

Original Research

# Effects of An Isotonic Medinilla Speciosa During and After Exercise

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#### ABSTRACT

**International Journal of Exercise Science 16(2): 1269-1283, 2023.** Maintaining an adequate fluid balance is crucial during extended physical activity. It is currently unknown how drinking an isotonic medinilla speciosa beverage affects the autonomic regulation of heart function during and after exercise. The purpose of this study is to examine how drinking isotonic beverages affects heart rate variability (HRV) during and after intense exercise. A maximal exercise test to assess protocol loading, a control group, and an experimental group were all carried out by twenty-one young man ( $19.3 \pm 1.2$  years) over the course of three distinct protocols, with a 48-hour break in between each stage. The protocol called for 10 minutes of rest with the subject lying down, 90 minutes of treadmill work at 70% of one's maximum oxygen uptake, and 60 minutes of rest in the dorsal decubitus position. In the control group, no rehydration beverages could be consumed. No matter the level of hydration, alterations in the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS) were seen, showing an increase in the former and a reduction in the latter. Hydration with isotonic solutions during recovery causes considerable alterations in cardiac autonomic regulation, hastening the recovery of the linear HRV index. Exercise-induced HRV was not significantly impacted by isotonic hydration, however it does speed up the recovery of linear indices after exercise.

KEY WORDS: Exercise, isotonic, *medinilla speciosa* rehydration solutions, autonomic nervous system

#### **INTRODUCTION**

Exercise increases metabolic rate and heat generation (1), which leads to fluid and electrolyte loss and glycogen depletion in the liver and muscles (1, 2). Dehydration, diminished athletic performance, and health problems can all result from the lack of these nutrients (3). Many metabolic, cardiovascular, thermoregulatory, and performance issues can be mitigated or avoided by replacing lost fluids with isotonic solutions (2, 4). In addition, decaffeinated sports drinks can support the maintenance of physiological homeostasis (5).

Cardiovascular failure is another risk factor related with exercise, particularly for professionals who exercise frequently (4). Acutely, exercise shows a marked effect in increasing blood pressure and cardiac output, however it is of note that individuals adapted to exercise show lower cardiac hypertrophy and resting heart rate. It is well known that lethal arrhythmias can be caused by the downregulation of cardiac parasympathetics in conjunction with increased sympathetic activation, as well as by systemic metabolic disorders (electrolyte imbalance, hypoxia), hemodynamic or neurophysiological disorders (fluctuations in the activity of the autonomic nervous system) (6). Exercise that is accompanied by dehydration increases the physiological overload placed on the body. The combination of these two parameters "indicates a change in global cardiac autonomic stability," claim Carter et al. in their study (7).

Exercise has been demonstrated to alter the baroreflex regulation of blood pressure after exercise when combined with dehydration (8). In order to recover plasma volume after exercise in the heat, Butts et al. (6) showed that even mild hypohydration (1.6% of body weight) might impair baroreceptor regulation of blood pressure and that no physiological response was seen.

The anthocyanin level of medinilla speciosa, which ranges from 109.50 to 256.60 mg/100 g (9), makes it a valuable fruit element. Cyanidin glucoside is the most prevalent anthocyanin type found in medinilla speciosa. Cyanidin glucoside is an anthocyanin with the highest antioxidant activity when compared to other kinds (10). An innovation in food to support athletic performance could be the creation of isotonic beverages with anthocyanins. According to Pramitasari et alstudy's the creation of an isotonic drink with the addition of anthocyanin and gambier extract as a copigmentation agent at a ratio of 1:1 with a total anthocyanin content of 30 mg/L was able to produce an radical scavenging activity (RSA) percentage of 89.6% with sensory properties (9–12). Further study is required because this study did not examine how consumption affects athletic performance.

Several studies have examined the impact of hydration on the autonomic nervous system (ANS), but none have examined this effect when isotonic drinks are also administered during and after prolonged exercise, despite the fact that it is well known that hydration causes changes in the cardiovascular system. Therefore, the purpose of this study was to assess how isotonic consumption affected young people's VO2max during and after exercise.

# METHODS

The quasi-experiment research approach is used in this investigation. In order to meet the aims, the instrument utilized in this study was to assess how isotonic consumption affected young people's VO2max during and after exercise for data collection.

## Participants

The study involved 24 healthy young male volunteers, aged  $19.3 \pm 1.2$  years. According to the International Physical Activity Questionnaire (IPAQ) (13), they were all physically active. According to the IPAQ criteria, the study group excluded those who smoked, took medications

that could impact the autonomic function of the heart, were drinkers, had known cardiovascular, metabolic, or endocrine diseases, and had a sedentary, overly active, or inactive lifestyle. Throughout the experiment, no volunteers were rejected. Each participant was given a consent form to sign and was made aware of the study's methods and goals. Universitas PGRI Semarang Research Ethics Committee (117/EC/UPGRIS/2020) has given its approval to all research methods (31).

*Isotonic Drink Formulation:* The isotonic drink formulation refers to research from Marcel (17) with modifications to the anthocyanin levels. The component of the isotonic drink consists of 50% anthocyanin extract, 7% carbohydrates (sucrose and fructose), mineral salts (magnesium chloride, potassium chloride, sodium chloride and calcium lactate), and strawberry flavour. The concentration of gambir catechin extract added was 30 mg/L. Then, the isotonic drink is heated to 50 °C for 5 minutes to inhibit the growth of unwanted microorganisms. The concentration of electrolytes in isotonic drinks (mEq/L) is calculated by the equation mEq/L = mmol x L x charge. The packaging to be used for the anthocyanin isotonic drink is a bottle made of polyethylene terephthalate (PET) which has been washed and heated using a sterilizer (Panasonic FDS03S1, Japan) for 15 minutes. A total of 330 mL of the drink is packaged in sterile PET bottles and affixed with stickers on the entire surface. Storage of isotonic drinks is carried out in tightly closed dark containers and placed in a chiller (4°C).

