 women) completed all experimental sessions of the current study. Their anthropometric characteristics, determined at the beginning of the experiments, are described in Table 1. No differences were observed in age, body mass, height, BMI, fat percentage, lean body mass, and Pmax between the individuals assigned to the two experimental groups ($P = 0.14$ to 0.77 ; Table 1). Our participants were classified as having a level 1 performance according to existing guidelines: women had a Pmax < 170 W (26) and men had a Pmax < 280 W (27).

Sb supplementation did not influence the performance variables measured in the first and sixth sprint training sessions, as evidenced by the non-significant main effects of experimental group ($F = 0.00$ to 2.51 ; $P = 0.137$ to 0.984) and non-significant group $\times$ time point interactions ($F = 0.00$ to 0.50 ; $P = 0.492$ to 0.945 ; Table 2). Therefore, the data of the two experimental groups were analyzed together (as a single group), thus allowing us to understand the performance changes across the six SIT sessions.

Data were compared between the first and second sessions to verify the reliability of performance measures. PPO, MPO, and FI, either in the first sprint or in the sprint with the highest values, did not differ between the first two training sessions ($P = 0.110$ to 0.738 ; Table 3). Moreover, ICC indicated moderate to good reliability for PPO (0.68 and 0.75 for the first and the sprint with the highest value, respectively) and moderate reliability for MPO (0.63 and 0.65). In contrast, ICC for FI indicated poor reliability (0.33 and 0.48 for the first sprint and the sprint with the highest value, respectively).

The PPO in the first sprint and the highest PPO attained were increased by $8 \pm 11\%$ and $7 \pm 7\%$, respectively, in the sixth compared to the first training session: first sprint ($+1.0 \pm 1.2$ W/kg; $t = -3.09$; $P = 0.008$) and sprint with the highest PPO ($+0.8 \pm 0.9$ W/kg; $t = -3.62$; $P = 0.003$). These differences in PPO were greater than SEM calculated from ICC values (Table 3) and classified as medium effects ($d = 0.67$ and 0.64 ; Figure 2A and B). In contrast, the short-term SIT did not affect MPO in the first sprint and the highest MPO (Table 4). As a result of an increased PPO and unchanged MPO, the FI in the first sprint and the highest FI were increased by $32 \pm 38\%$ and $24 \pm 31\%$, respectively, in the sixth compared to the first training session: first sprint ($t = -3.24$; $P = 0.006$) and sprint with the highest FI ($t = -2.73$; $P = 0.016$). These differences in FI were classified as medium effect sizes ($d = 1.13$ and 0.92 ; Figure 2C and D). As expected, total external work was higher (increased by $71 \pm 29\%$; $d = 2.68$) in the last than in the first training session (Table 4).

Lastly, we ran correlation analyses to understand whether the performance improvements induced by short-term SIT were associated with the initial performance level.

Table 1. Participants' anthropometric variables and maximal aerobic power before the first training session.

Variables	Age (years)	Body mass (kg)	Height (cm)	BMI (kg/m ²)	Body fat (%)	LBM (kg)	Pmax (W)
All (n=15)	26 (5)	75.9 (11.5)	175 (9)	24.6 (2.8)	22.3 (7.8)	58.9 (9.7)	200 (34)
PLA (n=8)	26 (5)	77.5 (12.9)	175 (11)	25.3 (3.1)	25.0 (7.5)	58.1 (10.7)	188 (38)
Sb (n=7)	25 (5)	74.1 (10.4)	176 (7)	23.8 (2.3)	19.3 (7.6)	59.8 (9.1)	214 (24)
<i>t</i> -test (PLA $\times$ Sb)	<i>t</i> =0.30	<i>t</i> =0.55	<i>t</i> =-0.32	<i>t</i> =1.08	<i>t</i> =1.47	<i>t</i> =-0.32	<i>t</i> =-1.55
<i>P</i> -value	<i>P</i> =0.77	<i>P</i> =0.59	<i>P</i> =0.75	<i>P</i> =0.30	<i>P</i> =0.17	<i>P</i> =0.75	<i>P</i> =0.14

Data are reported as means $\pm$ SD (Student's *t*-test). BMI: body mass index; LBM: lean body mass; PLA: placebo; Pmax: maximal aerobic power; Sb: *S. boulardii*.

