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Effects of a Fruit and Vegetable Sports Drink on Hydration and Oxidative Stress Recovery of Brazilian Professional Athletes

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Abstract

This pilot project aimed to investigate the hydration and recovery from oxidative stress in athletes from different sports, after hydration with water or with a natural sports drink made of fruit and vegetables, in addition to the supplementation effects caused by this drink, using the Electro Sensor Complex. The consumption of the natural sports drink provided all the athletes with better hydration levels when compared to hydration with water. To athletics practitioners, body mass loss (BML) was significantly lower after the consumption of the natural sports drink, ranging from -1.1 ± 0.4 (%) after supplementation and -1.77 ± 0.5 (%), without supplementation. The effects on the recovery from oxidative stress showed a significant difference in interstitial free-radical levels and cortisol for athletics practitioners as a result of the consumption of the natural sports drink. The consumption of the natural sports drink resulted in better responses regarding hydration and oxidative stress recovery of the athletes when compared to water intake.

Keywords: Bioactive compounds; Dehydration; Supplement; Oxidative Stress

Introduction

A healthy hydration status for athletes is the condition of healthy individuals who maintain their water balance at a relatively consistent level across exercise sessions, while a dehydration state, caused by a decrease of a 1% to 2% of body weight may cause poor performance and represents a risk to health. However, long before a risk to one's health is posed, dehydration can impair athletic performance [1,2].

Research examining the efficacy of sports beverages indicates that their intake results in better fluid balance restoration, following exercise in comparison to water [3]. For this reason, various products have been developed to, purportedly, enhance athletic performance to a greater extent than water alone [4-6].

Another recurring concern among athletes is the increased production of reactive oxygen species (ROS) induced by exercise [7], which can have adverse effects on performance and muscle recovery.

It is well known that excessive physical activity produces excess levels of ROS, especially in professional athletes after aerobic or anaerobic strenuous exercise, increasing oxidative stress biomarkers in blood and tissues [7-9]. Additionally, sex, age, and food intake directly affect ROS production and the balance between ROS and the antioxidant system capacity to preserve or restore homeostasis [10-13].

To ultra-endurance athletes, oxidative damage may persist for 1-month post-exercise, depending on the biomarker assessed. For these, it has been reported that reduced glutathione (GSH) levels remain depleted in blood after 28 days post- race [14]. Furthermore, the increase in the levels of inflammatory cytokines (IL-6), lactate production, associated with a decrease of both antioxidant capacity and renal function has also been observed [8].

The Electro Sensor Complex (ESC) has been used over the past decade as a method to assess, through sensors placed on the skin, biochemical data and body composition measures, providing physiological data. It is a fast (less than 3 minutes), cost-effective and non-invasive method widely used in medical practice around the world. It is a method approved by the Food and Drug Administration (FDA-US) and the National Health Surveillance Agency (Anvisa-Brazil) [15-17].

The consumption of sports drinks has become widespread both for recreational and professional athletes because of their proposed energetic effects, and their ability to replenish electrolytes and prevent dehydration [18]. In this context, a natural sports drink may be an effective way to promote adequate water intake, electrolyte replacement and the additional benefit of providing dietary antioxidants whose positive effect on physical activity after recovery is still unclear and divides opinions in literature [19,20].

Thus, the objective of this pilot study was to evaluate the effect of a natural sports drink, made from whole fruit and vegetables (edible and non-edible parts), in hydration and oxidative stress markers, using a non-invasive method of detection in Brazilian jiu-jitsu and athletics sportsmen.

Materials and Methods

Beverage preparation

The following species of fruits were used: Sweet orange (*Citrus sinensis*), passion fruit (*Passiflora edulis*) and watermelon (*Citrullus lanatus*). The following species of vegetables were used: lettuce (*Lactuca sativa*), courgette (*Cucurbita pepo*), carrot (*Daucus carota*), spinach (*Spinacea oleracea*), mint (*Mentha* s.p.), taro (*Colocasia esculenta*), cucumber (*Cucumis sativus*) and rocket (*Eruca sativa*) [4].