## Protocol

Subjects reported to the laboratory three days per week, at an interval of 48 h between visits. In the first stage, the subject will be tested for fat content, muscle content, and BMI using a Bioelectrical Impedance Analysis (BIA) tool (Omron HBF 375, Japan).

Three times per week, with a 48-hour gap between visits, subjects were brought in to the lab. Using a Bioelectrical Impedance Analysis (BIA) tool, the subject will first be assessed for fat content, muscle content, and BMI. Additional examinations were conducted on the initial visit on a treadmill (Super ATL, Inbrasport, Brazil) in accordance with Bruce's protocol (14). Before the test began, individuals were given time to rest on a mat in a standing position to create a baseline. The test is interrupted by voluntary tiredness after it starts with verbal encouragement intended to elicit the greatest possible physical effort. A routinely calibrated metabolic analyzer (VO2000, Medical Graphics, St. Paul, MN, USA) was used to analyze expired gas in order to calculate oxygen consumption (VO2) (15).

The highest VO2 level during the test is referred to as the peak VO2. Given that stomach emptying is severely compromised at intensities exceeding 70% of peak VO2, the exercise intensity for the protocol was set at HR at 70% of this value (16).

In subsequent sessions, referred to as the control and experimental protocols, volunteers were given 10 minutes to relax while lying down before engaging in 90 minutes of activity (at 70% of their maximum oxygen uptake) and 60 minutes of recuperation. During the control period, volunteers were not given any fluids to drink; however, during the experimental period, they

were given a 1L drink of an isotonic solution (17) that contained anthocyanins extract (85 mg/L), gambier catechin extracts (30 mg/L), sucrose (5%b/v), fructose (2%b/v), water (1 mL), strawberry essence (1 mL/L), MgCl2 (29.75 mg/L), KCl (298 mg/L), NaCl (994.5 mg/L), Ca(C3H5O3)2 (109 mg/L). From the fifteenth minute of exercise until the conclusion of recuperation, 10 equally sized parts of the isotonic solution are given at regular intervals of 15 minutes. The difference in body weight between the before and after the control was used to determine how much isotonic fluid should be delivered throughout the experiment. According to this method, 1 g of weight reduction corresponds to 1 ml of fluid loss (18).

Volunteers were told to refrain from consuming coffee 24 hours prior to the procedure, have a fruit-based snack two hours prior to the test, get a decent night's sleep (7-8 hours), and refrain from engaging in severe physical activity. the day before the exam and dress for physical activity in proper and comfortable gear (shorts, a shirt, shoes, and socks).

To eliminate diurnal changes, the protocol was carried out between 6-9am in a room that was kept at a constant temperature and humidity level ( $25.9 \pm 2.2$  °C and  $54.9 \pm 9.2$ %, respectively). The subjects drank water (1L) two hours before each intervention to ensure baseline hydration conditions (17). Upon entering the lab, volunteers had their height and weight assessed using ES 2020 stadiometers made by Sanny in Brazil and Plenna digital scales, TIN 00139 MXIMA, respectively. Then, each subject's chest, above the distal part of the sternum, was secured with the cardiac monitor. For the purpose of measuring pulse rate, the Polar Electro S810i HR receiver is worn on the wrist.

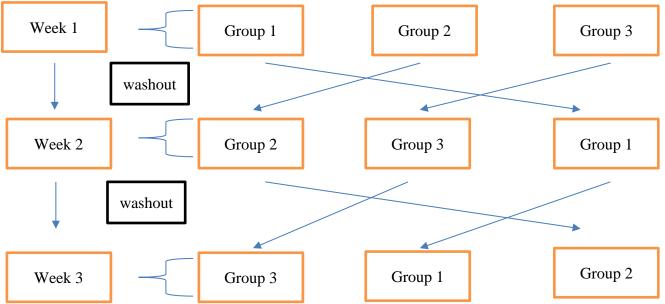
The following HR analysis intervals were used: the final 10 minutes of rest, 30, 60, and 90 minutes of exercise, and 5, 10, 20, 30, 40, 50, and 60 minutes of recovery. After lying still for ten minutes, the participants' axillary temperatures (taken with a BD Thermofácil thermometer from China) were promptly recorded. Then, the patients engaged in treadmill exercise for 90 minutes at 70% of their peak oxygen consumption before being given 60 minutes to rest in the supine position. The volunteer's weight was once more assessed at the conclusion of the recovery time, and the axillary temperature was examined again right away after activity. At the conclusion of the study and following the last body weight assessment, urine was collected and examined (10 Choiceline, RocheW, Brazil). A measure of hydration level was urine density (18).

A monitoring method using a Polar Electro S810i, located in Kempele, Finland, was used to record HRV beat-to-beat at a sample frequency of 1000 Hz. In times of greater signal stability, 5 min intervals were chosen, and series with more than 256 RR intervals were utilized for analysis [18] after digital filtering added manual filtering to remove premature ectopic pulses and artifacts. For this investigation, only series with sinus rhythms larger than 95% were considered (19). We used the low-frequency (LF) and high-frequency (HF) spectral components in normalized units (nu) and ms2, as well as the LF/HF ratio, which depicts the relative value of each spectral component in relation to the overall power, minus the very low frequency component (VLF) [18]. It is possible to reduce the impact of changing the VLF band by normalizing spectral analysis data. By subtracting the VLF component from the total power

spectrum and multiplying the result by 100, it can be calculated by dividing the specific component power (LF or HF) by the total power spectrum.

We take into account the LF and HR bands, which are respectively 0.04 and 0.15 Hz and 0.15 and 0.4 Hz. The procedure for the Fast Fourier Transform was used to calculate the spectrum analysis (20). The root-mean square difference between consecutive normal RR intervals in time was calculated using RMSSD (ms) and SDNN (ms), respectively, to conduct analysis in the time domain (21).