Table 2. Performance variables measured in the participants of the two experimental groups in the first and sixth (last) training sessions.

Variables	PLA 1st	Sb 1st	PLA 6th	Sb 6th	P (1)	P (2)	P (3)
First sprint							
PPO (W/kg)	13.9 (2.4)	13.4 (1.1)	14.8 (1.2)	14.4 (1.0)	0.570	0.011*	0.908
MPO (W/kg)	10.0 (1.6)	10.3 (0.9)	9.9 (1.3)	10.2 (0.9)	0.570	0.671	0.945
FI (%)	29 (6)	25 (5)	37 (9)	32 (6)	0.137	0.008*	0.726
Sprint with the highest value							
PPO (W/kg)	14.4 (1.9)	14.1 (1.2)	15.4 (1.3)	14.8 (1.0)	0.516	0.004*	0.694
MPO (W/kg)	10.1 (1.7)	10.3 (0.9)	9.9 (1.1)	10.2 (0.9)	0.644	0.666	0.934
FI (%)	44 (10)	41 (4)	51 (7)	54 (20)	0.984	0.018*	0.492
Whole training session							
Total work (kJ)	57 (14)	63 (10)	98 (19)	104 (18)	0.419	<0.001*	0.914

Data are reported as means $\pm$ SD. 1st: first training session; 6th: sixth (last) training session; FI: fatigue index; MPO: mean power output; PLA: placebo; PPO: peak power output; Sb: *S. boulardii*. PPO and MPO data were normalized by the participant's lean body mass. P-value (1) corresponds to the main effect of experimental group; P-value (2) corresponds to the main effect of time point; P-value (3) corresponds to the group $\times$ time interaction. *P < 0.05 indicates a significant main effect of time point (mixed-model two-way ANOVA).

Table 3. Reliability of performance variables measured in the first and second training sessions.

Variables	1st	2nd	P-value	d	ICC (3,1)	95%CI	SEM	MDD
First sprint								
PPO (W/kg)	13.7 (1.8)	14.2 (1.1)	0.110	0.34	0.68	0.28–0.88	0.83	2.31
MPO (W/kg)	10.2 (1.3)	10.2 (0.9)	0.704	0.00	0.63	0.20–0.86	0.68	1.87
FI (%)	27 (6)	30 (6)	0.142	0.50	0.33	–0.20–0.71	4.93	13.65
Sprint with the highest value								
PPO (W/kg)	14.2 (1.6)	14.6 (1.1)	0.181	0.29	0.76	0.42–0.91	0.67	1.86
MPO (W/kg)	10.2 (1.3)	10.3 (0.9)	0.738	0.09	0.65	0.22–0.87	0.66	1.84
FI (%)	43 (8)	46 (7)	0.111	0.40	0.48	–0.03–0.79	5.46	15.13

Data are reported as means $\pm$ SD (paired Student's *t*-test). 1st: first training session; 2nd: second training session; CI: confidence interval; d: Cohen's *d* effect size; FI: fatigue index; ICC: intraclass correlation coefficient; MDD: minimum detectable difference; MPO: mean power output; PPO: peak power output; SEM: standard error of measurement. P-values correspond to the comparisons between training sessions. PPO and MPO data were normalized by the participants' lean body mass.

Significant negative correlations were observed between PPO measured in the first training session and the change (delta) in performance from the first to the last session: PPO in the first sprint ($r = -0.818$; $P < 0.001$; Figure 3A) and sprint with the highest PPO ($r = -0.671$; $P = 0.006$; Figure 3B). However, no significant correlations were found between FI measured in the first session and the change in FI from the first to the last session: FI in the first sprint ($r = 0.063$; $P = 0.822$) and the sprint with the highest FI ($r = -0.213$; $P = 0.447$). In addition, there were no significant correlations between the total accumulated work of the six sessions and the gain in anaerobic power, either PPO in the first sprint ($r = 0.151$; $P = 0.590$) or highest PPO ($r = 0.167$; $P = 0.553$).

