ACUTE EFFECTS OF A TYPICAL RHYTHMIC GYMNASTICS TRAINING DAY ON PHYSIOLOGICAL PARAMETERS IN OLYMPIC ATHLETES

Raquel de Jesus Silva[1], Lysandro Pinto Borges[2], Lucindo José Quintans-Júnior[3,4], Camila Ferezin Resende[5], Flávio Adsuara Cadegiani[6], João Henrique Gomes[3], and Renata Rebello Mendes[1]

[1]*Nutrition Department, Federal University of Sergipe, São Cristóvão, Brazil*
[2]*Department of Pharmacy, Federal University of Sergipe, São Cristóvão, Brazil*
[3]*Health Sciences Graduate Program, Federal University of Sergipe (UFS), Aracaju, Brazil*
[4]*Laboratory of Neuroscience and Pharmacological Assays, Federal University of Sergipe (UFS), São Cristóvão, Brazil*
[5]*Brazilian Gymnastics Confederation (CBG), Aracajú, Brazil*
[6]*Adrenal and Hypertension Unit, Division of Endocrinology and Metabolism, Department of Medicine, Federal University of São Paulo Medical School, São Paulo, Brazil*

Abstract:

The aim of this study was to evaluate the effects of a day with two separate training sessions (morning and afternoon) of rhythmic gymnastics on erythrocytes, leukocytes, muscle damage, oxidative stress, and hydration of Brazilian team [age 17.7 (±1.1) years; body height 165 (±0.5) cm; body mass 49.7 (±4.2) kg]. Heart rate and session-ratings of perceived exertion were used to monitor training intensity. Blood samples were collected immediately before (M1) and after (M2) the training day for analyzing erythrocytes, leukocytes, plasma creatine kinase activity, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, ferric reducing ability plasma, thyroid-stimulating hormone, and free T4. Saliva was collected for cortisol analysis. After 24 hours rest (M3), blood collection was performed to analyze creatine kinase and lactate dehydrogenase. The moderate-intensity training day induced significant elevations of total leukocytes (5,163.3 to 9,617.8), lymphocytes (1,752.7 to 2,729.7), neutrophils (2,873.9 to 6,163.6), monocytes (255.7 to 519.1), platelets (280,000.0 to 300,666.7), aspartate aminotransferase (13.1 to 25.6), lactate dehydrogenase (102.5 to 249.1), thyroid-stimulating hormone (1.0 to 3.2), and ferric reducing ability plasma (136.8 to 165.4), as well as significant reductions in red cells (4,691,111.1 to 4,497,777.8), hematocrit (42.1 to 39.3), and hemoglobin (12.9 to 12.5) at M2. There were also significant increases in creatine kinase (144.2 to 519.3) and lactate dehydrogenase (102.5 to 538.2) at M3. The average dehydration rate was 1.3%. A moderate-intensity day of training in rhythmic gymnastics of 8h21min duration caused hemolysis, leukocytosis, muscle damage, redox status perturbations, and insufficient hydration status. These findings show that athletes are exposed to physiological vulnerabilities that can possibly harm their performance and health.

Key words: erythrocytes, sports, creatine kinase, metabolic response, leukocytes

Introduction

Competition-driven sports training programs require high volume and intensity training loads with aiming to continuously improve sports performance that must be balanced with strategies for appropriate recovery in order to optimize morphological and metabolic adaptive processes, eventually leading to the improvement of sports perfor-mance (Issurin, 2010; Meeusen, et al., 2013) However, there is an inherent complexity regarding the determination of the most appropriate proportion between training load and recovery aiming to achieve more robust improvements in performance (Kellmann, et al., 2018).

Boundaries between physiological adaptations that lead to further performance improve-

ments and pathological adaptations are harsh and not always feasible to be delimited precisely (Cadegiani & Kater, 2019b). Elite athletes with imbalanced routine and social and nutritional vulnerabilities (Cadegiani & Kater, 2019b; Tian, et al., 2015) are more likely to develop pathological adaptations that impair multiple systems, such as immune response (Walsh & Oliver, 2016), dehydration (Arnaoutis, et al., 2015), increased and unrepaired oxidative stress, overreactive muscle damage and impaired and prolonged muscle recovery (Owens, Twist, Cobley, Howatson, & Close, 2019), anemia and pathological hemolysis response to exercise (Kokubo, Yokoyama, Kotemori, & Kawano, 2020), menstrual or fertility disturbances (Cadegiani & Kater, 2019b), pathological impairment of hormonal responses to exercises, in addition to the more than 100 dysfunctions described to be directly resulted from unhealthy sports training regimens.

Rhythmic gymnastics (RG) has been considered a sport with high risk of exposure athletes to unhealthy training regimens, since it has high concurrent requirements, including flexibility, power, strength, coordination, balance, movement technical precision, body and facial expression, and rapid decision-making abilities for optimal performance (Flessas, et al., 2015). To meet all these demands, the gymnast's routine is characterized by unusually high training loads, leading to higher risk of dysfunctional adaptations than other sports (Antualpa, Aoki, & Moreira, 2018; Codonhato, et al., 2018; Debien, Miloski, Timoteo, Ferezin, & Bara Filho, 2019; Debien, et al., 2020)

Additionally, given the sports specialization and intense training begin very early in RG, and the time window of peak performance often coincides with adolescence, these athletes have to cope with both the demands of a high-performance environment and the changes associated with physical and sexual growth and maturation (Tan, Calitri, Bloodworth, & McNamee, 2016). Thus, exposing young gymnasts to unhealthy training regimens can be even more worrisome.

While the understanding of the expected specific physiological responses to an RG training session in elite athletes is critical to distinct physiological from pathological states, there are no investigations on the specific metabolic, hormonal, and biochemical physiological adaptations to RG training. Since conditioning processes and part of biochemical adaptations to exercise are sport-specific (Cadegiani & Kater, 2019a), the extrapolation of findings from other sport modalities when analyzing RG athletes may lead to imprecise conclusions.

