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Effects of Non-Alcoholic Beer after Running in Three Consecutive Days on Antioxidant Enzyme Activity and Muscle Damage Biomarkers

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Abstract: Running recovery is challenging for several body systems and can be improved by nutritional focus. Non-alcoholic beer is a widely used post-exercise beverage for its antioxidant and energetic properties. After three consecutive days of 1 h submaximal running (80% HRmax), antioxidant enzyme activity (glutathione peroxidase [GPx], glutathione reductase [GR], catalase), lactate dehydrogenase (LDH) activity as a muscle damage blood marker, and lower limb thermographic values were determined in order to observe possible changes in 20 subjects divided into two groups: control (n = 10) and NAB (n = 10). NAB drank 10 mL/kg of non-alcoholic beer post-exercise (both groups drank water ad libitum). Non-alcoholic beer did not show statistically significant changes compared to water. Regarding the effect size, the NAB group had a medium increase in thermography values (15′Post-15′Pre) on days 1 and 2 compared to the control group; a large increase in LDH activity (both 60′Post-0′Post and 60′Post-Pre) on day 2, and a medium increase (60′Post-0′Post) on day 3; a medium decrease in GR (60′Post-Pre) on days 1 and 3; and a large (60′Post-0′Post) and medium (60′Post-Pre) decrease in GPx on day 3. These findings support the idea that non-alcoholic beer is not an appropriate recovery beverage after 1 h running for three consecutive days.

Keywords: antioxidant capacity; lactate dehydrogenase; spectrophotometry; thermography; aerobic exercise; recovery beverage

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1. Introduction

Running is one of the most practiced exercises by healthy people in the world [1,2]. Nevertheless, a high incidence of injuries amongst the sports population of runners has been observed [3–5]. In addition to a correct technique and an appropriate workload, dietary supplementation is a factor that can be used as intervention in order to prevent injuries. For example, the rectus femoris damage level due to exercise was decreased by olive oil supplementation [6], and several indices of aerobic performance were improved thanks to quercetin supplementation [7]. Findings like these led us to think about using other supplements, with a more frequent presence in the diet, that may prevent musculotendinous injuries and reduce their incidence rate.

Within the common components of a diet, one of the most consumed by people, including athletes, is beer [8,9]. For the last few years, studies with contradictory results about its effects, both harmful and beneficial, on exercise performance, thermoregulation, and hydration have been published [10–12]. Its content, based on sodium, potassium, maltodextrins, vitamins (e.g., B, C, E), minerals, and antioxidant molecules, which allow recovery from hydroelectrolytic leak due to exercise and the replacement of used metabolic substrates, could justify its effectiveness as a rehydration beverage [9,11]. The moderate intake of beer as a post-exercise rehydrating supplement, alternative or complementary

to water, has been found in several works [13,14]. In addition, the exercise-produced inflammation after prolonged efforts at high intensity, as well as the respiratory disorder symptoms incidence rate, are both reduced by beer intake, as has been observed [15].

On the other hand, due to the alcohol content, some detrimental effects on performance have been reported in the last decades. Ethanol ingestion increases ATP degradation in the liver and could increase lactic acid blood concentration [16]. Furthermore, impairments in myofibrillar protein synthesis, neuromotor function, and fat distribution due to high doses of alcohol have been published, implying suboptimal training adaptations [17–19]. Regarding rehydration characteristics, alcohol negatively affects fluid balance restoration due to diuretic overstimulation [20,21], although the alcohol percentage in beer seems to influence its rehydrating potential [8,14].

Therefore, the selected drinkable supplement to carry out this study was non-alcoholic beer, which has a similar nutrient composition without the negative effects of alcohol, making it a potentially interesting rehydration drink [22,23]. Non-alcoholic beer has been used as a sports beverage for efforts recovery, even increasing the maximum oxygen consumption (VO_2max) during the post-exercise recovery period, which may have an influence on physical performance [24]. In addition, non-alcoholic beer intake has been shown to prevent exercise damage, probably due to the antioxidant molecules in beer, such as polyphenols (e.g., proanthocyanidins, flavonoids, and quercetin), which could minimize the oxidative stress induced by acute exercise [25–27]. In fact, some antioxidant and anti-inflammatory flavonoid effects have been proven, considering that quercetin is a flavonoid with high antioxidant capacity [28,29]. In relation to exercise, this beverage could act on increasing the amount of free radicals from reactive oxygen species (ROS) overproduction, leading to delayed onset muscle soreness (DOMS) prevention, a faster organism predisposition to tolerate subsequent efforts and recovery improvement [30].

