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Blood cardiac biomarkers responses are associated with 24 h ultramarathon performance

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ABSTRACT

Purpose: Clinical significance of cardiac biomarkers response in ultra-endurance runners are not completely elucidated because events vary in distance/duration and competitors modulate running intensity according to individual running capacity. The aim of this study was to examine the relationship between self-selected exercise intensity with cardiac biomarkers comparing experienced (EXP, N = 11) and novice (NOV, N = 14) runners able to finish a 24h ultramarathon (24UM) with significant differences in performance.
Methods: Cardiac biomarkers (i.e. CKMB/totalCK, cTnT and NT-proBNP), inflammatory markers (i.e. leukocytes and CRP) and cortisol were analyzed before and after a 24UM.
Results: EXP finished the race with significant ($p < 0.05$) longer distance than NOV (158.8 ± 15.8 vs 116.8 ± 10.3 Km). Two-way mixed ANOVA showed significant time × performance level interaction with greater increase of cTnT ($F(1,23) = 6.18$, $p = 0.021$), NT-proBNP ($F(1,23) = 9.27$, $p = 0.006$) and cortisol ($F(1, 23) = 5.13$, $p = 0.03$) in the EXP group. CKMB/totalCK ($F(1, 23) = 71.90$, $p < 0.0001$) decreased while leukocytes ($F(1, 23) = 100.06$, $p < 0.0001$) and CRP ($F(1, 23) = 93.37$, $p < 0.0001$) increased in both groups (main effect of time). Correlations were found between 24UM distance and cortisol (r = 0.58; $p = 0.002$), CKMB (r = 0.47; $p = 0.017$), cTnT (r = 0.44; $p = 0.027$) or NT-proBNP (r = 0.56; $p = 0.003$). Cortisol and NT-proBNP were also significantly correlated (r = 0.51; $p = 0.01$).
Conclusions: Although there is no clear evidence of cardiac risk when comparing cardiac biomarkers levels with clinical cut-off values, cardiac biomarkers are associated with running performance and pituitary-adrenocortical system response. In EXP runners, higher levels of cardiac biomarkers and cortisol suggest a more hemodynamically challenged heart during prolonged endurance exercise.

1. Introduction

The interest in ultra-endurance events has increased considerably in the last few decades, particularly in running. According to the International Association of Ultrarunners (IAU 2018), a running competition with distances beyond marathon (i.e. 42.195 Km) is considered an ultra-marathon. The increasing number of ultra-endurance running races worldwide (IAU 2018) upholds relevance for studies providing information about the benefits and risks of such physiological stress. Studies on prolonged strenuous exercise have highlighted cardiac risks, such as the transient loss of ventricular function, increased heart tissue damage and the subsequent appearance of myocardial injury biomarkers in the blood (George et al., 2008; Legaz-Arrese et al., 2011; Scott and Warburton, 2008; Shave et al., 2007).

A number of cardiac biomarkers have been used to understand the impact of acute exercise upon cardiomyocytes, including markers of necrosis, inflammation, cardiac function and ischemia (Shave et al., 2007). Historically, creatine kinase MB isoenzyme (CKMB) has been considered as the main biochemical marker of cardiomyocyte injury. Currently, the measurements of cardiac troponin T (cTnT) and troponin I (cTnI) are considered the "gold standard" tests for myocardial damage and ischemia (Regwan et al., 2010). Moreover, B-type natriuretic peptide

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(BNP) and N-terminal proBNP (NT-proBNP) have gained acceptance as markers of ventricular overload (Shave et al., 2007; Hammerer-Lercher et al., 2008).

Marathon and ultramarathon running have been associated with changes in cardiac markers. Several studies showed elevations in the CKMB (Smith et al., 2004; Kim et al., 2014), cardiac troponin T (Scherr et al., 2011; Kłapcińska et al., 2013) and NT-pro BNP (Scott et al., 2009; Scherr et al., 2011; Kłapcińska et al., 2013) after prolonged strenuous exercise. Nevertheless, exercise-induced elevations of cardiac markers do not necessarily represent permanent heart injury in ultrarunners. Cardiac biomarkers as NT-proBNP and cTnT peak after prolonged endurance exercise and return to normal values within 72 h (Scherr et al., 2011). Moreover, the clinical significance of changes in cardiac biomarkers after prolonged strenuous exercise is not clear and increases in those blood biomarkers may be a physiological signal for positive heart function adaptation when exercise and recovery are well balanced (Shave et al., 2007; Whyte, 2008).