Owing to the lack of understanding of what to be physiologically expected from RG elite athletes, the objective of the present study was to evaluate the acute effects of an RG training day with two sessions on multiple biochemical and metabolic parameters in professional athletes of the RG Brazilian national team of RG during the pre-Olympics intensified training period.

Methods

Participants

Participants were nine group athletes of the RG Brazilian national team, starters and non-starters, among which five participated in the Japan Olympic Games in 2020. For the present study, inclusion criteria were: 1. being part of the Olympic RG Brazilian national team, based in training camp in Aracaju, Sergipe, Brazil; 2. absence of major musculoskeletal injuries; 3. absence of infections, inflammations or other disturbances during the study; and 4. not consuming supplements that include antioxidants or probiotics.

Of 11 RG athletes of the RG Brazilian national team, nine were included and two were excluded due to musculoskeletal injuries. Baseline characteristics of participants are presented in Table 1.

Table 1. Baseline characteristics of the participants (n=9)

Variables (M ± SD)	
Age (years)	17.7 ± 1.10
RG experience time (years)	9.44 ± 2.8
Body height (m)	1.65 ± 0.05
Body mass (kg)	49.70 ± 4.20
Body mass index (kg/m²)	18.20 ± 1.20
Fat mass (kg)*	5.10 ± 0.80
Body fat (%)	10.30 ± 1.20

Note. M = mean; SD = standard deviation; m = meters; kg = kilograms.

Ethical approval was obtained from the local review board (Research Ethics Committee of Federal University of Sergipe, process No. 16452219.5.0000.5546, report No. 3.677.291). Participation was voluntary, and all participants signed an informed consent form before participating in the study. This study was conducted in accordance with the original Declaration of Helsinki and further amendment.

Study design (Figure 1)
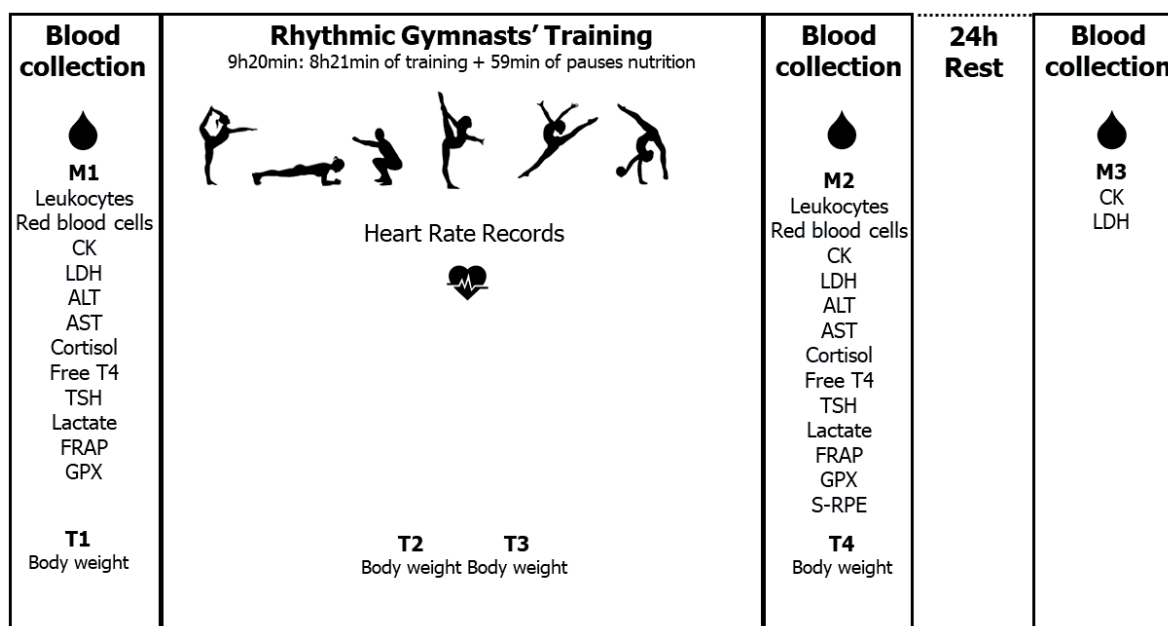
Note. M1 = moment 1; M2: moment 2; M3: moment 3. T1 = time 1 for body weight; T2 = time 2 for body weight; T3 = time 3 for body weight; T4 = time 4 for body weight; CK: creatine kinase concentrations; LDH: lactate dehydrogenase concentrations; ALT: alanine aminotransferase; AST: aspartate aminotransferase; FRAP: ferric reducing antioxidant power; GPx: glutathione peroxidase.

Figure 1. Time points of blood and salivary collection after exercise

Training characteristics

The overall intervention was a day with two separate training sessions (morning and afternoon), of 9h20min duration, including 8h21min of training and 59 minutes of intervals for meals, resting and diuresis. The RG training was coached by the technical staff, had not being interfered by any of the researchers, was recently intensified as part of a pre-season training routine, and consisted of non-standardized individual warm-up and low intensity jogging (5-min); ballet training included regular routine of classical ballet exercises on the bar, center, and floor (59 minutes); flexibility training included basic exercises for the trunk and lower limbs (15 minutes); physical training was based on resistance training exercises, such as squats, sit-ups, and trunk elevations (46 minutes); technical-driven training exercises were based on body difficulties and repetitions of isolated elements, such as dance steps, risks, exchanges, and collaborations (38 minutes), and practicing of parts of the routine training, with and without music (5h38min). This had been the same training loads as the two weeks previous to the study.