When exercising, some practitioners may suffer experiences of discomfort, which could be caused by musculoskeletal fiber damage related to the eccentric phase of the activity, since the negative work during muscle lengthening structurally affects the contractile protein elements located deep inside [31]. For example, acute downhill hiking may induce DOMS, which in turn is associated with muscle damage and inflammation, affecting in this way the muscle fibers' structure and the excitation–contraction coupling process [32,33]. In this sense, several biochemical markers have been used to assess the muscle tissue damage level considering the increase in their blood concentrations, such as the enzyme lactate dehydrogenase (LDH), which participates in the pyruvate–lactate conversion [34].

The skeletal muscle is a major source of free radicals and ROS during exercise, due to both contractile activity and mitochondrial oxidation, and their effort-related overproduction within muscle fibers could also contribute to a perception of DOMS and a feeling of discomfort [35]. The exercise-mediated inflammatory process in muscle may be one of the responsible factors contributing to oxidative stress due to free radical overproduction, especially when exercise is acute, since macrophages and other phagocytic cells invade the area involved [36]. Thus, it has been speculated that inflammation is a responsible process that leads to DOMS [37].

Infrared thermography is a non-invasive and low-cost assessment technique used in the detection of clinical changes, which can be applied for muscle injury prevention, although this function is not extensively known [38]. A good inter-examiner reliability to detect temperature changes in some body areas and in several sports has been described [39]. Body temperature behavior is considered pathological when differences > 6 °C appear among contralateral areas. This technique also allows the examiner to focus measurements on a concrete body area called the region of interest (ROI) [40]. Additionally, the adaptation degree to exercise intensity may be determined by this method since temperature decreases in anaerobic exercise but increases in aerobic exercise [41]. Thermography's applicability, just like its limitations, has been well reported [42]. In fact, its function has been shown to detect possible injuries in an efficient way by an infrared thermographic camera, which may be used before exercise to reduce the potential risk of muscle damage [43].

Therefore, our aim was to assess the effectiveness of non-alcoholic beer as a rehydrating and recovery supplement post-exercise, based on 1 h submaximal intensity running on several days, by observing possible alterations in the plasma markers of antioxidant capacity (the antioxidant enzyme activity of glutathione peroxidase, glutathione reductase, and catalase) and muscle damage (LDH enzyme activity), as well as changes in the lower limbs' thermographic values as a possible injury risk-predictor.

2. Materials and Methods

2.1. Subjects

Twenty healthy adult male volunteers, Sports Sciences Degree students in Universidad San Jorge (Villanueva de Gállego, Zaragoza, Spain), participated in a controlled trial over three months to assess the effects of non-alcoholic beer as a supplement on rehydration and post-exercise recovery, registering blood markers of antioxidant capacity and muscle damage after exercise, such as quadriceps and gastrocnemius thermography. Each subject reported a regular sport activity for 3 days/week at least, although none of them were runners. They also reported beer (alcoholic and/or non-alcoholic) consumption less than 500 mL/week. They were randomly distributed, by drawing, into two groups, n = 10/group: control (CON) and non-alcoholic beer supplementation (NAB).

The NAB group was instructed to drink 10 mL/kg alcohol-free beer (Ambar, Zaragoza, Spain) immediately after exercise. CON and NAB subjects could drink mineral water (Fontecabras, Zaragoza, Spain) ad libitum. Non-alcoholic beer ingredients were water, barley malt, rice, dietary fiber, hops, and scents, having a nutritional content as follows (per 100 mL): 18 kcal energy value, 0 g fats, 4 g carbohydrates (0 g sucrose), 0 g proteins, 1 g dietary fiber, and 0 g salt. In addition, water composition was 117 mg/L sulfates, 295 mg/L bicarbonates, 49.5 mg/L chlorides, 90.1 mg/L calcium, 37.5 mg/L magnesium, and 32.6 mg/L sodium.