The apparent lack of evidently unfavorable permanent effects of the ultra-endurance exercise may not reflect the potential cardiac risk in the long-term (Niemelä et al., 1984; Harper, 2010). There is a wide range of ultramarathon distances (e.g. 50, 100, 200 Km or more) or durations (e.g. 12, 24, 48 h) with recreational, amateur and professional runners (IAU 2018) able to complete the races performing according to their physiological capacity, training status and personal motivations(McCormick et al., 2015). Therefore, it is challenging for sports medicine to generalize about cardiac risks and biomarkers response in ultra-endurance runners because events vary widely in distance/duration and competitors modulate running intensity according to individual exercise tolerance and motivations to push themselves to evermore heights of physical endurance (St Clair Gibson et al., 2006; Tucker, 2009; Marcora and Staiano, 2010).

An ultra-endurance competition is a free paced time trial, that is, self-selected exercise intensity where the endpoint is previously set. Runners with differences in training experience and competitive performances may present differences in pace strategy and cardiac biomarkers responses. For instance, the running pace during a 24h ultra-endurance competition is roughly a reverse-J shape pattern where the fastest runners start at lower relative intensities and display a more even pacing strategy than slower runners (Bossi et al., 2017). Moreover, non-elite runners with less training showed increased cardiac biomarkers after a marathon compared to runners with greater training distances (Neilan et al., 2006). On the other hand, Hottenrott et al. (2016) compared "run/walk" with "running only" pacing strategies during a marathon and found a similar increment in cardiac biomarkers of both groups. However, "run/walk" and "running only" groups finished the marathon with similar times and the run/walk strategy was imposed by the research protocol design, therefore, pacing strategies may not reflect differences in overall physiological effort between groups and it may have also prevented self-selected exercise intensity of the "run/walk" group. Hereof, there is a need for studies relating self-selected running intensity with cardiac biomarkers comparing ultrarunners with differences in training levels and speed performance.

The aim of this study was to examine the relationship between self-selected exercise intensity with cardiac biomarkers comparing experienced (EXP) and novice (NOV) runners able to finish a 24h ultramarathon (24UM) with significant differences in running performance. We also aimed to discuss the clinical significance and potential risks related to cardiac biomarkers changes found in both groups of runners. A secondary purpose was to investigate potential differences among ultrarunners with differences in training levels and performance in cortisol, total CK, C-reactive protein (CRP) and leukocytes levels.

2. Material and methods

This is an observational study approved by the local Ethics Committee (proc. 1895–2011) affiliated with the National Commission of Ethics in Research and was performed in accordance with the principles of the Helsinki Declaration. The participants provided written informed consent before enrolment.

2.1. Subjects and participation criteria

On the day before the competition, a technical conference was held presenting to the participants the course of the race, medical team and the procedures regarding this present research. Volunteered runners were arbitrarily divided into two groups according to their training level and previous experience. EXP (N = 11) group consisted of volunteers training for endurance running for at least 5-years, with training frequency of 6-days/week, accumulated running distance of ≥100 km/week and participation of at least one 6 h-competitive race per year. NOV (N = 14) group consisted of volunteers who have run at least one marathon within the last twelve months prior to the 24UM but no ultramarathon.

The runners had to meet the following criteria to be included: 1) no smoking history during the last six months, 2) no cardiovascular or metabolic disease, 3) no systemic hypertension (≥140/90 mmHg) or use of antihypertensive medication, 4) no use of creatine supplementation, anabolic steroids, drugs or medication with potential effects on physical performance (self-reported), and 5) no musculoskeletal injury.

2.2. Anthropometric measures

Body mass was measured with runners dressed in their usual race clothes (accurate to 100 g), and height was determined without shoes (accurate to 0.1 cm) using a weight scale with a stadiometer (Detecto 439, New York, USA). Body mass measurements were carried out before and after the race.