All vegetable species were purchased in a local market in the city of Rio de Janeiro (Brazil), in January 2015.

The concentrated juice was prepared, as previously reported [4], using the whole fruits and vegetables, and kept at -18°C in plastic bags after pasteurization (60°C for 60 min). The final formulation of concentrated natural sports drink is described in table 1.

Finally, to prepare the natural sports drink (NSD) water was added using the proportion 1:2 of frozen concentrated juice, followed by the addition of natural green colorant (Chlorophyll), mint flavoring, xanthan gum (0.3%, w/w) and sodium citrate (0.1%, w/w) as previously reported [21].

The upper limit of carbohydrate concentration was established at around 8% (w/v) and the interval of sodium content 460–1,150 mg L^{-1} based on the suitable concentration for isotonic drinks [22].

Participants

A randomized pilot study was conducted on six healthy, non-smoking jiu-jitsu (n=3) and athletics practitioners (n=3) in Rio de Janeiro, Brazil, according to the guidelines laid down in the Declaration of Helsinki. All procedures were approved by the Ethics Committee of the Federal University of the State of Rio de Janeiro (CAS number: 0009.0.313.00008). All athletes were training for upcoming competitions when the experiment was performed and had not used supplements with antioxidants in the six months prior the intervention. Before the trials, all participants were informed about the aims of the study, potential risks and benefits, and how the intervention would be carried out. All participants signed an authorization form.

Procedure

To ensure a correct evaluation of the hydration status, the athletes received no orientation on pre-exercise hydration. At all steps, the athletes performed 60 minutes of aerobic exercises, with brief moments of anaerobic activity specific to their sports, considering competition preparation. There was no intervention into how the activities were usually carried out. Thus, intervention into the hydration of athletes was performed considering only the actual needs of each athlete for each sport during the training session. Immediately before and after each training, they were analyzed using the Electro Sensor Complex [15]. The interval between sessions was 5 to 7 days.

To supplementation assess, each athlete received two individual doses of the NSD (200 ml=1 dose) and was instructed to consume one in the morning and the other one in the evening for 5 days before trials.

Protocols: Control Assay (A): During exercise, they consumed water, freely, whenever they felt thirsty.

Hydration with an isotonic drink (NSD) without supplementation (B): During exercise, they consumed 3 $ml.kg^{-1}$ of isotonic drink every 15 minutes.

Vegetables	Amount ($g.L^{-1}$)	Fruits	Amount ($g.L^{-1}$)
Mint	20	Orange	110
Rocket	20	Passion fruit	190
Lettuce	55	Watermelon	220
Spinach	55		
Taro	55		
Cucumber	85		
Courgette	85		
Carrot	130		

Table 1: Final concentration of fruits and vegetables in concentrated natural sports drink.

Amounts defined after preliminary testes with different proportions of fruits and vegetables and sensory analysis.

(C): Hydration with water after 5 days of supplementation with the sports drink (NSD). During exercise, they consumed 3 $ml.kg^{-1}$ of water every 15 minutes.

(D): Hydration with an isotonic drink (NSD) after 5 days of supplementation with the same sports drink. During exercise, they consumed 3 $ml.kg^{-1}$ of isotonic drink every 15 minutes.

All measures were obtained using the Electro Sensor Complex to assess: (1) body composition: Body mass index (BMI), fat body mass (FBM), lean body mass (LBM), total body water (TBW), intracellular body water (IBW), extracellular body water (EBW) using ES-BC (Electro Sensor-Body Composition); (2) biochemistry parameters: electrolytes – sodium (Na); potassium (K), chloride (Cl), phosphorus (P), magnesium (Mg) and free ions of calcium (Ca); Oxidative stress biomarkers - Nitric oxide (NO·), Superoxide (O_2-·), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH-), peroxynitrite (ONOO-) ; Leptin, lactic acid and cortisol using ES-GS (Electro Interstitial Scan-Galvanic Skin); (3) Systemic Vascular Resistance (SVR), stroke volume (SV), cardiac output (CO), blood volume (BV), Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) using EIS (Electro Interstitial Scan) [16].