Training intensity

Objective and subjective measurement methods of training intensity were applied. Thirty minutes after the end of training sessions, gymnasts reported the self-perceived training intensity according to the session-Ratings of Perceived Exertion (s-RPE) (Foster, et al., 2001), on a scale from 0 to 10, in which zero corresponded to rest and 10 to maximal effort. Also, four of the athletes had their heart rate (HR) (beats per minutes – bpm) (Polar Team Pro, Kempele, Finland) monitored throughout the session, and training intensity was classified according to the American College of Sports Medicine (ACSM) six-intensity level category scale (Garber, et al., 2011): very light, < 57% of maximum HR; light, 57 – 63%; moderate, 64 – 76%; vigorous, 77 – 95%; near-maximal to maximal, >96%.

Blood and saliva measures

Biochemical data from blood and saliva samples were collected at three different time points: Moment 1 (M1) – immediately before the beginning of the training session used for the experiment, approximately 48 hours after the last training load was experienced; Moment 2 (M2) – immediately after the training sessions, at the end of the day, and Moment 3 (M3) – 24 hours after the training day (Figure 1).

Blood samples were collected by a qualified professional, and the materials used were adapters, 25x7 needles, and vacuum tubes; each blood sample contained 5ml of blood in tubes with EDTA (ethylenediaminetetraacetic acid) and separating gel for serum analysis. Saliva was stored in tubes specifically designed and prepared to receive saliva (Salivete®); disposable gloves; blood stop gauze dressing; and cotton and 70% alcohol for asepsis.

Samples were immediately stored in an ice cooler box at 4° C and transported for 15 minutes to the respective laboratories, where the necessary analyses were immediately performed. Athletes were required to avoid teeth brushing at least two hours before saliva collect in order to avoid microscopic blood contamination in saliva.

Biochemical parameters included red cells characterization, including erythrocytes ($*10^6/mm^3$), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume (MCV) (fl) and red cell distribution width (RDW) (%), leucocytes, including neutrophils, lymphocytes, eosinophils and monocytes, platelets ($*10^3/mm^3$) (Electrical Impedance Flow Cytometry), hormones, including salivary cortisol, thyroid-stimulating hormone (TSH) (chemiluminescence immunoassay – CLIA), free tetraiodothyronine (fT4) (CLIA), muscle- and liver-derived parameters, including serum lactate, creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and oxidative stress indicators, including ferric reducing ability of plasma (FRAP) and glutathione peroxidase (GSH-Px). Except for FRAP and GSH-Px, commercially available assays were used (LabTest®, COBAS MIRA, Roche, Germany). FRAP was determined according to the protocol by Benzie and Strain (1996) and GSH-Px according to Sies, Koch, Martino and Boveris (1979). Hormonal analyzes were carried out in a laboratory with ISO 9000-2015 certification, which guaranteed the reliability of the results. Neutrophil-to-lymphocyte ratio was also calculated for all time points.

Variations in fluid balance and biochemical parameters were assessed to determine the impact of a single RG training day on hematological, immune thyroid axis, muscle homeostasis and oxi-reductive state responses. T4 from the clinical characterization corresponds to M2 of biochemical measurements.

Hydration status

To estimate hydration status, body weight was measured at four time points of the training day: time 1 (T1), termed as initial weight 1, considered as the resting, basal body weight, immediately before the first training session started; time 2 (T2), termed as final weight 1, immediately after the morning training session (of 4h duration), before lunch interval; time 3 (T3), termed as initial weight 2, immediately before the afternoon training session, and time 4 (T4), immediately after the end of the afternoon training session (of 8h21min duration), while fluid balance was calculated by measuring oral fluid intake and estimated water content in foods as inputs, and urinary, fecal and estimated sweat volumes as outputs, and calculated for T2 and T4 of body weight.

Total sweating was estimated through an equation (Horswill, 1998), as follows:

sweating = (initial weight + quantity of liquids ingested) - (final weight - volumes of urine and fecal produced).

Hourly sweating rate was calculated through total sweating divided by the duration of the assessed period in hours. The estimated percentage of water loss was obtained by the following equation:

% dehydration = (change in body weight - urinary and fecal volume during training) / initial body weight x 100 (Burke & Hawley, 1997).

Diet

Ad libitum water intake was allowed throughout the intervention period. One day before the evaluation, athletes underwent anthropometric measurements (body mass, body height, and skinfold thicknesses) (Jackson, et al., 1980), and maintained their regular diet recommended for resting days, which consisted on average of 2031 kcal, 201g of carbohydrates (4g/kg), 86g of protein (1.7g/kg) and fat corresponding to 22% of the calories. On the day of the experiment, one hour before the beginning of training sessions, gymnasts had a controlled meal with 1.0g/kg, 0.3g/kg and 0.1g/kg of carbohydrate, protein and fat, respectively. Meals during intervals consisted of carbohydrate-electrolytes-based supplements containing ~0.3g CHO/kg of body weight/hour (~2.4g/kg for the 8h of training), while a full meal at lunchtime was provided with 1.0g/kg of carbohydrate, 0.33g protein/kg and 8g of fat. These meals reflected the usual caloric and macronutrient intake of a typical training day.

Statistical analyses

To obtain the results, descriptive statistics was first applied, with the presentation of the results in means and standard deviations. Normality and homoscedasticity were tested using Shapiro-Wilk and Levene tests, respectively. Subsequently, the t-test and ANOVA were employed to obtain statistical differences in normally distributed parameters, and results were shown as mean and standard deviation (SD), while Friedman test was employed for non-parametric parameters, and results were disclosed using medians and 95% confidence intervals (95% CI). Effect size (ES) was calculated using the following Hopkins criterion: 0-0.19 trivial; small 0.2-0.59; 0.6-1.19 moderate; 1.2-1.99 large; > 2 very large. Data were analyzed using SPSS version 21.0. The significance was p<.05.

Results

During all training stages performed in one day, with two separate training sessions, the average HR remained 129 (±11.3) bpm (64.0% maximum HR), corresponding to a moderate intensity as per the ACSM intensity level category scale. Athletes' average s-RPE corresponded to moderate-intensity training (3.2 [±0.4]).