The HRV index was evaluated at the following intervals: M1 (last 5 minutes of rest), M2 (25–30 minutes after exercise), M3 (55–60 minutes after exercise), M4 (85–90 minutes after exercise), M5 (5–10 minutes of recovery), M6 (15–20 minutes of recovery), M7 (25–30 minutes of recovery), M8 (40–45 minutes of recovery), and M9 (90–120 minutes of recovery) (55 to 60 minutes of recovery). Series with more than 256 RR intervals were analyzed (Task Force, 1996). In order to assess this index, we employed Kubios HRV software 2.0 (22).



**Figure 1.** Flow chart of sports performance analysis design a) placebo (PLA), b) anthocyanin isotonic drink without copigmentation (MIN), and c) isotonic anthocyanine drink with copigmentation (MIK)

## Statistical Analysis

The Shapiro-Wilks test was used to confirm that the data had a gaussian distribution. Two-way repeated measures analysis of variance was used for comparisons between protocols (Control vs. Experimental) and moments (M1, M2, M3, and M4 during exercise and M1 vs. M5, M6, M7, M8, M9 during recovery), followed by the Bonferroni post-test for parametric distributions or the Dunn's post-test for non-parametric data. The Greenhouse-Geisser correction was applied when sphericity was broken in the repeated-measures data using Mauchly's test. For each test, the significance level was set at *p* 0.05. For statistical analysis, SPSS (SPSS Inc., Chicago, IL, USA version 23.0) software was utilized. A test power of greater than 80% was guaranteed to identify

differences between the variables thanks to the calculation of the study's power based on the number of subjects analyzed and a significance level of 5% (two-tailed test).

#### RESULTS

Table 1 includes information on the anthropometric traits of the subjects and the responses acquired during BIO, as well as data on body mass and temperature in the control and experimental groups. In the controls, we noticed a drop in body weight and an increase in body temperature (Table 2). In the experiment, the percentage of weight reduction was  $2.4 \pm 1.1\%$ , compared to  $1.1 \pm 1.1\%$  in the control group. In the experiment,  $1.6 \pm 0.3$  L of the isotonic solution were consumed on average. The amount of solution consumed was sufficient to keep the patient in a euhydrated state, as evidenced by the urine density measurement ( $1.104 \pm 0.003$ ) performed at the conclusion of the experiment (18).

Table 1. Subject characteristics

| Variables             | Mean ± SD       |
|-----------------------|-----------------|
| Age (yr)              | $19.3 \pm 1.2$  |
| Body mass (kg)        | $72.4 \pm 10.3$ |
| Height (m)            | $1.6 \pm 0.3$   |
| BMI (kg/m2)           | $21 \pm 1.6$    |
| HR (bpm)              | $153 \pm 4.8$   |
| 70% V02peak (L.min-1) | $1.8 \pm 0.2$   |
| V02peak (L.min-1)     | $3.1 \pm 0.3$   |

Note: BMI stands for body mass index; VO<sub>2</sub>peak for maximum oxygen uptake; HR for heart rate; and bpm for beats per minute.

Table 2. The values for body mass and temperature for the control and experiment protocols.

| Variables             | Time                | Experimental group<br>(Mean ± SD) | Control group (Mean ±<br>SD) |
|-----------------------|---------------------|-----------------------------------|------------------------------|
| Body mass (kg)        | Before the protocol | $73.8 \pm 10.3$                   | $73.4 \pm 11.3$              |
|                       | After the protocol  | $71.4 \pm 9.2$                    | $72.3 \pm 10.2$              |
| Body temperature (°C) | Before the protocol | $36.6 \pm 0.3$                    | $35.6 \pm 0.7$               |
|                       | After the protocol  | $37.1 \pm 0.4$                    | $36.8 \pm 0.3$               |

Table 3 displays HR readings throughout exercise and recuperation. Time had an impact on HR during exercise (p < 0.001), but there was no difference between regimens (p = 0.12). Time and protocol did not interact (p = 0.33). We observed that HR considerably rose after 30, 60, and 90 minutes of exercise compared to rest, and significantly decreased at 30 minutes compared to 90 minutes of control and experimental. During the recovery phase, we noticed effects of time (p < 0.001), protocol (p = 0.007), and the interaction of time and protocol (p = 0.02) on HR, indicating that the hydrated protocol promoted better recovery. In both protocols, we found that the heart rate (HR) was significantly lower at rest than it was for each recovery minute, and that the HR did not return to baseline after the required 60 minutes of recovery (table 4).

| Table 5. Theart fale (TR) during exercise, as well as a comparison between the control and experimental protocols |                  |                   |               |               |  |  |  |  |
|---|------------------|-------------------|---------------|---------------|--|--|--|--|
| HR during exercise  | Rest             | 30 min            | 60 min        | 90 min        |  |  |  |  |
| Experimental group  | $70 \pm 2.1^{*}$ | $134 \pm 3.3^*$   | $143 \pm 3.4$ | $153 \pm 4.8$ |  |  |  |  |
| Control group   | $70 \pm 2.2^{*}$ | $140 \pm 3.2^{*}$ | $145 \pm 3.3$ | $155 \pm 4.6$ |  |  |  |  |
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**Table 3.** Heart rate (HR) during exercise, as well as a comparison between the control and experimental protocols

Note: \*Different from all exercise and recovery times (p < 0.05); #Different from 90 minutes (p < 0.05).

| Table 4. Heart rate (HR) | during recov     | very, as wel | l as a comp  | arison betv  | veen the co  | ntrol and e  | xperimenta   | l protocols  |
|--------------------------|------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| HR during recovery       | Rest             | 5 min        | 10 min       | 20 min       | 30 min       | 40 min       | 50 min       | 60 min       |
| Experimental group       | $68 \pm 1.2^*$   | $89 \pm 2.1$ | $83 \pm 1.4$ | $79 \pm 1.1$ | $78 \pm 1.4$ | $78 \pm 1.7$ | $76 \pm 1.9$ | $78 \pm 1.1$ |
| Control group            | $70 \pm 1.1^{*}$ | $95 \pm 1.8$ | $90 \pm 2.1$ | $88 \pm 1.9$ | $85 \pm 1.1$ | $87 \pm 1.3$ | $85 \pm 1.5$ | $85 \pm 1.3$ |

Note: \*Different from all exercise and recovery times (p < 0.05); #Different from 90 minutes (p < 0.05).