Every subject was informed orally and in writing by a participant information sheet and signed an informed consent by hand prior to his participation in this study. All personal data were recorded in a database specifically designed for the study and pseudo-anonymized. Age, weight, height, body mass index (BMI), and 1 h run distance were recorded with regard to descriptive statistics.

This study was conducted according to the Declaration of Helsinki principles (2008) and following the note for guidance on Good Clinical Practice in the European Union, CPMP/ICH/135/95. The intervention began after approval by the appropriate regional ethical committee: Comité de Ética de la Investigación de la Comunidad Autónoma de Aragón (CEICA), CP08/2014 (CP-CI-IP13/0175), according to valid legal regulations in Spain (RD 1090/2015).

2.2. Experimental Protocol

All participants started with a standardized warm-up and then carried out a 1 h running session for three consecutive days, at 80% maximum heart rate (maxHR) as submaximal intensity, which was calculated by the Karvonen method [44]. Heart rate (HR) was supervised by a Polar Heart Rate Monitor RS300X (Polar Electro Ibérica, Barcelona, Spain). Exercise protocol intensity was controlled by one researcher. As a running track, a grass soccer field perimeter was used. After 30 min running in one direction, participants were instructed to carry on in the opposite direction up to 1 h, registering reached distance. Blood samples, 10 mL antecubital vein-removed blood samples, were obtained in three stages: just before exercise (Pre); immediately after exercise although before rehydration (0′Post); 1 h after exercise (60′Post). Thermography images were taken as described below.

2.3. Thermography

Infrared thermography camera shots were used to observe muscle changes. Thermography was assessed by a FLIR Thermacam E60 infrared camera (FLIR Systems, Wilsonville, OR, USA). On each running day, images were taken 15 min before (15′Pre) and 15 min after

exercise (15′Post), according to the American Academy of Thermography guidelines [45]. Frontal plane images were taken from anterior and posterior views with both lower limbs visible on screen, and 2.5 m camera–participant distance. Quadriceps and gastrocnemius ROI temperature means were selected and analyzed with FLIRTools-Software (device software pack). Thermographic evaluation was carried out in a room at 21 °C steady temperature with 60% humidity, without metallic equipment to avoid thermographic image distortions. All subjects stood in their underwear on a step to avoid direct contact with the floor. A skin emissivity of 0.98 was assumed [46].

2.4. Plasma Samples Procedure

The antioxidant activity of catalase (Cat), glutathione peroxidase (GPx), and glutathione reductase (GR) enzymes were used to determine plasma antioxidant capacity [47]. Muscle damage was evaluated by plasma LDH enzyme activity [46]. Just after drawing them, blood samples were centrifuged at 1000× *g* for 10 min in a Biofuge Primo R refrigerated centrifuge (Thermo Scientific, Waltham, MA, USA). The 250 µL plasma aliquots were stored at −20 °C until LDH and antioxidant enzyme measurements were taken.

2.5. Antioxidant Capacity

Cat, GPx, and GR enzyme activity was determined with a spectrophotometric commercial kit (Cayman Chemical, Ann Arbor, MI, USA) to evaluate plasma antioxidant capacity [36]. Enzyme activity was expressed as enzyme units (U), which represent the enzyme amount required to catalyze the conversion of 1 substrate-µmol/min.

2.6. Skeletal Muscle Damage

Blood LDH activity was determined to assess the mechanical damage level on the skeletal muscle fibers. LDH activity was measured by spectrophotometry with a DGKC Kinetic UV kit (Spinreact, Barcelona, Spain) [48]. This method is based on LDH catalysis on pyruvate reduction by nicotinamide adenine dinucleotide + hydrogen (NADH). In a sample which is measured photometrically, the speed of the NADH concentration decrease is proportional to the LDH catalytic concentration.

2.7. Statistical Analysis

Statistical processing was performed using SPSS version 28.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics corresponding to participants (age, weight, height, BMI, and 1 h run distance) were presented as mean ± standard deviation (SD). The datasets' normality was checked with the Shapiro–Wilk test.