Table 2 presents the blood count data, muscle damage indicators, and markers of training stress and redox status for all the participants at the moments immediately before and after the training (M1 and M2, respectively). There were significant increases (p<.05) in total leukocytes, lymphocytes, neutrophils, monocytes, platelets, MCHC, AST, TSH, and FRAP, as well as significant reductions in erythrocytes, hemoglobin, hematocrit, MCV, and cortisol at M2. There were no significant changes at M2 for neutrophil-lymphocytes ratio, MCH, RDW, ALT, T4 free, lactate or GSH-Px; however, a very large effect was observed for GSH-px at M2.

Figure 2 shows markers of muscle damage (CK and LDH), and their variation from the analysis at three time points. There was a significant increase (p<.05) in CK only at M3 (p=.001), and in LDH at M2 (p=0) and M3 (p=0 in relation to M1 and p=.001 in relation to M2). There was no difference in CK at M2.

Table 2. Biochemical parameters observed immediately before (M1) and immediately after (M2) a training day of the Brazilian rhythmic gymnastics national team (M ± SD)

Parameters	M1	M2	p-value	ES
Total leukocytes (/mm³)	5163.3 ± 998.9	9617.8[a] ± 1883.8	0.001	4.5
Lymphocytes (/mm³)	1752.7± 498.0	2729.7[a]± 807.0	0.003	2.0
Neutrophils (/mm³)	2873.9 ± 826.9	6163.6[a] ± 1519.9	0.001	4.0
Neutrophils:Lymphocytes ratio	1,79±0,76	2,48±1,15	0.196	1.3
Monocytes (/mm³)	255.7 ± 172.6	519.1[a] ± 212.9	0.006	1.5
Platelets (/mm³)	280,000.0 ±76,767.5	300,666.7[a] ± 89,335.0	0.009	0.3
Erythrocytes (/mm³)	4,691,111.1 ±194,643.5	4,497,777.8[a] ±243,658.5	0.001	1.0
Hemoglobin (g/dl)	12.9 ± 1.0	12.5[a] ± 1.0	0.001	0.5
Hematocrit (%)	42.1 ± 2.1	39.3[a] ± 2.5	0.001	1.3
MCV (fL)	90.1 ± 6.8	87.7[a] ± 6.7	0.001	0.4
MCH (pg)	27.7 ± 2.6	27.7 ± 2.6	1.000	0.0
RDW (%)	11.1 ± 0.6	11.2 ± 0.4	0.695	0.1
MCHC (%)	30.8 ± 1.0	31.6[a] ± 1.1	0.008	0.8
AST (U/L)	13.1 ± 6.5	25.6[a] ± 5.9	0.007	1.2
ALT (U/L)	13.1 ± 4.7	10.9 ± 2.4	0.104	0.0
TSH (µIU/ml)	1.0 ± 0.2	3.2[a] ± 2.8	0.008	9.5
Free T4 (ng/dL)	0.9 ± 0.1	0.9 ± 0.1	0.117	0.4
Lactate (mmol/L)	1.7 ± 0.2	1.8 ± 0.8	0.648	0.8
Salivary cortisol (µg/dL)	0.5 ± 0.2	0.2[a] ± 0.1	0.001	2.8
FRAP (µM)	136.8 ± 29.3	165.4[a] ± 32.1	0.001	1.0
GSH-Px (µM)	4.8 ± 4.7	15.0 ± 9.4	0.051	2.1

Note. M = mean; SD = standard deviation; M1 = moment 1; M2: moment 2; ES: effect size; AST = aspartate aminotransferase; ALT = alanine aminotransferase; TSH = thyroid-stimulating hormone; FREE T4 = free tetraiodothyronine; FRAP = ferric reducing ability of plasma; GSH-Px = glutathione; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width; MCHC = mean corpuscular hemoglobin concentration.
[a] Statistically significant difference in relation to M1 (p≤.05).

Table 3. Body hydration indicators observed between the beginning of the training and the lunch break (PRE-LUNCH), and between the end of the lunch break and the end of the training day (POST-LUNCH) of the Brazilian rhythmic gymnastics national team (M ± SD)

Parameters	Morning training session	Afternoon training session	p-value
Average liquid intake (mL)	1150.0 ± 291.0	1123.3 ± 358.7	0.781
Total sweating (mL)	1720.0 ± 520.1	1760.0 ± 540.0	0.641
Sweating rate per hour (mL/hour)	390.1 ± 120.2	340.0 ± 110.0	0.104
Percentage of dehydration (%)	1.16 ± 0.85	1.44 ± 0.65	0.496
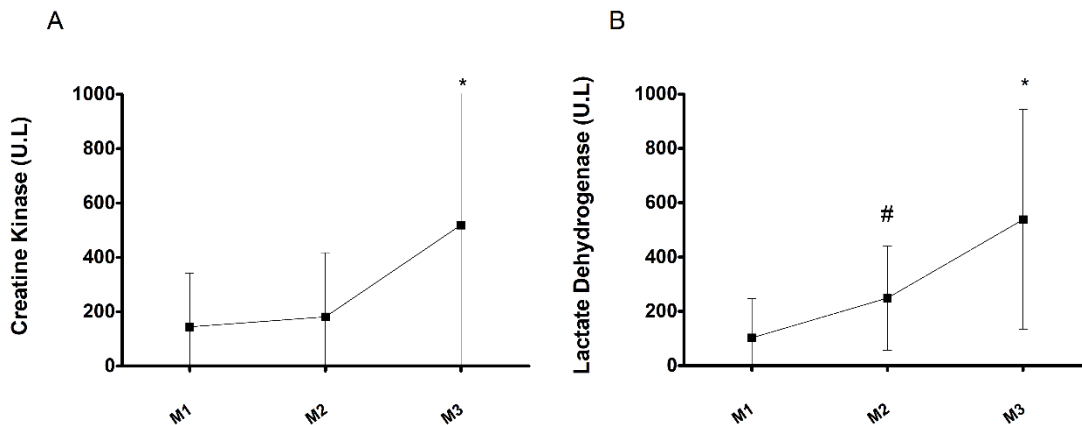
Note. *Statistically significant difference in relation to M1 (p≤.05); #Statistically significant difference in relation to M3 (p≤.05).