2.3. Race conditions

The competition started at 10:00 am. Prior to the race, the runners were instructed to ingest their usual breakfast as well as to maintain hydration and feeding procedures *ad libitum* during the race (no interference of researchers). The air temperature during the race measured every 2 hours was 23 ± 3 °C (range 21–26 °C) and the relative humidity was 71 ± 4% (range 61–79%). The weather was mostly cloudy with short periods of sun exposure during the day, drizzle rain between the 12th and 13th hour and heavy rain between the 13th and 17th hours of the race. The race loop was 2725 m long and the surface was dirt ground with slight slopes (accumulated altitude difference 27 m per loop). All runners received an electronic chip which was fixed to running shoes to record the total distance. A medical team and physiotherapy staff were available to runners during the race. Solid meals were offered during the race at 2–4 h (pasta with tomato sauce), 8–10 h (mashed potatoes) and 14–16 h (sandwiches). Beverages (such as various fruits, water, isotonic beverages, and soda) were offered during the 24h. Meals were eaten on the move or in accordance with each runner's planning (researchers and/or organizers did not interfere in the plan).

2.4. Blood samples and analyses

Blood samples (20 mL) were collected by a professional nurse in EDTA (ethylenediaminetetraacetic acid) tubes prior to (8:00–10:00 am) and after (10:00–11:00 am) the 24UM. Test tubes were carefully inverted vertically to ensure thorough mixing of the anticoagulant (i.e. EDTA) with blood to prevent clot formation. Samples were stored in an insulated box (- 8° to +2 °C) and sent to the Laboratory of Clinical Analysis of the Military Hospital (Brasilia, Federal District, Brazil) certified by the National Program of Quality Control in Clinical Analysis (Brazilian Society of Clinical Analysis). All analytical kits were provided by Roche Diagnostics (Rotkreuz, Switzerland). Total CK was measured in serum using a kinetic UV method (Hitachi Modular® Analytics; Roche-Hitachi

Diagnostics, Tokyo, Japan). Serum CKMB, cTnT, cortisol, and plasma NT-proBNP levels were measured by electrochemiluminescence immunoassay method (Elecsys® analyzer, Roche Diagnostics, Rotkreuz, Switzerland). The technical limit of cTnT detection was 0.003 ng/mL. Serum CRP was analyzed by the nephelometric method (Array 360 System Beckman, Abbott Laboratories, North Chicago, IL, USA). Blood leukocyte counts were analyzed using an automated hematology analyzer (Horiba ABX, São Paulo, SP, Brazil).

3. Analysis

3.1. Statistical analysis

All figures, descriptive and inferential statistics were performed using GraphPad Prism 6.0 (San Diego, USA). The Shapiro-Wilk test was applied to verify the distribution of the data. Unpaired Student's *t*-test was used to compare the variables shown in Table 1. Two-way mixed ANOVA was used for the analysis of the main effects of time, performance level and time × performance level interaction on blood biomarkers followed by Bonferroni *post hoc* test (Table 2, Figs. 1 and 2). The independent paired variable was time (i.e. two levels: pre- and post-24UM) and the unpaired variable was a competitive running level (i.e. two levels: EXP and NOV).

Pearson's correlation coefficient (r) was used to analyze the relationship between blood biomarkers levels after 24UM and running distance (i.e. performance) or cortisol. Correlations were reported when significant. The level of significance was set at $p < 0.05$.

4. Results

All runners successfully completed the 24UM. Participant characteristics and performance are shown in Table 1. NOV and EXP were similar in age, BMI, body mass, relative loss (%) in body mass after race and years of dedication in endurance competitions. Hemoglobin and hematocrit levels did not change after the race in both groups. EXP runners finished the race with a significantly longer distance than NOV ($p < 0.001$).

Table 1
Characteristics of Novice (NOV) and Experienced (EXP) groups.