Discussion

Our findings indicated that Sb supplementation for 14 days did not impact the changes in anaerobic performance induced by short-term SIT. When combining the data from both groups (PLA and Sb), we found that only

six training sessions increased anaerobic power, as evidenced by higher PPO in the last than in the first training session. FI increased, whereas MPO did not change across the short-term SIT. Finally, significant correlations were found between initial performance levels (PPO measured during the first training session) and change in PPO between the last and first sessions. Therefore, the hypothesis that Sb supplementation enhances SIT-induced improvements in anaerobic performance was rejected, and the hypothesis that initial anaerobic conditioning correlates negatively with performance gains induced by training was accepted.

Sb supplementation did not enhance the performance improvements caused by short-term SIT, thus not matching our expectations based on previous research reporting the beneficial effects of probiotics on performance. Although the mechanisms linking probiotics/microbiota to modifications in the central nervous system and to improvements in muscle power and strength remain inconclusive, the literature has shown some mechanisms

that deserve further investigation. For example, probiotics can potentially enhance protein digestion and absorption within the body, as suggested in previous research with human individuals (8). Furthermore, the production of short-chain fatty acids (SCFAs) from dietary fibers is a recognized function of various bacterial species in the microbiota; SCFAs have diverse physiological effects on the host, including improvements in insulin sensitivity (31). Insulin inhibits muscle protein breakdown and seems to have a permissive role in muscle protein synthesis in the presence of elevated amino acid availability (32). Probiotic supplementation could also enhance performance by reducing muscle damage during recovery, as reported in triathlon athletes (5), and by increasing muscle glycogen

storage, as reported in mice (7). The lack of effects of Sb supplementation on the performance variables measured in the current study may be attributed to the relatively short duration of supplementation (14 days), to the specific probiotic strain administered (a yeast probiotic strain), and to a possible ceiling effect in performance improvements, thus preventing the observation of a synergistic effect between SIT and Sb supplementation in PPO. Similarly, Ibrahim et al. (33) did not find extra gains in isokinetic knee strength and power when probiotic supplementation (multi-strain probiotics containing 3×10^{10} colony-forming units of *Lactobacillus acidophilus*, *Lactococcus lactis*, *Lactocaseibacillus casei*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, and *Bifidobacterium infantis* twice daily for 12 weeks) was combined with circuit training compared to the group subjected only to circuit training.

Only six training sessions increased the PPO, but not the MPO of our participants, in line with the findings reported by Barker et al. (20), who also conducted six training sessions over two weeks. Beyer et al. (21) used an SIT protocol similar to ours but lasting four weeks and with 20-s sprints, and they reported significant increases in PPO and MPO in peripubescent and postpubescent male athletes. These findings stress the efficacy of SIT in enhancing anaerobic performance, particularly by increasing anaerobic power (PPO). Furthermore, Scribbans et al. (34) demonstrated that efforts at intensities surpassing $\dot{V}O_{2\max}$, although not reaching PPO levels achieved with a sprint, can also effectively increase anaerobic power. In their study, 32 weekly sprints (20-s sprints at 170% of the maximum aerobic power with 10 s of recovery; 8 sprints per training session) for 6 weeks resulted in increased PPO and MPO measured in a Wingate test. Therefore, vigorous training, not necessarily at intensities required during all-out sprints, can improve anaerobic performance.