Values differences obtained in the "60′Post-Pre" and "60′Post-0′Post" time intervals were calculated to study possible changes in Cat, GPx, GR, and LDH for 1 h after the non-alcoholic beer supplementation moment. The thermographic variable differences in quadriceps (ThQ) and gastrocnemius (ThG) were calculated in "15′Post-15′Pre" time intervals. Regarding these data, means comparison tests for independent samples were performed (Student's *t* test for normal distributed variables and Mann–Whitney U test for non-normal distributed variables) for the 3 running days of the NAB and CON groups. These tests were conducted with a significance level $p < 0.05$.

To assess possible changes in the variables of this study over time depending on the group, a two-way repeated measures ANOVA test was performed (time: day 1, day 2, and day 3; and group: NAB vs. CON). Those changes that reached a significance value $p < 0.05$ and a statistical power 1-SS > 0.80 were considered as significant. The η^2 value was also reported to assess the effect size in this test.

In these comparisons, Cohen's d effect size with 95% confidence interval (ES, 95% CI) was calculated to study possible differences, clinically relevant but statistically not significant, establishing d = 0.20 as the minimum relevant clinical effect. The CI results without 0 (zero) value included were considered for discussion; i.e., <5% probability for

null/trivial ES. The magnitude of ES was considered small ($0.2 \leq |d| < 0.5$), medium ($0.5 \leq |d| < 0.8$), or large ($0.8 \leq |d|$) [49].

Regarding clinical effect, negative results indicated a harmful effect (beneficial effect when positive) in Cat, GPx, and GR; whereas negative results indicated a beneficial effect (harmful effect when positive) in LDH, ThQ, and ThG. For these calculations, Hopkins spreadsheets for independent samples comparisons were used [50].

3. Results

A total of 20 subjects participated in this study. Their main characteristics (anthropometric parameters and 1 h run distance) are shown in Table 1. No intergroup significant differences were observed ($p > 0.05$), neither in the anthropometry values nor in the run distance for 1 h.

Furthermore, there were no intragroup performance differences among the three running days, independent of supplementation post-exercise (=NAB) or without supplementation (=CON).

Table 1. Anthropometric characteristics and run distance. CON, n = 10; NAB, n = 10. Data are expressed as mean ± SD.

Variable		CON	NAB
Age (years old)		23.40 ± 4.79	21.30 ± 3.27
Body mass (kg)		75.75 ± 10.09	68.47 ± 6.13
Height (cm)		179.50 ± 7.68	178.20 ± 7.60
BMI (kg/m^2)		23.57 ± 3.38	21.55 ± 1.21
Run distance (m)	Day 1	10,035.61 ± 2099.24	8620.70 ± 1332.68
	Day 2	10,218.70 ± 1998.32	8968.80 ± 1563.72
	Day 3	10,383.70 ± 1855.97	9382.26 ± 1535.27

CON = control group; NAB = non-alcoholic beer group; SD = standard deviation; BMI = body mass index.

None of the variables has a significance value $p < 0.05$, a statistical power 1-SS > 0.45, or an effect size $\eta^2 > 0.25$ for the repeated measures ANOVA test. Therefore, it could be concluded that there are no changes in the variables in relation to the three moments of evaluation or to the interaction between the moment of evaluation and the group of belonging.

The effects analysis of non-alcoholic beer as a rehydrating beverage immediately post-exercise (NAB group), in comparison to water normal use (CON group), after 1 h submaximal intensity running for three days, is reflected in the following tables: first day results in Table 2; second day results in Table 3; third day results in Table 4. The study variables on each variable are as follows: plasma antioxidant enzyme activity (GPx, GR, Cat) (U), plasma LDH enzyme activity (U), lower limb thermographic register (quadriceps, gastrocnemius) (°C).

As can be seen, no intragroup significant difference (60′Post-Pre interval vs. 60′Post-0′Post interval) was produced, neither in CON nor in NAB. There was also no intergroup significant difference (CON vs. NAB), neither in the 60′Post-Pre interval nor in the 60′Post-0′Post interval. This was observed in the three running days. Therefore, based on these results, to drink non-alcoholic beer did not imply statistically significant changes regarding the study variables.

Considering this, clinical relevance by ES was then investigated, comparing the NAB group in relation to the CON group and establishing a 95% IC, with 0.20 being the minimum relevant value. This threshold value was set to negative for antioxidant enzymes (so that if there is a positive ES, the result = beneficial), and was set to positive for the muscle damage (LDH) and muscle injury risk (thermography) indicators (so that if there is a positive ES, the result = harmful).