	NOV	EXP
Subjects (N)	**14**	**11**
Age (Years)	**43.1 ± 8.7**	**41.4 ± 9.1**
(min-max)	(31–58)	(26–57)
BMI (Kg/m^2)	**25.1 ± 2.5**	**24.3 ± 2.2**
(min-max)	(21.2–31.3)	(20.4–27.4)
Body mass (Kg)	**75.7 ± 9.4**	**73.7 ± 9.4**
(min-max)	(62.4–95.9)	(57.6–87.8)
Decrease in BM after race (%)	**2.5 ± 0.4**	**3.6 ± 0.6**
(min-max)	(-0.4 - 6.2)	(1.1–7.8)
Experience in competitions (years)	**4.5 ± 5.8**	**5.4 ± 4.0**
(min-max)	(0.1–22)	(1–15)
Distance covered (Km)	**116.8 ± 10.3**	**158.8 ± 15.8** *
(min-max)	(100.8–130.8)	(138.9–188.0)
Hemoglobin (g/dL)		
pre-race	**15.0 ± 0.4**	**14.5 ± 0.5**
post-race	**15.2 ± 0.6**	**14.7 ± 0.5**
Hematocrit (%)		
pre-race	**46.6 ± 1.4**	**45.3 ± 1.7**
post-race	**47.5 ± 2.6**	**46.5 ± 1.4**

Data are expressed as mean ± SD.
Unpaired t-test *$p < 0.001$.
BMI, Body mass index.

4.1. Cortisol, leukocytes and CRP

Fig. 1 shows a significant time × performance level interaction for cortisol ($F(1, 23) = 5.13$, $p = 0.03$).

Significant main effect of time for leukocytes ($F(1, 23) = 100.06$, $p < 0.0001$) and CRP ($F(1, 23) = 93.37$, $p < 0.0001$) shows increment in both groups.

4.2. CK, CKMB and CKMB/total CK

Table 2 shows tendency of time × performance level interaction ($p = 0.07$) for Total CK and significant interaction ($p < 0.0001$) for CKMB. Significant differences in post-race Total CK and CKMB comparing EXP and NOV were found after *post hoc* test ($p < 0.05$ and $p < 0.001$, respectively).

Significant main effect of time was observed for CKMB/Total CK % ($p < 0.0001$) with no interaction. Of note, whereas total CK and CKMB increased after the race, CKMB/Total CK % decreased in both groups.

4.3. cTnT and NT-pro BNP

Most of the subjects presented cTnT values below the detection limit of 0.003 ng/mL before the race. For statistical analysis, samples below the detection limit were considered as 0.003 ng/mL. Fig. 2A and B show significant interaction for cTnT ($F(1,23) = 6.18$, $p = 0.021$) and NT-proBNP ($F(1,23) = 9.27$, $p = 0.006$), respectively. Significant differences in post-race cTnT and NT-proBNP comparing EXP and NOV were found after *post hoc* test ($p < 0.01$ and $p < 0.05$, respectively).

4.4. Correlations

Significant correlations were found between running distance and cortisol ($r = 0.58$; $p = 0.002$, Fig. 3A), CKMB ($r = 0.47$; $p = 0.017$, Fig. 3B), cTnT ($r = 0.44$; $p = 0.027$, Fig. 3C) and NT-pro BNP ($r = 0.56$; $p = 0.003$, Fig. 3D). Cortisol and NT-proBNP were also significantly correlated ($r = 0.51$; $p = 0.01$, Fig. 4). No significant correlations were found between other blood biomarkers measured in this study with running distance or cortisol (data not shown).

5. Discussion

The main findings of this study were that (i) greater increase of cardiac biomarkers in the EXP group compared to NOV and (ii) significant correlations between cardiac biomarkers or cortisol with the 24UM running performance. In general, our findings indicate that running performance is associated with cardiac biomarkers and with a physiological response of the pituitary-adrenocortical system during a 24h self-paced running competition. This confirms that cortisol is a good predictor of speed during a 24 h ultra-endurance competition (Tauler et al.,

Table 2
Total CK and CKMB pre and post 24h ultramarathon in novice (NOV) and experienced (EXP) groups (mean ± SEM).

		Pre-race	Post-race		Time effect	Interaction	Differences post-race EXP x NOV
Total CK (U/L)	**EXP**	133.7 ± 18.0	6859.3 ± 2176.1	*p*	0.0002	0.07	<0.05
	NOV	140.5 ± 41.3	2895.9 ± 855.1	F (1, 23)	20.83	3.65	
CKMB (U/L)	**EXP**	3.0 ± 0.3	91.1 ± 22.0	*p*	<0.0001	<0.0001	<0.001
	NOV	2.8 ± 0.6	28.3 ± 8.7	F (1, 23)	27.72	8.40	
CKMB/Total CK (%)	**EXP**	2.5 ± 0.1	1.6 ± 0.2	*p*	<0.0001	0.39	ns
	NOV	2.4 ± 0.2	1.2 ± 0.1	F (1, 23)	71.90	0.76	

ns: non-significant; Bonferroni *post hoc* test.