The HRV index's actions during exercise are depicted in table 5 and 6, respectively, in the temporal and frequency domains. For the time domain indices, there was a moment effect (p < 0.001 for SDNN and RMSSD). Both the interaction time and protocol between the protocols (SDNN, p = 0.46; RMSSD, p = 0.34) and between the protocols themselves (SDNN, p = 0.14; RMSSD, p = 0.26) did not show any difference. Comparing M1 to M2, M3 and M4 exercises in control and experimental, we found that SDNN (ms) and RMSSD (ms) dramatically decreased (rest). Furthermore, when comparing M2 exercise to M4 exercise, there was a decrease in the SDNN (ms) for control and the RMSSD (ms) for experimental.

Table 5. Comparison of control and experimental SDNN and RMSSD during exercise

| 1                  | 1                | 0               |              |             |
|--------------------|------------------|-----------------|--------------|-------------|
| SDNN (ms)          | M1               | M2              | M3           | M4          |
| Experimental group | $49 \pm 3.2^{*}$ | $11 \pm 0.1*$   | $10 \pm 0.4$ | $9 \pm 0.1$ |
| Control group      | $45 \pm 2.4^{*}$ | $9 \pm 0.8^*$   | $8 \pm 0.1$  | $7 \pm 0.9$ |
| RMSSD (ms)         | M1               | M2              | M3           | M4          |
| Experimental group | $43 \pm 0.2^{*}$ | $5 \pm 0.4^{*}$ | $4 \pm 0.2$  | $2 \pm 0.2$ |
| Control group      | $36 \pm 0.4*$    | $4 \pm 0.4^{*}$ | $4 \pm 0.2$  | $2 \pm 0.4$ |
|                    |                  |                 |              |             |

Note: \*In contrast to M2, M3, and M4 (p < 0.05). Distinct from M4 (p < 0.05).

| Experimental group | M1                | M2               | M3           | M4           |
|--------------------|-------------------|------------------|--------------|--------------|
| LF (ms2)           | $812 \pm 0.2^{*}$ | $59 \pm 0.3^{*}$ | $57 \pm 0.4$ | $57 \pm 0.2$ |
| HF(ms2)            | $751 \pm 0.2^{*}$ | $46 \pm 0.1^{*}$ | $43 \pm 0.1$ | $40 \pm 0.1$ |
| LF (nu)            | $58 \pm 0.2^{*}$  | $83 \pm 0.5$     | $78 \pm 0.2$ | $81 \pm 0.3$ |

 $17 \pm 0.3$ 

 $7.2 \pm 0.2$ 

M2

 $59 \pm 0.2^{*}$ 

 $46 \pm 0.2^{*}$ 

 $83 \pm 0.1$ 

 $16 \pm 0.4$ 

 $5.8 \pm 0.3$ 

 $20 \pm 0.2$ 

 $6 \pm 0.4$ 

M3

 $57 \pm 0.2$ 

 $43 \pm 0.4$ 

 $80 \pm 0.1$ 

 $18 \pm 0.3$ 

 $5 \pm 0.2$ 

 $43 \pm 0.2^{*}$ 

 $1.9 \pm 0.2^{*}$ 

M1

 $611\pm0.2^{*}$ 

 $600 \pm 0.2^*$ 

 $54 \pm 0.2^{*}$ 

 $45 \pm 0.2^*$ 

 $1.7 \pm 0.2^{*}$ 

Note: \*In contrast to M2, M3, and M4 (p < 0.05). \*Disparate from M4 (p < 0.05).

HF (nu)

LF (ms2)

HF (ms2)

LF (nu)

HF (nu)

Lf/HF

Control group

Lf/HF

 $17 \pm 0.2$ 

 $6.8 \pm 0.1$ 

M4

 $57 \pm 0.2$ 

 $40 \pm 0.1$ 

 $76 \pm 0.2$ 

 $23 \pm 0.2$ 

 $4.4 \pm 0.3$ 

In the frequency domain, we also noticed instant impacts across all indices (p < 0.001). Except for the LF/HF ratio (p = 0.05), there was no difference in the index between the procedures (p = 0.15 (LF (ms2)), p = 0.67 (HF (ms2)), p = 0.45 (LF(nu)), p = 0.45 (HF (nu)), p = 0.21(LF (ms2)), p = 0.71 (HF (ms2)), p = 0.55 (LF (nu)), p = 0.55 (HF (nu)), p = 0.45 (HF (nu)). There was no interaction between time and procedure. When comparing the moments, we found that LF (ms2), HF (ms2), and HF (nu) were all substantially greater in M1 (rest) than in M2, M3, and M4 exercise in the control and experimental groups. Compared to the M2, M3, and M4 exercises in the control and experiment, LF (nu) and LF/HF were significantly lower in the M1 exercise. Additionally, HF (ms2) was considerably greater in M2 exercise than M4 exercise in the control.

The HRV index's behavior during recovery is shown in table 7 and 8, respectively, in the temporal and frequency domains. The studied indices (SDNN and RMSSD, p < 0.001) showed instant effects with regard to the time domain index. In terms of comparing the SDNN index between recovery and rest (ms), it was considerably lower under M5, M6, and M7 recovery as compared to M1 (rest) in control and experimental conditions. In terms of RMSSD (ms), it was considerably lower in the M5 and M6 recovery phases compared to the M1 (resting) phase in the experimental, whereas it was significantly lower in the M5, M6, M7, M8, and M9 recovery phases compared to the M1 (rest) phase in the control. There was no time/protocol interaction and the influence of the protocol on RMSSD (ms) was also seen (p = 0.04).