Table 2. Values variation of plasma enzyme activity and lower limb thermography after non-alcoholic beer supplementation on the first running day. CON, n = 10; NAB, n = 10. Data in CON and NAB are expressed as mean ± SD.

Interval Time Comparison	Variable	Threshold Value	Day 1		
			CON (Mean ± SD)	NAB (Mean ± SD)	ES (CI 95%)
60′Post-0′Post	GPx (U)	−0.20	71.52 ± 698.25	3.72 ± 754.20	−0.09 (−0.99; 0.81)
	GR (U)	−0.20	−19.56 ± 633.77	−88.84 ± 240.35	−0.10 (−0.78; 0.58)
	Cat (U)	−0.20	0.47 ± 12.18	−1.38 ± 5.72	−0.14 (−0.91; 0.64)
	LDH (U)	0.20	5.67 ± 66.20	8.91 ± 50.72	0.04 (−0.73; 0.82)
60′Post-Pre	GPx (U)	−0.20	−1.94 ± 727.11	−66.17 ± 748.06	−0.08 (−0.96; 0.79)
	GR (U)	−0.20	56.89 ± 342.68	−143.75 ± 574.94	−0.54 (−1.75; 0.68)
	Cat (U)	−0.20	3.59 ± 11.94	2.20 ± 8.14	−0.10 (−1.02; 0.82)
	LDH (U)	0.20	19.70 ± 83.42	36.16 ± 53.11	0.18 (−0.55; 0.91)
15′Post-15′Pre	ThQ (°C)	0.20	0.37 ± 0.66	0.69 ± 1.12	0.44 (−1.03; 1.91)
	ThG (°C)	0.20	0.41 ± 0.79	1.00 ± 0.77	0.68 (−0.29; 1.65)

CON = control group; NAB = non-alcoholic beer group; SD = standard deviation; ES = effect size; CI = confidence interval; GPx = glutathione peroxidase; GR = glutathione reductase; Cat = catalase; LDH = lactate dehydrogenase; ThQ = thermography in quadriceps; ThG = thermography in gastrocnemius.

Paying attention to the ES in Table 2, there was only a single result more relevant than the rest: non-alcoholic beer intake turned out to be likely harmful in thermography (ThG) vs. CON, since d = 0.68 is a positive medium ES, closer to a large ES than a small ES. Regarding the rest of the data on the first day, except for GR in 60′Post-Pre (d = −0.54, which suggests possibly harmful), the others were clinically irrelevant.

On the second running day (Table 3), the clinically relevant results appeared unequivocally in the LDH activity, since when comparing the difference in NAB vs. CON results, both just before supplementation (60′Post-0′Post) and before running (60′Post-Pre), the ES was large in both cases (d = 0.80 and d = 1.59, respectively), not including the 0 value within its IC. Qualitatively, this could be interpreted as most likely harmful because of the positive ES for LDH.

Table 3. Values variation of plasma enzyme activity and lower limb thermography after non-alcoholic beer supplementation on the second running day. CON, n = 10; NAB, n = 10. * Large ES, '0' value is not included in CI.

Interval Time Comparison	Variable	Threshold Value	Day 2		
			CON (Mean ± SD)	NAB (Mean ± SD)	ES (CI 95%)
60′Post-0′Post	GPx (U)	−0.20	150.49 ± 400.30	−37.03 ± 662.86	−0.42 (−1.62; 0.77)
	GR (U)	−0.20	−182.87 ± 311.50	−42.79 ± 489.40	0.41 (−0.75; 1.56)
	Cat (U)	−0.20	4.54 ± 13.78	−2.18 ± 6.66	−0.44 (−1.17; 0.29)
	LDH (U)	0.20	−28.18 ± 44.97	11.33 ± 33.65	0.80 (0.01; 1.59) *
60′Post-Pre	GPx (U)	−0.20	32.26 ± 417.05	−124.95 ± 463.97	−0.34 (−1.27; 0.59)
	GR (U)	−0.20	−37.52 ± 677.05	−116.70 ± 856.56	−0.11 (−1.10; 0.89)
	Cat (U)	−0.20	7.38 ± 15.86	−0.16 ± 6.41	−0.43 (−1.21; 0.36)
	LDH (U)	0.20	−51.87 ± 40.49	19.43 ± 48.68	1.59 (0.62; 2.56) *
15′Post-15′Pre	ThQ (°C)	0.20	0.76 ± 0.42	1.09 ± 0.49	0.72 (−0.39; 1.82)
	ThG (°C)	0.20	0.37 ± 0.50	0.44 ± 0.71	0.12 (−1.14; 1.39)