The percentage changes were calculated according to the formula $\{[(B \times 100) \div A] - 100\}$ where, A was obtained before practice and B after practice.

Statistical analysis

The statistical program ASSISTAT 7.7 beta was used for the statistical analyses in addition to the ANOVA (Tukey's test). P<0.05 was considered significant in all analyses.

Results and Discussion

Body composition and hydration status

In the present study, we initially evaluated the body composition of all athletes. All measures were taken before and after a regular practice day, before the competition. Therefore, the average age and height (means ± SD) of athletics and jiu-jitsu sportsmen were 22.0 ± 3.41, 38.33 ± 2.14 (years) and 1.66 ± 0.05, 1.77 ± 0.08 (m), respectively. Their body characteristics (mean ± SD) can be found in table 2. All athletes were considered eutrophic according to their BMI, and the average total body water content was 60.04 ± 3.95 and 67.68 ± 1.09 (%) for jiu-jitsu and athletic practitioners, respectively, indicating that they were all normally hydrated before the trials, and this status was maintained across all protocols.

Percentage of body mass (BM) loss in the control assay was -2.3 ± 0.6 for athletics practitioners, while no BM loss was observed in jiu-jitsu athletes. Although there was no difference in body composition among protocols, a significantly smaller body weight loss when rehydrated with the NSD (B) and supplemented with the NSD followed by hydration with water (C) and the NSD (D) was observed for athletics practitioners, as in table 3.

Regarding jiu-jitsu athletes, only supplementation (C and D) ensured rehydration, although in this sport the athletes seem to present a lower loss in body water through sweat when compared to athletics.

During practice, jiu-jitsu athletes showed a higher loss of electrolytes, which hydration with water was not able to replace, as shown in table 4. It was found that rehydration with the NSD (B) promoted a positive percentage change for all electrolytes, except for phosphorus (-7.14 ± 1.23; %), ensuring an adequate electrolyte replacement. The effects of supplementation were also observed, where chlorine was the exception (-18.88 ± 2.0; %) in that it presented a higher loss after supplementation (D) than the one seen in rehydration with water after supplementation.