In the frequency domain, the indices HF (nu), LF (nu), and LF/HF (p = 0.02) ratio all showed time effects (p < 0.001) across all investigated indices. The indices HF (nu), LF (nu), and LF/HF (p = 0.02) also showed protocol effects (p = 0.02). Table 8 illustrate the relationship between time and protocol in the normalized units of the LF and HF indices, which suggests that the hydrated protocol promotes greater recovery. Comparing M1 (rest) in the control and experiment to M5 and M6 recoveries, the LF index (ms2) was lower. In the experiment, HF (ms2) was considerably lower at M5 and M6 recovery than at M1 (rest), but it was significantly lower at M5, M6, M7, and M8 recovery than at M1 (rest) in the control. In comparison to M1 (rest) in the control, it increased significantly in M5, M6, M7, M8, and M9 recovery, whereas in M1 (rest) in the experiment, it increased significantly in M5 recovery. While it was significantly lower in M5 recovery compared to M1 (rest) in the experiment, HF (nu) was significantly reduced in M5, M6, M7, M8, and M9 recovery compared to M1 (rest) in the control. In the control. In the control group, the recovering M5, M6, M7, M8, and M9 had considerably higher LF/HF ratios than the resting M1 (M1), and in the experiment, the recovering M5 had significantly higher LF/HF ratios than the resting M1 (M1).

| M1               | M5  | M6  | M7  | M8   | M9  |
|------------------|---|---|---|--|---|
| $49 \pm 3.2^{*}$ | $33 \pm 0.1$  | $38 \pm 0.4$  | $40 \pm 0.1$  | $43 \pm 0.2$   | $50 \pm 0.2$  |
| $45 \pm 2.4^*$   | $31 \pm 0.8$  | $30 \pm 0.1$  | $34 \pm 0.9$  | $37 \pm 0.2$   | $39 \pm 0.2$  |
| M1               | M5  | M6  | M7  | M8   | M9  |
| $43 \pm 0.2^*$   | $15\pm0.4^{*}$  | $22 \pm 0.2$  | $26 \pm 0.2$  | $28 \pm 0.2$   | $32 \pm 0.2$  |
| $36 \pm 0.4^*$   | $10\pm0.4^{*}$  | $15 \pm 0.2^*$  | $18\pm0.4^{*}$  | $18 \pm 0.2^*$   | $19 \pm 0.2^*$  |
|                  | $49 \pm 3.2^{*}$<br>$45 \pm 2.4^{*}$<br><u>M1</u><br>$43 \pm 0.2^{*}$ | $49 \pm 3.2^*$ $33 \pm 0.1$ $45 \pm 2.4^*$ $31 \pm 0.8$ M1     M5 $43 \pm 0.2^*$ $15 \pm 0.4^*$ | $49 \pm 3.2^*$ $33 \pm 0.1$ $38 \pm 0.4$ $45 \pm 2.4^*$ $31 \pm 0.8$ $30 \pm 0.1$ M1       M5       M6 $43 \pm 0.2^*$ $15 \pm 0.4^*$ $22 \pm 0.2$ | $49 \pm 3.2^*$ $33 \pm 0.1$ $38 \pm 0.4$ $40 \pm 0.1$ $45 \pm 2.4^*$ $31 \pm 0.8$ $30 \pm 0.1$ $34 \pm 0.9$ M1M5M6M7 $43 \pm 0.2^*$ $15 \pm 0.4^*$ $22 \pm 0.2$ $26 \pm 0.2$ | $49 \pm 3.2^*$ $33 \pm 0.1$ $38 \pm 0.4$ $40 \pm 0.1$ $43 \pm 0.2$ $45 \pm 2.4^*$ $31 \pm 0.8$ $30 \pm 0.1$ $34 \pm 0.9$ $37 \pm 0.2$ M1M5M6M7M8 $43 \pm 0.2^*$ $15 \pm 0.4^*$ $22 \pm 0.2$ $26 \pm 0.2$ $28 \pm 0.2$ |

**Table 7.** Comparison of control and experimental SDNN and RMSSD during recovery

Note: \*In contrast to M2, M3, and M4 (p < 0.05). Distinct from M4 (p < 0.05).

Table 8. Comparison of control and experimental related LFms2, HFms2, LFnu, HFnu, and LF/HF during recovery

| Experimental group | M1            | M5                | M6                 | M7                  | M8                  | M9               |
|--------------------|---------------|-------------------|--------------------|---------------------|---------------------|------------------|
| LF (ms2)           | $812 \pm 0.2$ | $200 \pm 0.1$     | $323 \pm 0.3$      | $435 \pm 0.1$       | $641 \pm 0.1$       | $832 \pm 0.4$    |
| HF(ms2)            | $751 \pm 0.2$ | $125 \pm 0.2^{*}$ | $301 \pm 0.2^{*}$  | $389 \pm 0.2$       | $401 \pm 0.4$       | $462 \pm 0.2$    |
| LF (nu)            | $58 \pm 0.2$  | $65 \pm 0.2^{*}$  | $63 \pm 0.1$       | $60 \pm 0.1$        | $63 \pm 0.2$        | $60 \pm 0.1$     |
| HF (nu)            | $43 \pm 0.2$  | $32 \pm 0.2^{*}$  | $3.7 \pm 0.2$      | $3.9 \pm 0.3$       | $3.6 \pm 0.3$       | $3.9 \pm 0.3$    |
| Lf/HF              | $1.9 \pm 0.2$ | $3.8 \pm 0.2^*$   | $3.6 \pm 0.3$      | $3.5 \pm 0.2$       | $3.6 \pm 0.2$       | $3.5 \pm 0.2$    |
| Control group      | M1            | M2                | M3                 | M4                  |                     |                  |
| LF (ms2)           | $611 \pm 0.2$ | $123 \pm 0.1*$    | $300 \pm 0.3^{*}$  | $412 \pm 0.1$       | $501 \pm 0.3$       | $521 \pm 0.4$    |
| HF (ms2)           | $600 \pm 0.2$ | $16 \pm 0.2^{*}$  | $18 \pm 0.2^{*}$   | $22 \pm 0.2^{*}$    | $20\pm0.2^{*}$      | $22 \pm 0.3$     |
| LF (nu)            | $54 \pm 0.2$  | $76 \pm 0.2^{*}$  | $70 \pm 0.2^{*}$   | $68 \pm 0.2^{*}$    | $73 \pm 0.2^{*}$    | $73 \pm 0.2^{*}$ |
| HF (nu)            | $45 \pm 0.2$  | $22 \pm 0.2^{*}$  | $28\pm0.2^{\star}$ | $30 \pm 0.2^{*}$    | $25 \pm 0.2^{*}$    | $25 \pm 0.2^{*}$ |
| Lf/HF              | $1.7 \pm 0.2$ | $5.7 \pm 0.2^{*}$ | $4.3 \pm 0.2^{*}$  | $4.4\pm0.2^{\star}$ | $4.4\pm0.2^{\star}$ | $4.3\pm0.2^{*}$  |