CON = control group; NAB = non-alcoholic beer group; SD = standard deviation; ES = effect size; CI = confidence interval; GPx = glutathione peroxidase; GR = glutathione reductase; Cat = catalase; LDH = lactate dehydrogenase; ThQ = thermography in quadriceps; ThG = thermography in gastrocnemius.

Furthermore, this was accompanied by other medium ES values in thermography, closer to large than to small (as in Table 2), since in ThQ, NAB vs. CON d = 0.72 (suggesting likely harmful).

In the rest of results, the ES was small or trivial, without clinical relevance.

On the third and last running day (Table 4), the only clinically relevant result was in the GPx antioxidant enzyme, since when comparing the non-alcoholic beer supplementation effect 1 h after its intake (60′Post-0′Post) in this enzyme activity relative to the CON group, the ES was large (d = −1.02). Considering the ES negative sign and that the IC was quite far from '0′, this suggests that non-alcoholic beer intake could be very likely harmful as regards its clinical interpretation.

Table 4. Values variation of plasma enzyme activity and lower limb thermography after non-alcoholic beer supplementation on the third running day. CON, n = 10; NAB, n = 10. * Large ES, '0′ value is not included in CI.

Interval Comparison	Variable	Threshold Value	Day 3		
			CON (Mean ± SD)	NAB (Mean ± SD)	ES (CI 95%)
60′Post-0′Post	GPx (U)	−0.20	179.47 ± 615.45	−515.57 ± 462.73	−1.02 (−1.87; −0.17) *
	GR (U)	−0.20	−311.18 ± 1406.37	−1218.37 ± 1639.07	−0.58 (−1.68; 0.52)
	Cat (U)	−0.20	3.45 ± 12.19	2.08 ± 13.44	−0.10 (−1.15; 0.95)
	LDH (U)	0.20	−23.39 ± 56.78	13.49 ± 78.70	0.59 (−0.66; 1.84)
60′Post-Pre	GPx (U)	−0.20	172.91 ± 1029.69	−515.57 ± 462.73	−0.60 (−1.35; 0.14)
	GR (U)	−0.20	277.22 ± 2061.09	−83.61 ± 2221.46	−0.16 (−1.19; 0.88)
	Cat (U)	−0.20	2.40 ± 15.90	4.54 ± 11.01	0.12 (−0.71; 0.95)
	LDH (U)	0.20	−8.69 ± 72.42	−2.31 ± 62.67	0.08 (−0.83; 0.99)
15′Post-15′Pre	ThQ (°C)	0.20	0.69 ± 0.83	0.46 ± 0.65	−0.26 (−1.13; 0.61)
	ThG (°C)	0.20	0.23 ± 0.70	0.46 ± 0.70	0.30 (−0.69; 1.28)

CON = control group; NAB = non-alcoholic beer group; SD = standard deviation; ES = effect size; CI = confidence interval; GPx = glutathione peroxidase; GR = glutathione reductase; Cat = catalase; LDH = lactate dehydrogenase; ThQ = thermography in quadriceps; ThG = thermography in gastrocnemius.

This is also strengthened by the other interval results (60′Post-Pre), where for the same GPx enzyme, the ES was medium and negative (d = −0.60), with one of the IC limits quite close to '0′ (0.14).

Other results with a medium ES in the 60′Post-0′Post difference were observed in the GR antioxidant enzyme (d = −0.58, according to Table 2) and in the LDH enzyme (d = 0.59, as in Table 3), suggesting a harmful action probability with NAB vs. CON.

The rest of the results showed a small ES (e.g., ROI temperature means by thermographic assessment) or a clinically irrelevant ES for all other enzymatic activities.