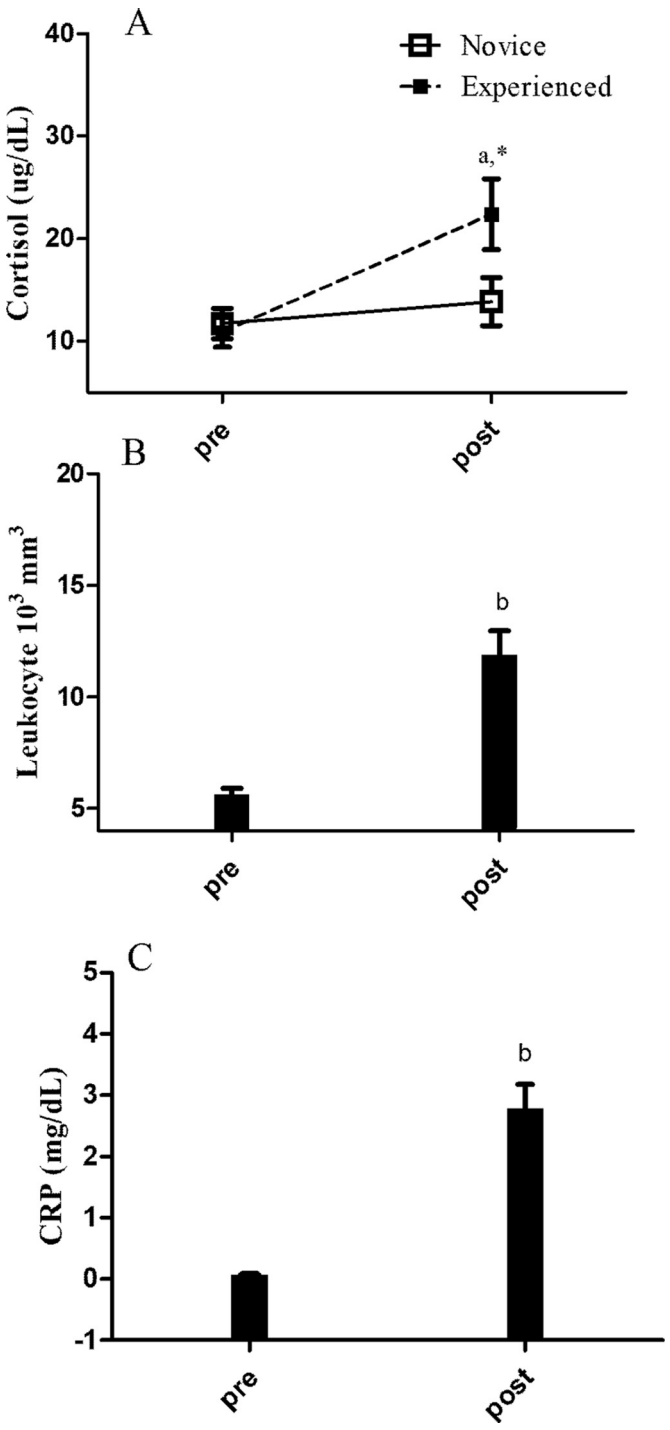

Fig. 1. Cortisol (panel A), leukocyte count (panel B) and C-reactive protein (CRP, panel C) responses *pre-* and *post* 24h ultra-marathon (mean ± SEM) in novice (N = 14) and experienced (N = 11) runners. Two-way mixed measures ANOVA: [a] significant interaction (time × performance level) and [b] main effect of time. [*] significant difference between novice and experienced; Bonferroni *post hoc* test. $p < 0.05$.

2014) and that the activity of the pituitary-adrenocortical system may be also a good indicator of the effort during prolonged running (Vuorimaa et al., 2008).