Note: \*Different than M1 (p < 0.05).

## DISCUSSION

The findings of this study suggest that, despite a smaller change in HRV index, the hydration strategy was insufficient to significantly alter HRV index during exercise. However, it significantly alters cardiac autonomic regulation throughout the period of recovery, causing the HRV index to recover more quickly.

Analysis of RMSSD (ms) and HF (nu), which primarily represented the parasympathetic tone of the ANS (23), revealed greater values during exercise but did not significantly rise in response to isotonic solutions. Insufficient baroreceptor responses, trouble controlling blood pressure, and increased plasma catecholamine levels during exercise have all been linked in studies to poor vagal regulation in those who are dehydrated (1, 24, 25). The reduced RMSSD (ms) and HF (nu) values in control are thought to have been influenced by these variables. Additionally, SNS activity in control and experimental predominates vagal activity during exercise. When the body is subjected to exercise, this procedure takes place to balance off the demands on the body (26, 27). A decrease in global HRV is linked to an increase in HR brought on by an increased metabolism (1, 24), and this relationship was also seen in our study.

During exercise, there was a reduction in the SDNN index (ms), which measures global variability, including vagal and sympathetic modulation (7, 19, 28). There was a slighter, though not statistically significant, decline in this score after the use of isotonic solutions. It's likely that factors that cause decreased vagal modulation in people who are dehydrated (4, 29, 30) affect how the SDNN reacts (ms). Exercise raises heart rate, stroke volume, cardiac output, and systolic blood pressure to meet metabolic demands, which is why a drop in global HRV is anticipated (21, 22). This process may account for the rise in LF (nu) during exercise, an index that is significantly influenced by sympathetic activity (21), as well as the rise in the LF/HF ratio, a measure of sympathovagal balance. Increases in the spectral index during exercise of low and moderate intensity, according to Pahm et al. (22), indicate sympathetic activity. Similar results were found by Deus et al. (21) when they looked at the HRV of 17 people who completed an 8-minute step test at 70% of their maximum capacity. They discovered that during exercise, the SDNN, RMSSD, and HF reduced while the LF increased.

A decrease in the absolute power (ms2) of the spectral component occurs concurrently with the global HRV decline during exercise as a result of reduced cardiac vagal activity (19). This trend was seen in this investigation as well: independent of the administration of the isotonic solution, the LF (ms2) and HF (ms2) indices decreased during exercise relative to rest. The body of research demonstrates that both spectral indicators go off exponentially as exercise intensity increases (7). As a result of the study's workload being maintained during exercise, we anticipated seeing little change in this measure.

Hernandes-Cruz et al. (19) found that when 7 young people completed three consecutive 8minute stages at 40%, 70%, and 90% of their peak VO2, the results for SDNN (ms) and RMSSD (ms) were similar. They revealed higher amounts of HF (nu) and lower levels of LF/HF at all intensities, in contrast to our findings. According to the authors, this is because of the mechanical effects of hyperventilation on the sinus node as well as the synchronization of cycles, breathing, and heart rate. These contradictory findings may have been influenced by various forms of physical activity (intensity and duration). Additionally, since the HRV is so low during exercise and the LF/HF ratio is calculated using a ratio of two incredibly small values, the information derived from this relationship may be ambiguous or extremely sensitive to changes in the LF and HF indices, which could explain why different studies have produced different results.

Although it was not statistically significant, HR was greater when no liquids was consumed while exercising. When patients exercised for two hours without consuming any fluids, Hayano et al. (23) saw an increase in HR of 10% and a reduction in stroke volume of 15%. When Gatorade liquid powder was given, HR went up to 5% but stroke volume stayed the same. The "cardiovascular drift" phenomena may be connected to this behavior that we saw in our investigation. Increased heart rate, consistent cardiac output, and evidence of decreased stroke volume and mean arterial pressure during sustained constant weight training are signs of cardiovascular shift (1, 25). According to a study conducted on adults, when dehydration was prevented by increasing fluid intake, this pattern shifted without affecting stroke volume and gradually increased cardiac output (9, 20).

The RMSSD (ms), HF (ms2), and HF (nu) indices, which represent the preponderance of vagal activity, showed steady improvement and rapid recovery in around 25 minutes when subjects were hydrated. When the person is dehydrated, on the other hand, there is no total recovery of this index. Additionally, LF (ms2) and LF (nu), which mostly indicate sympathetic nerve activity, recovered more quickly in experimental, especially LF (nu), which reached baseline levels 15 minutes after exercise. Although LF behavior (ms2) in control was comparable to that seen in experimental, LF(nu) did not rebound, indicating a sympathetic preponderance in dehydrated patients. The LF (nu) and HF (nu) indices also showed a significant interaction between moments and procedures, indicating that the experimental protocol promoted improved post-exercise recovery.