Several other studies support that SIT increases anaerobic performance (17,35). PPO, as measured by the Wingate test, increased after two weeks in a protocol similar to that in our study (17), while peak running speed was faster during 30-s efforts after six weeks of training with a frequency of four weekly sessions (35). SIT also

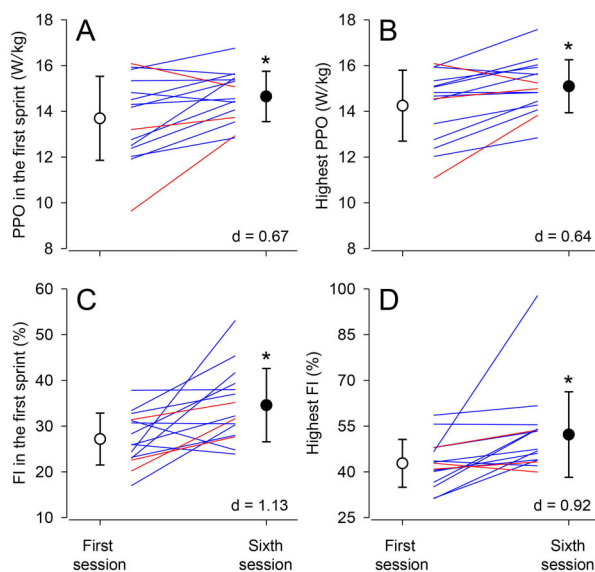


Figure 2. Peak power output (PPO) in the first sprint (A), highest PPO during the training session (B), fatigue index (FI) in the first sprint (C), and highest FI during the training session (D). The symbols correspond to group data reported as means $\pm$ SD, whereas the lines correspond to individual data. The blue and red lines represent male and female participants, respectively. PPO data were normalized by the participants' lean body mass. * $P < 0.05$ compared to first session (paired Student's *t*-test).

Table 4. Performance variables measured in the first and sixth (last) training sessions.

Variable	1st	6th	<i>t</i>	P-value	d
MPO in the first sprint (W/kg)	10.2 (1.3)	10.0 (1.1)	0.45	0.662	0.17 ^T
Sprint with highest MPO (W/kg)	10.2 (1.3)	10.0 (1.0)	0.45	0.657	0.17 ^T
Total work (kJ)	60 (12)	101 (18)*	-12.41	<0.001	2.68 ^L

Data are reported as means $\pm$ SD. * $P < 0.05$ compared to the 1st session (paired Student's *t*-test). d: Cohen's d effect size; L: large effect size; MPO: mean power output; t: *t*-test statistics; T: trivial effect size. MPO data were normalized by the participants' lean body mass. P-values for the comparisons between training sessions.

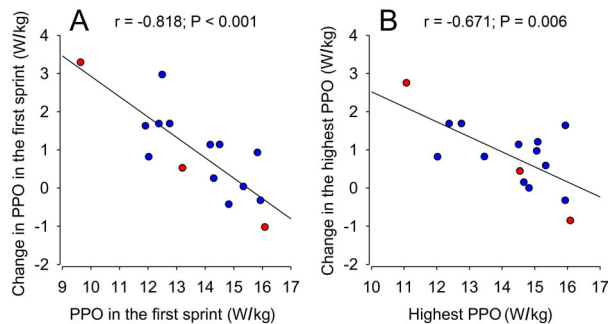


Figure 3. Negative correlations between peak power output (PPO) in the first training session and change in PPO induced by the six training sessions. **A**, PPO in the first sprint, and **B** highest PPO during the training session. The blue and red circles represent male and female participants, respectively. PPO data were normalized by the participant's lean body mass (Pearson's correlation).

improved performance in various test protocols, including incremental treadmill tests and 50-m sprint times, highlighting its efficacy as a time-efficient strategy to induce favorable adaptations in anaerobic power, isokinetic strength, and sprint performance (36). Moreover, a study involving judokas who engaged in 8 weeks of SIT revealed significant improvements in PPO and MPO, which increased by 16 and 17% in the fourth week and by 17 and 22% in the eighth week, compared to baseline levels (19). It should be noted that both the training duration and the sample characteristics seem to influence the SIT-induced changes in anaerobic performance. While PPO increases after a few sessions, more than two weeks of training may be required to improve MPO.