4. Discussion

The aim of this study was to assess the non-alcoholic beer effectiveness as a recovery supplement after 1 h submaximal exercise on several days, observing alterations in plasma antioxidant enzyme activity (GPx, GR, Cat), and in muscle damage levels (LDH enzyme activity), as well as changes in lower limb thermographic values.

One of the main findings in this research is that non-alcoholic beer does not show statistically significant changes in the study variables compared to water as a rehydrating beverage. Furthermore, regarding the ES, the NAB group has a medium increase in thermography values (15′Post-15′Pre) on days 1 (ThG) and 2 (ThQ) compared to CON; a large increase in LDH activity (both 60′Post-0′Post and 60′Post-Pre) on day 2, and a medium increase (60′Post-0′Post) on day 3; a medium decrease in GR (60′Post-Pre) on days 1 and 3; and a large (60′Post-0′Post) and medium (60′Post-Pre) decrease in GPx on day 3. Even with the rehydration properties of non-alcoholic beer, which are sometimes better than water [23], the findings presented here support the idea that it is not an appropriate recovery beverage after 1 h running for three consecutive days.

Acute changes in thermography after exercise are previously studied, but not after running exercises or in consecutive training days. After an isometric and auxotonic training (4 × 10 repetitions of 70% maximal isometric squat strength), skin temperature decreased

and recovered baseline values within 15 min of completion [51], similarly to the data obtained in the three days of our study in the control group. Non-alcoholic beer consumption maintained a skin temperature above basal level after 15′ on days 1 and 2. After plyometric training, the skin temperature was elevated after 48 and 72 h, suggesting a possible relation with markers of muscle damage [52]. It is possible that non-alcoholic beer contributes to an increase in muscle damage instead of preventing it by blunting normal muscle response after running, as a non-potentially muscle-damaging exercise. This affirmation is supported by the observed increase in the LDH activity in the blood, which is a marker of cell damage [34]. After a marathon run, this enzyme activity could be doubled, and remain high after two weeks [53]. As shown in the results section (Tables 3 and 4), the LDH activity was higher, which was not expected because submaximal exercises do not appear to increase it [54,55].

Furthermore, antioxidant enzymes are described as increasing their plasma activity after submaximal efforts. In a study of 30 min at 70% of maximal work capacity, GR and GPx activities were statistically higher after the exercise [45]. In a recent study, running at 75% of VO_2max for 25 min resulted in the GPx activity remaining unchanged [56], but there are no studies, to the best of our knowledge, where exercise induces a decrease in antioxidant enzyme activity in the blood. Observing the exercise effect in blood cells, a single bout of exercise (75–80% of maximal capacity) induced a decrease in GPx activity in human leukocytes [57]. On the other hand, exercise induced a GR activity increase in red blood cells after a long ride cycling [58]. Previous studies showed an increase in GPx and GR activities in the blood, but there are unclear results when blood cells are analyzed. The present study shows different responses in the CON and NAB groups. The CON results are similar to those previously described in other studies, while non-alcoholic beer decreases the GPx and GR activities in plasma. Related to the glutathione system, selenium may play a role. Selenium content in beer has been considered to improve the glutathione antioxidant system, although there is not clear evidence. Some studies show no effects [59], or small increases in GPx or GR activities [60]. In this study, NAB has shown a decrease in both GPx and GR enzyme activity, maybe because selenium levels are too low due to the brewing process [61]. The CO_2 present in NAB may have some effect on antioxidant activities as shown in a recent study [62]. The authors found slight decreases in CAT activity due to the ingestion of soda in resting rats, but it is not clear its effect after exercise or in humans.

Finally, it is necessary to expose the limitations of this study. First of all, the sample size is small and composed only of young men, and that makes the results not widely applicable. More studies with males and females of different ages, and more numerous subjects, will make the study more consistent. In these future studies, it will be interesting to assess the hydration levels of both groups, to compare the hydration potential of both drinks, which is also a limitation of the present study.

5. Conclusions

Non-alcoholic beer has not shown benefits when used as a recovery drink compared to water in three consecutive days of 1 h running in amateur athletes. Furthermore, it has shown some detrimental effects in antioxidant enzyme activities (GR and GPx) and in muscle damage (as an increase in blood LDH activity).

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