	Jiu-jitsu (n=3)									
	Pre-exercise		A		B		C		D	
		CV(%)		CV(%)		CV(%)		CV(%)		CV(%)
Weight (Kg)	80.31 ± 10.21	12.72	80.24 ± 12.42	15.48	80.03 ± 11.50	14.38	80.9 ± 12.75	15.77	80.03 ± 11.66	6.56
BMI[1] (Kg/m²)	25.49 ± 1.47	5.78	25.46 ± 1.70	6.69	25.43 ± 1.56	6.14	25.7 ± 2.03	7.90	25.43 ± 1.69	14.58
FBM[2] (%)	17.96 ± 5.40	30.09	18.33 ± 5.70	31.14	18.3 ± 5.58	30.53	18.66 ± 4.89	26.22	18.3 ± 4.34	29.16
LBM[3] (%)	82.03 ± 5.40	6.59	81.66 ± 5.70	6.99	81.7 ± 5.58	6.83	81.33 ± 4.89	6.02	81.6 ± 4.34	6.54
TBW[4] (%)	60.04 ± 3.95	6.59	59.76 ± 4.19	7.02	59.8 ± 4.10	6.87	59.53 ± 3.57	6.00	59.8 ± 3.92	6.56
EBW[5] (%)	40.0 ± 3.24	8.12	40.66 ± 2.30	5.68	39.9 ± 2.64	6.78	38.66 ± 1.15	2.99	41.0 ± 2.64	6.45
IBW[6] (%)	60.0 ± 3.24	5.41	59.33 ± 2.30	3.90	61.0 ± 2.64	4.33	61.33 ± 1.15	1.88	59.0 ± 2.64	4.48
	Athletics (n=3)									
	Pre-exercise		A		B		C		D	
		CV(%)		CV(%)		CV(%)		CV(%)		CV(%)
Weight (Kg)	59.63 ± 5.83	9.78	57.9 ± 6.39	11.05	58.2 ± 6.58	11.31	58.93 ± 6.70	11.37	59.43 ± 6.84	11.51
BMI[1] (Kg/m²)	21.44 ± 1.07	4.70	20.96 ± 1.09	5.23	21.06 ± 1.18	5.62	20.77 ± 0.96	4.63	21.46 ± 1.18	5.52
FBM[2] (%)	7.54 ± 1.52	19.07	8.56 ± 0.75	8.76	8.33 ± 0.58	7.03	7.8 ± 1.37	17.63	6.16 ± 2.33	37.87
LBM[3] (%)	92.45 ± 1.52	1.55	91.43 ± 0.75	0.82	91.66 ± 0.58	0.64	92.2 ± 1.37	1.49	93.83 ± 2.33	2.49
TBW[4] (%)	67.68 ± 1.09	1.53	66.93 ± 0.57	0.86	67.1 ± 0.43	0.65	67.5 ± 1.01	1.50	68.66 ± 3.78	2.51
EBW[5] (%)	44 ± 1.39	4.78	44.33 ± 3.78	8.54	43.0 ± 1.73	4.03	42.33 ± 2.52	5.94	45.66 ± 3.78	8.29
IBW[6] (%)	56 ± 1.39	3.73	55.66 ± 3.78	6.80	57.0 ± 1.73	3.04	57.66 ± 2.52	4.36	54.33 ± 3.78	6.97

Table 2: Athletes body characterization pre-exercise and after hydration with different protocols.

[1]Body Mass Index; [2]Fat body mass; [3]Lean body mass; [4]Total body water; [5]Extracellular body water; [6]Intracellular body water.
Mean ± SD obtained from triplicate. No statistically significant difference was observed ($p<0.05$). A, B, C and D are the means obtained after each hydration protocol.

	Athletics (n=3)	Jiu-Jitsu (n=3)
A	-2.3 ± 0.6 a	0.00 ± 0.00 c
B	-1.77 ± 0.5 b	-0.031 ± 0.24 d
C	-1.57 ± 0.5 b	0.23 ± 0.13 a
D	-1.1 ± 0.4 c	0.19 ± 0.09 b

Table 3: Weight percentage changes in athletes, following different hydration protocols.

Different letters in the same column mean significant difference ($p<0.05$). Mean ± SD obtained from triplicate.

For athletics practitioners, rehydration with the NSD (B) also seems to have potentially positive effects, since it was able to replace all electrolytes. Supplementation with the NSD (D) promoted an increase in the interstitial sodium content of 32.5 ± 3.03 (%), and an increased loss of potassium, magnesium, and phosphorus of -21.59 ± 1.93 (%) each.

All kinds of exercise cause an increased rate of metabolic heat production so, body temperature, especially muscle temperature, will rise during intense exercise if this heat is not dissipated fast enough [2]. Thus, to ensure an adequate body temperature during exercise, proper hydration strategies must be developed for each athlete, considering their requirements for water and nutrients [23]. This can be observed by the different levels of water body losses in jiu-jitsu athletes and athletics practitioners and their responses to each hydration protocol.

It has been reported that severe hyperthermia may be more common in high-intensity, short duration exercise, when thermal equilibrium is less likely to be achieved, especially in hot humid environments where water body loss is increased. In this study, however, all athletes were euhydrated and their body water losses during exercise were not characteristic of dehydration. Likewise, it is important to know the initial hydration status of these athletes, given the possibility that a fluid deficit incurred before exercise can increase physiological strain and reduce performance, although this effect has not been reported in all studies of the area [24].