The higher cortisol level observed in the EXP group (Fig. 1A) is in accordance with other studies on 24UM. Fellmann et al. (1988) showed ~3 times increased cortisol levels after 24UM with distances ranging from 132.13 to 187.75 Km (mean ± SD, 166.36 ± 16.23 Km) in runners used to training distances above 80 Km per week. Kupchak et al. (2014) found 4 fold increase in cortisol after 161 Km with a mean finish time of 25.08 h (range 22.53 h–27.62 h) in runners used to weekly training distances of ~100 Km. Previous (Fellmann et al., 1988; Millet et al., 2011; Kupchak et al., 2014) and present data on experienced ultra-runners showed a consistent increase in blood cortisol after 24UM. On the other hand, the cortisol level found in the NOV group was not reported by previous studies.

Moderate to high intensity exercise that exceeds 60% of VO_2max cause intensity-dependent elevations in blood cortisol levels (Brandenberger and Follenius, 1975; Luger et al., 1987; Hill et al., 2008). When exercise intensity is below the threshold, a reasonably larger amount of exercise has to be performed to achieve the threshold by duration (Viru, 1992). Cortisol release presents a biphasic behavior during prolonged exercise with an initial increase, that is substituted by a decrease to pre-exercise levels followed by a secondary increase during the second hour of exercise (Viru, 2004). The secondary increase is much more pronounced in endurance-trained athletes than in less trained athletes during 2h of exercise at ~60% VO_2 max (Viru et al., 1992). Despite the large difference in exercise duration between 24UM and the previous studies that evaluated the cortisol response during exercise, we speculate that differences in cortisol levels observed after the 24UM may be related with differences in training status between NOV and EXP groups. Considering this finding, the clinical significance of cardiac blood biomarkers within the context of 24UM is discussed herein.

5.1. Total CK, CKMB and CKMB/Total CK analysis

Despite differences in running performance, EXP and NOV groups showed increased inflammatory markers (Fig. 1B and C) which are related to skeletal muscle injury and repair in ultra-endurance(Kim et al., 2007). Moreover, the total CK also increased in both groups with greater increment in total CK observed in the EXP group, adding evidence of greater skeletal muscle damage in this group after 24UM. In this sense, the interpretation of CKMB should be contextualized in prolonged exercise when muscle damage influences the total CK level in blood. The concentration of CKMB isoform in skeletal muscle may be approximately 4% of total CK, furthermore, trained athletes can present greater concentration of CKMB in skeletal muscle compared to less trained counterparts (i.e. 5–6% and 2-1%, respectively) (Sylven et al., 1983). Therefore, within the context of ultra-endurance exercise, the ratio of CKMB to total CK is a more accurate marker of cardiac risk when the clinical threshold of 5–6% is achieved (Shave et al., 2007) representing additional CKMB leakage from cardiomyocytes. Our data shows that none of the runners exceeded 2% CKMB/Total CK, an indicative of non-pathological cardiac CKMB release (Lott and Stang, 1980). The decrease in CKMB/Total CK observed in both groups (Table 2) also supports that CKMB leakage from cardiomyocytes is unlikely to be significant in this present study. Of note, we found elevated CKMB levels above clinical cut-off for cardiac risk (Apple et al., 2008) in both EXP and NOV groups, however, the assumption of cardiac risk interpreting only the CKMB is not straightforward for clinical assessment when total CK and CKMB increases due to skeletal muscle injury in ultra-endurance exercise context.

5.2. Cardiac troponin T analysis

Considered as a gold standard for acute myocardial infarction (Collinson et al., 2003), cTnT elevation occurs when there is myocyte injury (Baig et al., 2006). However, during prolonged exercise, cTnT increment may be expected. Shave et al. (2007) presented a theoretical explanation for the exercise-induced cTnT release, that is, transient disruption of plasma membrane due to mechanical stimuli and stress-induced overload of free radicals causing a transient membrane permeability and, therefore, leakage of troponin from the cytosol to blood. Considering this context, both EXP and NOV groups were well below the cut-off values of