Figure 2. Muscle damage marker changes at three time points.

Table 3 presents parameters related to the athletes' body hydration status in the two training periods, pre-lunch and post-lunch. There was no statistical difference between the two evaluated moments in any of the parameters.

Discussion and conclusions

The main findings of the present study demonstrate that a day of RG training of moderate intensity lasting 8 hours and 21 minutes caused: 1. Increase of overall leukocytes, neutrophils, lymphocytes and monocytes; 2. Reduction in hematocrit, erythrocytes, hemoglobin and MCV; 3. Increase of platelets; 4. Elevation in muscle-derived markers, including AST immediately after it, in LDH immediately after it and 24h after it, and in CK only 24h after it; 5. Increase of TSH; and 6. Increase in oxidative-stress markers, including FRAP increase, and large effect size for GSH-Px after training; 7. Average dehydration rate of 1.3%, despite the average intake of 2.3 L of liquids during the training day.

Considering a wide variety of types of training adopted during the periodization of an elite team, it is essential to identify the intensity of a training day before evaluating its impact on physiological parameters of athletes (Zimmermann, et al., 2022). In our study, during all training stages, the intensity was moderate; nonetheless, moderate but to an extreme prolonged RG exercises probably lead to distinct responses from moderate but shorter physical efforts.

In our study, there was no significant change in lactate levels, possibly because it is better correlated with training intensity rather than training duration. Also, it is likely that intermittency, a common feature of RG training sessions (Flessas, et al., 2015), justifies the stability of lactate in our study, especially because increased speed of lactate clearance is a physiological adaptation found in athletes (Ferguson, et al., 2018). So, it would be more effi-

cient for this analysis to be carried out at specific moments of the training.

Salivary cortisol is a stress marker inherent in training; however, the present study revealed lower cortisol levels at the end of the training day. The reduction of cortisol at M2 may be explained by a long duration of training (>8h), since cortisol is highly influenced by the circadian cycle. To overcome this limitation, sequential cortisol measurements throughout the day are necessary (Silva, Silva, & Enumo, 2017).

In the present study, as expected, significant changes were observed in the total and differential leukocyte count at the end of the training day. The total number of leucocytes circulating in peripheral blood is strongly influenced by physical exercise (Gleeson, 2006). This acute increase can be perceived as a response of the innate system (neutrophils and monocytes) to tissue damage and its need for repair and remodeling, and this hypothesis is reinforced by the significant elevation of platelets (Keaney, Kilding, Merien, & Dulson, 2018), which was observed in the present study as well. Additionally, the stress arising from exercise stimulates the neuro-endocrine axis, promoting greater synthesis and secretion of catecholamines by the adrenal glands. Such hormones are responsible for the migration of these cells into the circulation, causing an acute effect of increased absolute counts of circulating leukocytes, lymphocytes, neutrophils, and monocytes (Dias, et al., 2017), just as occurred in the present study. While catecholamines induce acute rise in leucocytes, cortisol has a late effect of inhibiting mitogenesis and lymphocyte functionality, promoting immunosuppression and increasing the incidence of URIs, and consequently, lowering the volume and quality of training among athletes (Keaney, et al., 2018; Pedersen & Hoffman-Goetz, 2000). However, in the present study, besides the fact that no increase in cortisol was observed at M2, it is also noteworthy that

leukocyte analyses were only performed immediately after training, which prevented the investigation of the delayed effects of this training on leukocytes and their likely implications for the athletes' immunity. This temporal limitation in the analysis of leukocyte counts may also justify the absence of statistical difference between the values of the neutrophils-lymphocytes ratio before and immediately after the training. Both values were within the normal range.

The acute leukocytosis detected in the present study corroborates the findings of Bessa et al. (2016) and Gomes et al. (2020), who also found leukocytosis at the moment immediately after the exercise in addition to a greater contribution of neutrophils in this increase. Nevertheless, in these studies, a drop to baseline levels of most markers was also noticed after 24 hours and 30 minutes, respectively, confirming the need for these markers to be analyzed later.

In the present study, despite significant changes resulting from training, hemoglobin values were within the normal range considering reference standards for healthy individuals according to the age and sex of women athletes, thus ruling out the diagnosis of anemia. Nonetheless, it is relevant to highlight that nearly all counts related to erythrocytes and their integrity (erythrocytes, hematocrit, and MCV) presented significant decreases at the end of the training day. Elevation of these compounds is expected, especially when there is insufficient fluid replacement (Logan-Sprenger, Heigenhauser, Killian, & Spriet 2012), as observed in our study. So, in our study, the unexpected increase in erythrocytes, hematocrit, and MCV demonstrates the presence of hemolysis.

MCV should be attentively regarded, as this marker tends to increase in physiological adaptation to exercise. But we found unexpected reduction of MCV, that indicated hemolysis, reinforced by the elevation in MCHC, which revealed a reduction in viable erythrocytes compensated by a hyperchromia in the organic attempt to continue delivering oxygen efficiently to the tissues (Sureira, Amancio, & Braga, 2012).

While training was considered as being of moderate intensity, there were significant immediate elevations in LDH and AST and late elevations in LDH and CK levels, demonstrating muscle damage, in a similar manner of what was typically found in high-intensity training sessions in other sports (Gomes, et al., 2020; Harty, Cottet, Malloy, & Kerksick, 2019; Naderi, Rezvani, & Degens, 2020). CK and LDH has been correlated to bone injurie (Miyamoto, et al., 2018), and the risk of bone fractures is particularly high in aesthetic modalities such as RG (Hassmannová, Pavlů, & Nováková, 2019); therefore, assessment of bone health status and markers deserves more attention.