In this study, the athletics practitioners present an average dehydration of -2.3% of body weight, after hydration with water (A), and smaller losses in the following protocols. A similar body weight loss (>2%) has been reported as enough to compromise physiologic function and negatively influence performance [25]. It has also been reported, however, that a body mass reduction of more than 2% has no significant effect on sprint performance, suggesting that sprinting would be "easier" with a lower body mass [2]. Despite this, to prevent excessive dehydration (>2% body weight loss), it is also necessary to minimize electrolyte imbalance [26], as well as strength and power losses that seem to be related to alterations in total body water. These alterations affect certain aspects, which are yet to be determined, of strength generation, which in turn may cause an adverse effect on performance [24].

Biochemical markers

As shown in table 5, jiu-jitsu athletes presented an increase in lactic acid when hydrated with water (20. 0 ± 1.73; %) and the NSD (30.0 ± 0). A decrease was observed only after supplementation (C and D). This differed from the behavior observed in athletics practitioners, when a better response was observed only after supplementation (D)-this was not, however, better than rehydration with water (A)-with a reduction in lactic acid of -9.67 ± 1.73 (%).

	Jiu-jitsu (n=3)				
	Pre-exercise	A	B	C	D
SVR1 (SRU)*	1323.50± 179.73a	1120.10± 116.06d	1197.10± 86.28b	1105.53± 139.76e	1130.86± 19.33c
SV2 (ml/beat)	89.16 ± 18.64a	77.36 ± 17.50c	69.53 ± 7.00e	72.7 ± 15.05d	78.9 ± 15.95b
CO3 (L/min)	6.13 ± 1.00d	6.80 ± 1.08bc	6.9 ± 0.75ab	6.96 ± 0.91a	6.73 ± 0.46c
BV4 (L)	5.065 ± 0.56d	5.39 ± 0.73a	5.30 ± 0.63b	5.29 ± 0.61b	5.26 ± 0.58c
SBP5 (mmHg)	138.25 ± 11.78b	128.33 ± 8.50e	142.33 ± 17.03a	131.0 ± 5.29d	134.0 ± 1.73c
DBP6 (mmHg)	80.66 ± 8.70b	77.33 ± 8.50c	84.33 ± 18.02a	77.0 ± 5.56d	75.66 ± 10.41e
Na and Ca (%)		-12.5 ± 2.16c	22.22 ± 3.84a	13.13 ± 4.83b	-12.5 ± 2.16c
K and Mg (%)		-12.5 ± 2.16d	0 ± 0b	-7.14 ± 1.23c	29.09 ± 3.00a
Cl (%)		-8.33 ± 1.44b	6.66 ± 1.15a	8.33 ± 1.44a	-18.88 ± 2.00c
P (%)		12.5 ± 2.16a	-7.14 ± 1.23b	12.85 ± 4.22a	12.5 ± 2.16a
	Athletics (n=3)				
	Pre-exercise	A	B	C	D
SVR1 (SRU)*	995.89 ± 19,78b	1286.56 ± 83.22ab	1295.9 ± 37.51ab	1118.97 ± 233.68ab	1383.73 ± 96.62a
SV2 (ml/beat)	120.45 ± 5,37a	59.66 ± 5.65e	63.13 ± 7.88d	63.33 ± 6.29c	66.13 ± 12.14b
CO3 (L/min)	7.09 ± 0.35a	5.66 ± 0.40b	5.4 ± 0.17c	5.43 ± 0.47c	5.46 ± 0.49c
BV4 (L)	4.08 ± 0.46d	4.34 ± 0.45a	4.17 ± 0.58c	4.33 ± 0.43a	4.27 ± 0.50b
SBP5 (mmHg)	119.83 ± 4.14a	112.33 ± 7.50b	108 ± 5.29c	112.66 ± 10.97b	119.66 ± 6.03a
DBP6 (mmHg)	72.25 ± 4.33e	79.66 ± 5.85b	76.66 ± 0.57c	74.0 ± 4.58d	81.0 ± 5.29a
Na and Ca (%)		0 ± 0b	1.81 ± 5.72b	1.81 ± 5.72b	32.5 ± 3.03a
K and Mg (%)		-12.5 ± 2.16c	14.31 ± 6.06b	80 ± 6.93a	-21.59 ± 1.93d
Cl (%)		2.77 ± 2.09c	8.88 ± 5.38b	-10.55 ± 3.87d	26.11 ± 0.67a
P (%)		0 ± 0b	30.90 ± 7.83a	30.90 ± 7.83a	-21.59 ± 1.93c