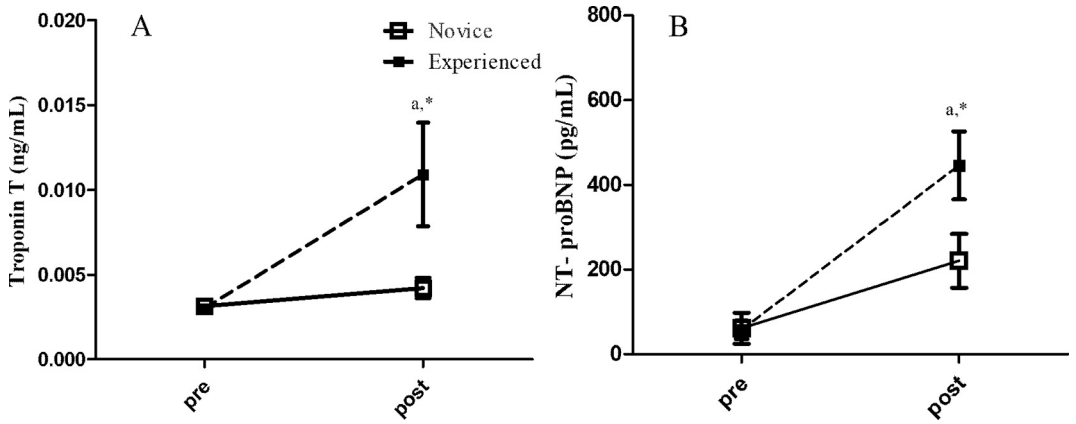

Fig. 2. Troponin T (panel A) and NT-proBNP responses (panel B) *pre-* and *post* 24h ultra-marathon (Mean ± SEM) in novice (N = 14) and experienced (N = 11) runners. Two-way mixed measures ANOVA: [a] significant interaction (time × performance level). [*] significant difference between novice and experienced; Bonferroni *post hoc* test. $p < 0.05$.

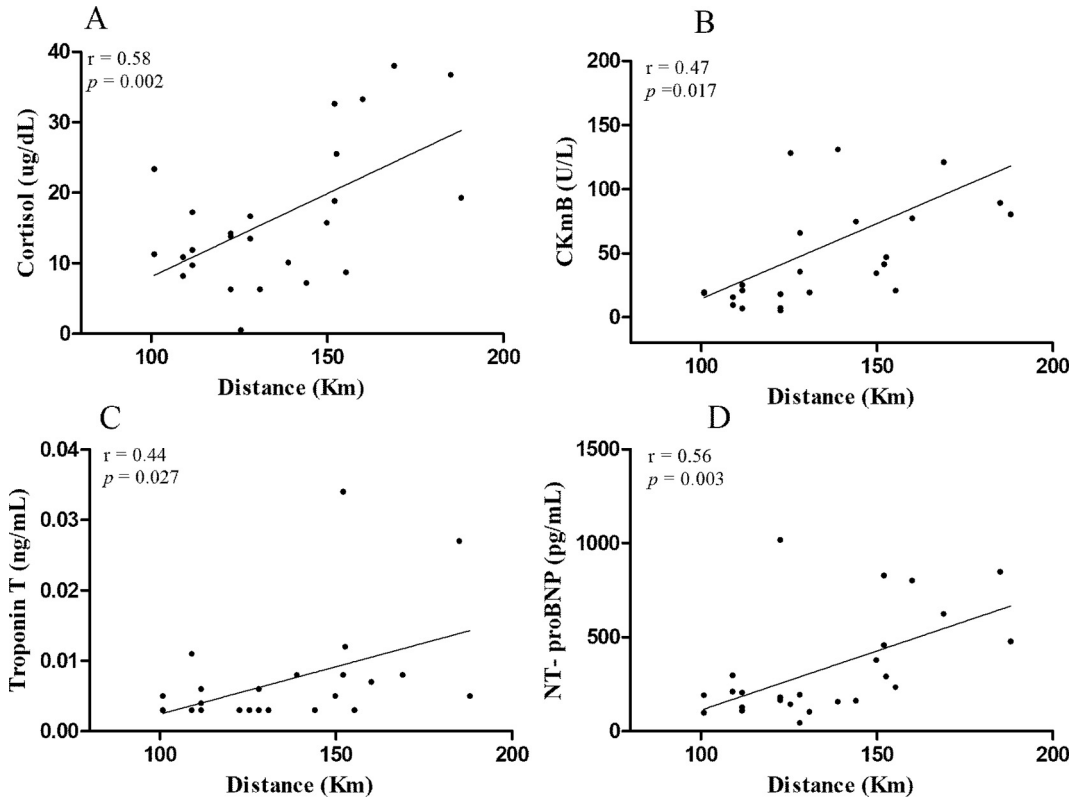

Fig. 3. Correlations between cortisol and cardiac biomarkers with 24h ultra-marathon running distances (Km). *r*, Pearson's correlation coefficient.