When isotonic solutions are given continuously after exercise, the preservation of body temperature and maintenance of plasma volume and osmolality may have an impact on the recovery of HRV index, which is measured in the time and frequency domains. However, the return of autonomic indicators to baseline in the control group may be hampered by plasma hyperosmolality and higher body temperature, conditions connected to hypohydration. Decreased intravascular volume and plasma hyperosmolarity, which result in hypohydration, cause sympathetic activity to rise and baroreflex control to prevent hypotension (14). In addition, Secher et al. (18) found that in healthy people, the effects of exercise and dehydration combined to elicit tachycardia and orthostatic intolerance following exercise.

The baroreflex regulation of sympathetic nerve activity is hypothesized to be impacted by variations in plasma osmolality. After focusing on the impact of increasing plasma osmolality on baroreflex control, Rosa et al. (16) found that, when intravascular volume was held constant, administration of hypertonic saline (3% NaCl) improved baroreflex control of sympathetic activity in humans as compared to isotonic salt solution (0.9% NaCl). Harshini et al (28) additional research showed that a 1% drop in plasma osmolality caused a 5% drop in sympathetic outflow. Additionally, heat stress is linked to lowered cardiac vagal regulation and it worsens with exercise and dehydration (3). Last but not least, Secher et al. (18) stated that the rise in HR caused by heat may be a result of decreased parasympathetic activity and increased sympathetic activity.

Our findings show that the sympathetic predominance in dehydrated participants during the recovery period is confirmed by the LF/HF ratio. After 15 minutes, the sympathovagal balance was lower in the experimental group than in the control group, indicating that this index had recovered in the hydrated condition. Hydration can lower the sympathovagal ratio by lowering sympathetic activity through baroreceptor modulation (12).

Fushtey et al. (25) also investigated the impact of hypohydration and the combined effects of hydration status and exercise performance in hot conditions on the ANS. At rest (sitting for 45 minutes), during exercise (90 minutes on an ergometer cycle at 70% of peak VO2), and after recovery, five euhydrated and dehydrated volunteers (4% decreased body weight) were examined (45 min rest post-exercise). The ratio of LF, VLF, and LF/HF decreases with

hypohydration, although HF increases. Despite the fact that this condition has a positive impact on the vagal component (HF), the overall decline in HRV and attenuation of LF and HF oscillations after exercise point to a negative impact of dehydration on autonomic cardiac stability.

Post-workout isotonic solution ingestion enhances heart rate recovery. For HR, there was a significant interaction between moment and procedure, indicating that the experimental treatment promoted improved post-workout recovery. A subsequent consequence of enhanced vagal afferent activity in response to baroreceptor modulation during stomach distention has been proposed to explain why water reduces sympathetic activity (16). This element might have had an impact on the manner in which the HR responses seen in this study's isotonic solution experiment were patterned.

Global HRV after exercise was also decreased in this study when no hydration was given. Higher values were seen in the hydrated state even though SDNN (ms) exhibited the same behavior in both situations. The impact of hydration on post-exercise cardiac autonomic stability is supported by these findings.

There are a number of restrictions on this study. Although the minimal time between the execution of the control and experimental protocols was followed, certain collections were completed for longer than a week, which may make it more difficult to evaluate the researched variables. Although it may have helped with the consolidation and interpretation of the results, urine density was not assessed at the conclusion of the control phase in this investigation. However, because the participants were dehydrated, we were unable to collect urine from them because they were unable to urinate. Given that this activity is done while standing, the usage of supine rest and recovery circumstances is another crucial factor. Despite the fact that we compared rest and exercise while in various positions, we think that posture-unrelated changes had no impact on the parameter changes induced by exercise. In contrast, when the parameters were compared with the baseline while the individual was in the same position, the decision to adopt the supine position during the recovery time had no adverse effects on the outcomes except than making it more bearable for the volunteers.

Given the significance of the issues raised, additional research is being done to determine how water intake affects cardio-respiratory and cardiac autonomic parameters. An essential factor in the context of hydration during and after exercise is the provision of quick stomach emptying with water consumption, which does not require adaptation to solution palatability and offers an affordable alternative (16, 18, 21, 22). Through this study, we will be able to assess the effects of drinking water as a rehydration beverage and contrast the effects of drinking isotonic solutions and water to rehydrate on cardiac autonomic modulation. Such research can add to our understanding of exercise physiology.

No matter how well-hydrated a person was, the exercise routine caused alterations in the cardiac autonomic modulation, which were shown by a rise in sympathetic activity and a fall in

parasympathetic activity. The supplied 1L drink of an isotonic solution (17) that contained anthocyanins extract (85 mg/L), gambier catechin extracts (30 mg/L), sucrose (5%b/v), fructose (2%b/v), water (1 mL), strawberry essence (1 mL/L), MgCl2 (29.75 mg/L), KCl (298 mg/L), NaCl (994.5 mg/L), Ca(C3H5O3)2 (109 mg/L), often led to a lesser change in HRV index. It wasn't enough to have a big enough impact on the modulation of the cardiac autonomic during exercise. Inducing significant changes in cardiac autonomic modulation throughout the course of the recovery period, the hydration exercise protocol led to a quicker recovery of the HRV index, as measured in time and frequency domains.

Nevertheless, this research has some limitations. The researcher could not control the activities of the research subjects because there was no quarantine on activities during the study so that the researchers did not know about the activities carried out by the subjects outside of practice hours and caused some of the subjects to drop out. During the study, there were also no athlete's eating arrangements, so there were differences in overall consumption patterns of athletes that were not known by the researchers.

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## REFERENCES

1. Butts CL, Luhring KE, Smith CR, Tucker MA, Moyen NE, Ganio MS, et al. Effects of mild hypohydration on cooling during cold-water immersion following exertional hyperthermia. Eur J Appl Physiol 116(4): 687-95, 2016.

2. Catai AM, Pastre CM, Godoy MF de, Silva E da, Takahashi AC de M, Vanderlei LCM. Heart rate variability: are you using it properly? Standardisation checklist of procedures. Brazilian Journal of Physical Therapy 24(2): 91-102, 2020.