In our study, a very large effect size for GSH-Px and significant increase in FRAP observed immediately after the exercise reflect the effort and ability to keep balance between oxidants and antioxidants, and to prevent excessive exposure to oxidative stress, or even the ability to repair damage caused when the production of oxidants is found in greater proportions (Bellafiore, et al., 2019; Finaud, Lac, & Filaire, 2006; Fisher-Wellman & Bloomer, 2009; Petry, Alvarenga, Cruzat, & Tirapegui, 2010; Pisoschi & Pop, 2015; Savasky, Mascotti, Patel, & Rodriguez-Collazo, 2018).

In the present study, there was a significant increase in TSH immediately after the training, yet it remained within normal values, according to the reference population (Sgarbi, et al., 2013), and similar to those found in response to other sports, in an attempt to optimize the obtainment of energy for the practice of sports (Arkader, Rosa, & Moretti, 2016; Bogdanis, Philippou, Stavrinou, Tenta, & Maridaki, 2022).

To the best of our knowledge, this is the first study to analyse the acute effects of one RG training day on immune parameters, red cells, hormonal concentrations, muscle damage, oxidative stress, and hydration. The results found can be useful faced with the increasing number of very young RG practitioners, which makes it essential for the interdisciplinary health team to establish strategies ducts.

In conclusion, a day of RG training of moderate intensity and duration of 8h and 21min caused hemolysis, leukocytosis, muscle damage, perturbations in redox status, and insufficient fluid replacement during training.

References

Antualpa, K., Aoki, M.S., & Moreira, A. (2018). Intensified training period increases salivary iga responses but does not affect the severity of upper respiratory tract infection symptoms in prepuberal rhythmic gymnasts. *Pediatric Exercise Science*, *30*(2), 189-197. https://doi.org/10.1123/pes.2017-0079

Arkader, R., Rosa, M.R., & Moretti, G. (2016). Physiological changes of exercise of thermogenesis, thyroid homeostasis and inflammation. *Endocrinology and Metabolism International Journal*, *3*(4), 85-88. https://doi.org/10.15406/emij.2016.03.00055

Arnaoutis, G., Kavouras, S.A., Angelopoulou, A., Skoulariki, C., Bismpikou, S., Mourtakos, S., & Sidossis, L.S. (2015). Fluid balance during training in elite young athletes of different sports. *Journal of Strength and Conditioning Research*, *29*(12), 3447-3452. https://doi.org/10.1519/JSC.0000000000000400

Bellafiore, M., Bianco, A., Battaglia, G., Naccari, M.S., Caramazza, G., Padulo, J., Chamari, K., Paoli, A., & Palma, A. (2019). Training session intensity affects plasma redox status in amateur rhythmic gymnasts. *Journal of Sport and Health Science*, *8*(6), 561-566. https://doi.org/10.1016/j.jshs.2016.04.008

Benzie, I.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Bessa, A.L., Oliveira, V.N., Agostini, G.G., Oliveira, R.J.S., Oliveira, A.C.S., White, G.E., Wells, G.D., Teixeira, D.N.S., & Espindola, F.S. (2016). Exercise intensity and recovery: Biomarkers of injury, inflammation, and oxidative stress. *Journal of Strength and Conditioning Research*, *30*(2), 311-319. https://doi.org/10.1519/JSC.0b013e31828f1ee9

Bogdanis, G.C., Philippou, A., Stavrinou, P.S., Tenta, R., & Maridaki, M. (2022). Acute and delayed hormonal and blood cell count responses to high-intensity exercise before and after short-term high-intensity interval training. *Research in Sports Medicine (Print)*, *30*(4), 400-414. https://doi.org/10.1080/15438627.2021.1895783

Burke, L.M., & Hawley, J.A. (1997). Fluid balance in team sports. *Sports Medicine*, *24*(1), 38-54. https://doi.org/10.2165/00007256-199724010-00004

Cadegiani, F.A., & Kater, C.E. (2019a). Enhancement of hypothalamic-pituitary activity in male athletes: Evidence of a novel hormonal mechanism of physical conditioning. *BMC Endocrine Disorders*, *19*(1), 117. https://doi.org/10.1186/s12902-019-0443-7

Cadegiani, F.A., & Kater, C.E. (2019b). Novel causes and consequences of overtraining syndrome: The EROS-DISRUPTORS study. *BMC Sports Science, Medicine and Rehabilitation*, *11*(1), 21. https://doi.org/10.1186/s13102-019-0132-x

Codonhato, R., Rubio, V., Oliveira, P.M.P., Resende, C.F., Rosa, B.A.M., Pujals, C., & Fiorese, L. (2018). Resilience, stress and injuries in the context of the Brazilian elite rhythmic gymnastics. *PloS One*, *13*(12), e0210174. https://doi.org/10.1371/journal.pone.0210174

Debien, P., Miloski, B., Timoteo, T., Ferezin, C., & Bara Filho, M. (2019). Weekly profile of training load and recovery in elite rhythmic gymnasts. *Science of Gymnastics Journal*, *11*(1), 23-35.