Table 4: Athletes cardiac parameters before and after exercise, and interstitial electrolytes percentage changes after hydration with different protocols
[1]Systemic Vascular Resistance; [2]Stroke Volume; [3]Cardiac output; [4]Blood Volume; [5]Systolic blood pressure; [6]Diastolic blood pressure.
Different letters in the same line mean significant difference ($p<0.05$). Mean ± SD obtained from triplicate.

All acid-base indicators and nitric oxide (NO) of jiu-jitsu athletes and athletics practitioners presented a better response after protocols B and C (-19.97 ± 0.57; %). A significant reduction was observed in superoxide, hydrogen peroxide, hydroxyl radical and peroxynitrite radical after supplementation (D), as shown in table 5.

Jiu-jitsu athletes presented a higher production of leptin in all protocols except with supplementation (D), where the percentage changes were significantly lower. A greater change was observed in athletics practitioners with an increase of 41.86 ± 1.15 (%) after rehydration with NSD (B).

Interstitial production of cortisol was higher in athletics practitioners than jiu-jitsu athletes. Cortisol changes were only significant after supplementation protocols, presenting a percentage increase (3.96 ± 1.15; %) similar to water intake in jiu-jitsu athletes. For athletics practitioners, all interventions made with the NSD caused a lower increase in cortisol levels (30.03 ± 1.15; %).

It is well known that fruit and vegetables are the main source of antioxidant compounds in human diets [25-28]. Among them, phenolic compounds seem to play a major role in the prevention of oxidative stress-related diseases as recently reviewed by Haminiuk et al. [29]. A decrease in oxidative stress markers and muscle recovery as an effect of an antioxidant supplementation has also been reported in several studies [28-32].

Although most of these studies were conducted following protocols that tested blood or tissue samples, data obtained from ESC in this study presents a very similar response to stress markers (nitric oxide (NO·), superoxide (O_2-·), hydrogen per/oxide (H_2O_2), hydroxyl radical (·OH-), peroxynitrite (ONOO-). After supplementation, as observed in athletics practitioners, proper regulation in NO levels is particularly important for the maintenance of cardiovascular signaling activity during exercise. While the reduction in all other radicals suggests that the supplement provided anti-oxidant protection and that the type of the protocol and intake of polyphenols from the NSD had an impact on free-radical scavenging [33,34].

Regardless of exercise intensity, cortisol concentration is expected to be greater during exercise, especially in hypohydration [35]. Similar results were observed in this study where, after hydration with water (A), athletics practitioners presented a higher level of interstitial cortisol, while a smaller increase was observed after hydration with the NSD (B, C, and D).

It was highlighted that the differences between sports were a limiting factor since they spawned many variables intrinsic to each athlete and each physical activity. However, the effects obtained in both hydration, cardiac and biochemical markers assessed are of great importance to determine the effective dose-response, and the optimal intervention time for each athlete.

Regardless of this, the natural sports drink showed great potential to replenish electrolytes and as a supplement with a natural antioxidant capacity (from fruits and vegetables) that play an important role in good physical performance and recovery.