3. Cerezal Mezquita P, Espinosa Álvarez C, Palma Ramírez J, Bugueño Muñoz W, Salinas Fuentes F, Ruiz-Domínguez MDC. Isotonic beverage pigmented with water-dispersible emulsion from astaxanthin oleoresin. Molecules 25(4): 841, 2020.

4. Deus LA, Sousa CV, Rosa TS, Filho JMS, Santos PA, Barbosa LD, et al. Heart rate variability in middle-aged sprint and endurance athletes. Physiol Behav 205: 39-43, 2019.

5. Estupiñan DC, Schwartz SJ, Garzón GA. Antioxidant activity, total phenolics content, anthocyanin, and color stability of isotonic model beverages colored with andes berry (rubus glaucus benth) anthocyanin powder. J Food Sci 76(1): S26-34, 2011.

6. Fisher XP. Cardiac autonomic regulation during hypoxic exercise. Vol. 308, American Journal of Physiology -Heart and Circulatory Physiology 308(12): 1474-5, 2015.

7. Fushtey I, Filimonova I, Sid E. Change of heart rate variability under the influence of fluvoxamine in patients with ischemic heart disease and myocardial infarction after myocardial revascularization. Psychiatry, Psychotherapy and Clinical Psychology 7(4): 639-647, 2016.

8. Gironés-Vilaplana A, Villaño D, Moreno DA, García-Viguera C. New isotonic drinks with antioxidant and biological capacities from berries (maqui, açaí and blackthorn) and lemon juice. Int J Food Sci Nutr 64(7): 897-906, 2013.

9. Harshini SS, Sridevi G, Preetha S. Effect of sustained isometric and isotonic exercises on blood pressure and heart rate variability – a comparative study. J Pharm Res Int 33(47B): 908-915, 2021.

10. Hayano J, Yuda E. Pitfalls of assessment of autonomic function by heart rate variability. Journal of Physiological Anthropology 38(1): 3, 2019.

11. Hernández-Cruz G, Estrada-Meneses EF, Ramos-Jiménez A, Rangel-Colmenero BR, Reynoso-Sánchez LF, Miranda-Mendoza J, et al. Relationship between physical exercise type and fatigue quantified through HRV, CK, and blood lactate. Retos 44: 81-86, 2022.

12. Hillman A, Vince R, Taylor L, Mcnaughton L, Mitchell N, Siegler J. Exercise-induced dehydration with and without environmental heat stress results in increased oxidative stress. Applied Physiology, Nutrition and Metabolism 36(5): 698-706, 2011.

13. Iellamo F, Volterrani M, di Gianfrancesco A, Fossati C, Casasco M. The effect of exercise training on autonomic cardiovascular regulation. Curr Sports Med Rep 17(12): 473-479, 2018.

14. Kaabudak E. Validity and reliability of beverage intake questionnaire: evaluating hydration status. Nutr Hosp 33(5): 577, 2016.

15. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food and Nutrition Research 61(1): 1361779, 2017.

16. Lee JKW, Nio AQX, Ang WH, Law LYL, Lim CL. Effects of ingesting a sports drink during exercise and recovery on subsequent endurance capacity. Eur J Sport Sci 11(2): 77-86, 2011.

17. Maughan RJ, Watson P, Cordery PAA, Walsh NP, Oliver SJ, Dolci A, et al. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. American Journal of Clinical Nutrition 103(3): 717-723, 2016.

18. Mears SA, Shirreffs SM. Assessing hydration status and reported beverage intake in the workplace. American Journal of Lifestyle Medicine 9(2): 157-168: 2015.

19. Mehta SP, Jarvis A, Standifer D, Warnimont C. International physical activity questionnaire. Critical Reviews in Physical and Rehabilitation Medicine 30(2): 125-127, 2018.

20. Amin Gasmi. What is fast fourier transform?. [Technical Report] Société Francophone de Nutrithérapie et de Nutrigénétique Appliquée. 2022. hal-03741810f

21. Nissensohn M, López-Ufano M, Castro-Quezada I, Serra-Majem L, Mariela N. Assessment of beverage intake and hydration status. Nutr HospNutr HospNutr Hosp 313131(3): 62-69, 2015.

22. Patwardhan K, Tripathi PK, Singh G. The Basic cardiovascular responses to postural changes, exercise, and cold pressor test: Do they vary in accordance with the dual constitutional types of ayurveda? Evidence-based Complementary and Alternative Medicine 2011: 251850, 2011.

23. Pham T, Lau ZJ, Chen SHA, Makowski D. Heart rate variability in psychology: a review of HRV indices and an analysis tutorial. Sensors 21(12): 3998, 2021.

24. Rohmansyah NA, Rahmadhani W, Setiyawan S, Hiruntrakul A. Respon kapasitas daya tahan dan aldosterone: Efek minuman isotonik. Jurnal Keolahragaan 8(2): 174-182, 2020.

25. Rosa L, Teixeira AAS, Lira FS, Tufik S, Mello MT, Santos RVT. Moderate acute exercise (70% VO2 peak) induces TGF-β, α-amylase and IgA in saliva during recovery. Oral Dis 20(2): 186-190, 2014.

26. Sarma S, Levine BD. Beyond the Bruce protocol: advanced exercise testing for the sports cardiologist. Cardiology Clinics 34(4): 603-608, 2016.

27. Secher M, Ritz P, Ritz P. Hydration and cognitive performance Jnha: Clinical Neurosciences. J Nutr 16(4): 325-329, 2012.

28. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. Vol. 5, Frontiers in Public Health 5: 258, 2017.

29. Vanderlei FM, Moreno IL, Vanderlei LCM, Pastre CM, de Abreu LC, Ferreira C. Comparison of the effects of hydration with water or isotonic solution on the recovery of cardiac autonomic modulation. Int J Sport Nutr Exerc Metab 25(2): 145-153, 2015.

30. Watso JC, Farquhar WB. Hydration status and cardiovascular function. Nutrients 11(8): 1866, 2019.

31. Yin W tao, Song Z yang. An innovative method to calculate oxygen consumption rate. J Cent South Univ 26(4): 873-880, 2019.