Debien, P., Miloski, B., Werneck, F., Timoteo, T., Ferezin, C., Bara Filho, M., & Gabbett, T. (2020). Training load and recovery during a pre-Olympic season in professional rhythmic gymnasts. *Journal of Athletic Training*, *55*(9), 977-983. https://doi.org/10.4085/1062-6050-402.19

Dias, R., Baganha, R.J., Cieslak, F., Krinski, K., Camarço, N.F., Verlengia, R., Lopes, C.R., Prestes, J., Silva, C.A. da, & Cavaglieri, C.R. (2017). Parâmetros imunológicos e infecções do trato respiratório superior em atletas de esportes coletivos. [Immunological parameters and upper respiratory tract infections in team sports athletes.] *Revista Brasileira de Medicina do Esporte*, *23*(1), 66-72. https://doi.org/10.1590/1517-869220172301149299

Ferguson, B.S., Rogatzki, M.J., Goodwin, M.L., Kane, D.A., Rightmire, Z., & Gladden, L.B. (2018). Lactate metabolism: Historical context, prior misinterpretations, and current understanding. *European Journal of Applied Physiology*, *118*(4), 691-728. https://doi.org/10.1007/s00421-017-3795-6

Finaud, J., Lac, G., & Filaire, E. (2006). Oxidative stress: Relationship with exercise and training. *Sports Medicine (Auckland, N.Z.)*, *36*(4), 327-358. https://doi.org/10.2165/00007256-200636040-00004

Fisher-Wellman, K., & Bloomer, R.J. (2009). Acute exercise and oxidative stress: A 30 year history. *Dynamic Medicine : DM*, *8*, 1. https://doi.org/10.1186/1476-5918-8-1

Flessas, K., Mylonas, D., Panagiotaropoulou, G., Tsopani, D., Korda, A., Siettos, C., di Cagno, A., Evdokimidis, I., & Smyrnis, N. (2015). Judging the judges' performance in rhythmic gymnastics. *Medicine and Science in Sports and Exercise*, *47*(3), 640-648. https://doi.org/10.1249/MSS.0000000000000425

Foster, C., Florhaug, J.A., Franklin, J., Gottschall, L., Hrovatin, L.A., Parker, S., Doleshal, P., & Dodge, C. (2001). A new approach to monitoring exercise training. *Journal of Strength and Conditioning Research*, *15*(1), 109. https://doi.org/10.1519/1533-4287(2001)015<0109:ANATME>2.0.CO;2

Garber, C.E., Blissmer, B., Deschenes, M.R., Franklin, B.A., Lamonte, M.J., Lee, I.-M., Nieman, D.C., & Swain, D.P. (2011). Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults. *Medicine and Science in Sports and Exercise*, *43*(7), 1334-1359. https://doi.org/10.1249/MSS.0b013e318213fefb

Gleeson, M. (2006). Immune system adaptation in elite athletes. *Current Opinion in Clinical Nutrition and Metabolic Care*, *9*(6), 659-665. doi: 10.1097/01.mco.0000247476.02650.18

Gomes, J.H., Mendes, R.R., Franca, C.S., da Silva-Grigoletto, M.E., Pereira da Silva, D.R., Antoniolli, A.R., de Oliveira E Silva, A.M., & Quintans-Júnior, L.J. (2020). Acute leucocyte, muscle damage, and stress marker responses to high-intensity functional training. *PloS One*, *15*(12), e0243276. https://doi.org/10.1371/journal.pone.0243276

Harty, P.S., Cottet, M.L., Malloy, J.K., & Kerksick, C.M. (2019). Nutritional and supplementation strategies to prevent and attenuate exercise-induced muscle damage: A brief review. *Sports Medicine – Open*, *5*(1), 1. https://doi.org/10.1186/s40798-018-0176-6

Hassmannová, K., Pavlů, D., & Nováková, T. (2019). Most common injuries of the musculoskeletal system among children of elementary school age who engage in gymnastic sports (aerobics, artistic or rhythmic gymnastics) at an elite level. *Acta Universitatis Carolinae Kinanthropologica*, *55*(1), 10-20. https://doi.org/10.14712/23366052.2019.2

Horswill, C.A. (1998). Effective fluid replacement. *International Journal of Sport Nutrition*, *8*(2), 175-295. https://doi.org/10.1123/ijsn.8.2.175

Issurin, V.B. (2010). New horizons for the methodology and physiology of training periodization. *Sports Medicine (Auckland, N.Z.)*, *40*(3), 189-206. https://doi.org/10.2165/11319770-000000000-00000

Jackson, A.S., Pollock, M.L., & Ward, A. (1980). Generalized equations for predicting body density of women. *Medicine and Science in Sports and Exercise*, *12*(3), 175-181.

Keaney, L.C., Kilding, A.E., Merien, F., & Dulson, D.K. (2018). The impact of sport related stressors on immunity and illness risk in team-sport athletes. *Journal of Science and Medicine in Sport*, *21*(12), 1192-1199. https://doi.org/10.1016/j.jsams.2018.05.014

Kellmann, M., Bertollo, M., Bosquet, L., Brink, M., Coutts, A.J., Duffield, R., Erlacher, D., Halson, S.L., Hecksteden, A., Heidari, J., Kallus, K.W., Meeusen, R., Mujika, I., Robazza, C., Skorski, S., Venter, R., & Beckmann, J. (2018). Recovery and performance in sport: Consensus statement. *International Journal of Sports Physiology and Performance*, *13*(2), 240-245. https://doi.org/10.1123/ijspp.2017-0759

Kokubo, Y., Yokoyama, Y., Kisara, K., Ohira, Y., Sunami, A., Yoshizaki, T., Tada, Y., Ishizaki, S., Hida, A., & Kawano, Y. (2016). Relationship between dietary factors and bodily iron status among japanese collegiate elite female rhythmic gymnasts. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(2), 105-113. https://doi.org/10.1123/ijsnem.2015-0123

Kokubo, Y., Yokoyama, Y., Kotemori, A., & Kawano, Y. (2020). Seasonal changes in body iron status including erythropoiesis and hemolysis and dietary intakes among Japanese collegiate elite female rhythmic gymnasts. *The Journal of Physical Fitness and Sports Medicine*, *9*(4), 149-156. https://doi.org/10.7600/jpfsm.9.149

Logan-Sprenger, H.M., Heigenhauser, G.J.F., Killian, K.J., & Spriet, L.L. (2012). Effects of dehydration during cycling on skeletal muscle metabolism in females. *Medicine and Science in Sports and Exercise*, *44*(10), 1949-1957. https://doi.org/10.1249/MSS.0b013e31825abc7c