Cardiac parameters

When analyzing cardiac parameters (Table 4), the only difference observed was a small alteration in the stroke volume of jiu-jitsu athletes after hydration with the NSD (B), when compared to water intake (A) and supplementation (C and D). There was also a significant effect of the NSD

	Jiu-Jitsu (n=3)			
	A	B	C	D
Lactic acid	20.0 ± 1.73b	30.0 ± 1.73a	10.0 ± 0c	10.0 ± 3.00c
NO[1]	-15.01 ± 0.57a	-19.97 ± 0.57b	-19.97 ± 0.57b	-15.01 ± 0.57a
O_2[2]	6.93 ± 0.57b	10.35 ± 0.57a	10.35 ± 0.57a	6.93 ± 0.57b
H_2O_2[3]	6.93 ± 0.57b	10.35 ± 0.57a	10.35 ± 0.57a	6.93 ± 0.57b
OH-[4]	-0.77 ± 0.57b	10.35 ± 0.58a	10.35 ± 0.58a	-0.77 ± 0.58b
ONOO-[5]	-0.77 ± 0.57b	10.35 ± 0.58a	10.35 ± 0.58a	-0.77 ± 0.58b
Leptin	22.16 ± 1.15a	22.16 ± 1.15a	22.16 ± 1.15[a]	11.0 ± 2.30b
Cortisol	3.96 ± 1.15b	12.0 ± 1.15a	3.96 ± 1.15b	3.96 ± 1.15b
	Athletics (n=3)			
	A	B	C	D
Lactic acid	-9.67 ± 3.46c	3.22 ± 3.0b	29.03 ± 1.73a	-9.67 ± 1.73c
NO[1]	12.41 ± 2.88a	-1.07 ± 2.31c	-10.12 ± 1.15d	7.96 ± 2.0b
O_2[2]	-0.88 ± 1.73b	2.75 ± 1.15a	2.75 ± 1.15a	-4.62 ± 1.15c
H_2O_2[3]	-0.88 ± 1.73b	2.75 ± 1.15a	2.75 ± 1.15a	-4.62 ± 1.15c
OH-[4]	-0.88 ± 1.73b	2.75 ± 1.15a	2.75 ± 1.15a	-4.62 ± 1.15c
ONOO-[5]	-0.88 ± 1.73b	2.75 ± 1.15a	2.75 ± 1.15a	-4.62 ± 1.15c
Leptin	16.27 ± 0.0b	41.86 ± 1.15a	3.10 ± 0.58c	-9.68 ± 0.58d
Cortisol	45.04 ± 1.53a	30.03 ± 1.15b	30.03 ± 1.15b	30.03 ± 1.15b

Table 5: Biochemical markers percentage changes after rehydration following different protocols.

[1]Nitric oxide (NO·); [2]Superoxide (O_2-·); [3]Hydrogen peroxide (H_2O_2); [4]Hydroxyl radical (·OH-); [5]Peroxynitrite (ONOO-).

Different letters in the same line mean significant difference ($p<0.05$). Mean ± SD obtained from triplicate.

(B, C, and D) on the stroke volume of athletics practitioners that, despite not having reached the standard average (120.45 ± 5.37), was significantly higher than after rehydration with water (A). The same effect was observed in jiu-jitsu athletes, only after supplementation with the sports drink (D).

Cardiac output, blood volume, and blood pressure were significantly different at all steps for both sports. However this difference was not relevant since rehydration with water caused similar effects.

It is also known that dehydration affects cardiovascular functions by causing a decrease in blood and plasma volumes, as well as in the stroke volume, which reduces cardiac output and impairs overall endurance capacity [36]. Thereby, an effective hydration protocol can influence the behavior of cardiac parameters, as observed by Moreno et al. [37], who studied the influence of hydration with water in cardiorespiratory parameters in 31 healthy young male volunteers. Thus, it is possible that hydration and supplementation with a NSD (C and D) had a positive effect on athletics practitioners' hydration status which could impact performance.

Based on these results, we conclude that the natural sports drink was able to improve the hydration of all athletes at different levels, regarding their needs during exercise. Also, supplementation has a positive effect on stress markers, causing a reduction in radical and cortisol levels. These findings may benefit the sporting community who should consider this natural sports drink as a supplement and as a dietary intervention that could improve health and performance. Moreover, it is important to emphasize that no side effects were observed or reported by participants in this pilot study.

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