The enhanced anaerobic power found in our participants could be explained by an augmented reserve of energy substrates, such as muscle glycogen, which may facilitate the rapid supply of ATP through the anaerobic glycolytic pathway. Indeed, just two weeks of SIT increased the resting muscle concentration of glycogen by 26%, albeit without changing the concentrations of ATP, phosphocreatine, or creatine (17). Furthermore, performance improvements could result from an increased buffering capacity within skeletal muscles, encompassing alterations in carnosine content, protein composition, and inorganic phosphates, which prevent the accumulation of hydrogen ions (H^+) (37). Although the ability to buffer H^+ contributes to maintaining performance during brief, repeated sprints (38), this mechanism seems unlikely to explain our findings. A better buffering capacity would be expected to increase anaerobic capacity rather than anaerobic power, but we failed to observe a training-induced effect on MPO. Other factors that may have contributed to the improved anaerobic power include central adaptations, such as the recruitment rate of muscle fibers, firing rate, and synchronization of motor units (34).

Significant negative correlations were observed between PPO measured during the first training session and change in PPO across the six sessions. This finding reinforces the concept of trainability, as evidenced by greater anaerobic power improvements resulting from SIT in individuals with lower initial anaerobic conditioning, thus agreeing with a previous study about training effects on $\dot{V}O_{2\max}$ (24). Interestingly, the current findings contradict those reported by Medbø and Burges (6), who showed that women had 17% less accumulated oxygen deficit than men before training and that the response of female participants to training was attenuated compared to those of male participants. However, these comparisons should be viewed with caution because the exercise mode, method of determining anaerobic performance, and training duration differed between the previously published study and our study.

This study was the first to address the effects of yeast supplementation on human performance. Additionally, the current investigation assessed the effects of SIT on anaerobic performance, which has been little studied in the context of interval training. However, our research is not free of limitations. First, the experimental design did not include sedentary, non-trained groups treated with PLA or Sb. Among other reasons, these control groups would be relevant to identify possible learning effects on the performance-related variables. However, the moderate to good reproducibility of PPO data ($0.68 \leq ICC \leq 0.76$) and the lack of significant differences in this variable between the first and second training sessions (Cohen's $d \leq 0.34$; small effects sizes) suggest that the improved performance did not result from learning by repeating the Wingate tests. Second, performance was measured during training sessions, and since there were multiple Wingate tests within a session, we cannot rule out that the participants were saving energy in the first sprints as a strategy to maintain high levels of power output during the last sprints. Burgomaster et al. (17) also measured performance during training sessions and found improved PPO, as in the current study. Third, evidence indicates that a resistance of 11.0% (i.e., 0.11 kg/kg of body mass) rather than 8.7% is ideal for measuring PPO in physically active individuals subjected to Wingate tests (39). This limitation regarding the selection of a load that does not induce PPO values was minimized by conducting within-subject analyses. Fourth, the unequal distribution of women between the Sb and PLA groups could also weaken our conclusions. We addressed this issue by normalizing the power output data by the body mass of the participant, thus allowing more precise intergroup comparisons. Moreover, the individual data in Figures 2 and 3 clearly show that the three female participants did not respond differently to SIT than the 12 male participants. Although Medbø and Burges (6) reported greater trainability in men than in women when anaerobic capacity was assessed in

2- to 3-min exhaustive runs, this finding was not reproduced in 30-s exhaustive runs. Considering these equivocal observations, the authors did not draw any conclusion about the different effects of training on men and women (6). Finally, Sb supplementation was initiated two days before the first session of SIT. The fact that supplementation was initiated earlier may not have interfered with performance in the first training session, as a pharmacokinetic study showed that, after repeated oral administration (two capsules per day), Sb achieves steady-state concentrations in human feces within three days (40).

In conclusion, the current findings emphasized the potential benefits of six sessions of SIT on the performance of physically active individuals. Specifically, short-term SIT improved the participants' anaerobic power, as evidenced by increased PPO. This improvement in PPO was more apparent in individuals with worse than better performance levels at training initiation. Regarding probiotic supplementation, the 14-day Sb ingestion did not influence the improved anaerobic power caused by SIT. Therefore, our research provides valuable insights into the

interplay between Sb supplementation, SIT, and anaerobic performance.

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