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., Raglin, J., Rietjens, G., Steinacker, J., Urhausen, A., European College of Sport Science, & American College of Sports Medicine. (2013). Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the European College of Sport Science and the American College of Sports Medicine. *Medicine and Science in Sports and Exercise*, *45*(1), 186-205. https://doi.org/10.1249/MSS.0b013e318279a10a

Miyamoto, T., Oguma, Y., Sato, Y., Kobayashi, T., Ito, E., Tani, M., Miyamoto, K., Nishiwaki, Y., Ishida, H., Otani, T., Matsumoto, H., Matsumoto, M., & Nakamura, M. (2018). Elevated creatine kinase and lactic acid dehydrogenase and decreased osteocalcin and uncarboxylated osteocalcin are associated with bone stress injuries in young female athletes. *Scientific Reports*, *8*(1), 18019. https://doi.org/10.1038/s41598-018-36982-0

Naderi, A., Rezvani, M.H., & Degens, H. (2020). Foam rolling and muscle and joint proprioception after exercise-induced muscle damage. *Journal of Athletic Training*, *55*(1), 58-64. https://doi.org/10.4085/1062-6050-459-18

Owens, D.J., Twist, C., Cobley, J.N., Howatson, G., & Close, G.L. (2019). Exercise-induced muscle damage: What is it, what causes it and what are the nutritional solutions? *European Journal of Sport Science*, *19*(1), 71-85. https://doi.org/10.1080/17461391.2018.1505957

Pedersen, B.K., & Hoffman-Goetz, L. (2000). Exercise and the immune system: Regulation, integration, and adaptation. *Physiological Reviews*, *80*(3), 1055-1081. https://doi.org/10.1152/physrev.2000.80.3.1055

Petry, É., Alvarenga, M., Cruzat, V., & Tirapegui, J. (2010). Exercício físico e estresse oxidativo: mecanismos e efeitos. [Exercise and oxidative stress: Mechanisms and effects.] *Revista Brasileira de Ciência e Movimento*, *18*(4), 90-99. https://doi.org/10.18511/rbcm.v18i4.1363

Pisoschi, A.M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, *97*, 55-74. https://doi.org/10.1016/j.ejmech.2015.04.040

Savasky, B.M., Mascotti, D.P., Patel, N., & Rodriguez-Collazo, E. (2018). Nutritional and pharmacological effects on oxidative stress in soft tissue and bone remodeling. *Journal of Nutrition and Metabolism*, *2018*, 4183407. https://doi.org/10.1155/2018/4183407

Sgarbi, J.A., Teixeira, P.F.S., Maciel, L.M.Z., Mazeto, G.M.F.S., Vaisman, M., Montenegro Junior, R.M., & Ward, L.S. (2013). Consenso brasileiro para a abordagem clínica e tratamento do hipotireoidismo subclínico em adultos: recomendações do Departamento de Tireoide da Sociedade Brasileira de Endocrinologia e Metabologia. [The Brazilian consensus for the clinical approach and treatment of subclinical hypothyroidism in adults: Recommendations of the thyroid Department of the Brazilian Society of Endocrinology and Metabolism.] *Arquivos Brasileiros de Endocrinologia & Metabologia*, *57*(3), 166-183. https://doi.org/10.1590/S0004-27302013000300003

Sies, H., Koch, O.R., Martino, E., & Boveris, A. (1979). Increased biliary glutathione disulfide release in chronically ethanol-treated rats. *FEBS Letters*, *103*(2), 287-290.

Silva, A.M.B. da, Silva, M.L.B. da, & Enumo, S.R.F. (2017). Relações entre o hormônio cortisol e comportamentos de adolescentes: Uma revisão sistemática. [Relationship between cortisol hormone and adolescent behavior: A systematic review.] *Psicologia Revista*, *26*(2), 337. https://doi.org/10.23925/2594-3871.2017v26i2p.337-362

Sureira, T.M., Amancio, O.S., & Braga, J.A.P. (2012). Influence of artistic gymnastics on iron nutritional status and exercise-induced hemolysis in female athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(4), 243-250. https://doi.org/10.1123/ijsnem.22.4.243

Tan, J.O.A., Calitri, R., Bloodworth, A., & McNamee, M.J. (2016). Understanding eating disorders in elite gymnastics: Ethical and conceptual challenges. *Clinics in Sports Medicine*, *35*(2), 275-292. https://doi.org/10.1016/j.csm.2015.10.002

Tian, Y., He, Z., Zhao, J., Tao, D., Xu, K., Midgley, A., & McNaughton, L. (2015). An 8-year longitudinal study of overreaching in 114 elite female Chinese wrestlers. *Journal of Athletic Training*, *50*(2), 217-223. https://doi.org/10.4085/1062-6050-49.3.57

Walsh, N.P., & Oliver, S.J. (2016). Exercise, immune function and respiratory infection: An update on the influence of training and environmental stress. *Immunology and Cell Biology*, *94*(2), 132-139. https://doi.org/10.1038/icb.2015.99

Zimmermann, P., Schöffl, I., Schöffl, V., Zimmermann, L., Eckstein, M.L., Moser, O., & Wüstenfeld, J. (2022). Physiological effects of training in elite german winter sport athletes: Sport specific remodeling determined using echocardiographic data and CPET performance parameters. *Journal of Cardiovascular Development and Disease*, *9*(8), 235. https://doi.org/10.3390/jcdd9080235

Correspondence to:
João Henrique Gomes, Ph.D.
Health Sciences Graduate Program,
Federal University of Sergipe
Rua Cláudio Batista s/n, Centro de Pesquisas
Biomédicas, Hospital Universitário. Bairro Cidade
Nova – Aracaju – SE. CEP: 49060-108
E-mail: profjhgomes@gmail.com