0.03 ng/mL (Dolci et al., 2006) for myocardial necrosis and 0.05 ng/mL for diagnosis of acute myocardial infarction (Collinson et al., 2003) using an Elecsys® analyzer. Specifically, two EXP subjects showed cTnT levels equal to 0.03 ng/mL and the remaining runners (N = 23) presented values below 0.01 ng/mL. Of note, when the two subjects mentioned were withdrawn from two-way ANOVA analyses, the interaction was still significant ($F(1,21) = 6.50$, $p = 0.02$) and a difference in post-race cTnT comparing EXP and NOV was also observed ($p < 0.01$).

The increment in blood cTnT may be considered an acute response that should return to baseline values within 24h (Shave et al., 2007). It can be speculated that the cTnT response found herein is related to the greater cardiac mechanical stimuli in the EXP group than in the NOV group due to higher running speed and cardiac output.

5.3. NT-pro BNP analysis

Clinically, NT-proBNP is accepted as a marker of left ventricular volume overload primarily stimulated by myocyte stretch (Shave et al., 2007). The NT-proBNP is frequently used as a marker for the diagnosis of chronic heart failure (Al-Mohammad et al., 2010). According to the National Institute for Health and Clinical Excellence, a diagnosis of heart failure is unlikely when the NT-proBNP level is below 400 pg/ml (Al-Mohammad et al., 2010). In this present study, none of the NOV runners were above 300 pg/ml whereas six EXP runners were found with values above 400 pg/ml [range: 457–848 pg/ml]. However, both pathologic (i.e. volume overload associated with heart failure) and physiological stimuli (e.g. exercise) result in the release of NT-proBNP (Shave

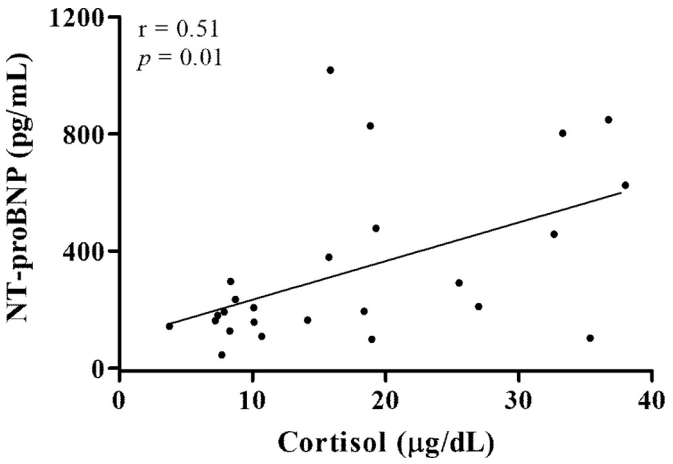

Fig. 4. Correlation between cortisol and NT-pro BNP. *r*, Pearson's correlation coefficient.

et al., 2007). The NT-proBNP is a hormone of natriuresis, blood vessel expansion, and the interception of sympathetic nerves to reduce the preload and afterload to decrease the myocardial wall stress (Scharhag et al., 2008; Kim et al., 2012). During a 24h ultra-marathon, preload increases due to enhanced venous return causing increased wall stretch during an extended period of time (Kim et al., 2012), which may affect the left ventricular function. Niemelä et al. (1984) showed that a competitive 24h run impaired left ventricular performance due to possible cardiac fatigue reversed in 2–3 days. Furthermore, increased NT-proBNP level similar to this present study was previously found after prolonged endurance exercise (Neumayr et al., 2005; Kłapcińska et al., 2013) that is expected to decrease to basal levels in 1–2 days of rest (Shave et al., 2007). For that reason, Neumayr et al. (2005) concluded that transient increases in NT–pro-BNP and also cTnT are more likely to reflect cardiac fatigue than injury. In this regard, all pre-race cardiac biomarkers levels were well below clinical cut-off values and EXP subjects had similar baseline levels of these markers compared to NOV runners, suggesting no cumulative effects of repeated UM regime of training.

Hydration during the race causing exercise-induced changes in blood volume could interfere in the comparison of NT-proBNP responses between EXP and NOV groups since plasma volume interferes in cardiac preload. Our data suggest that a significant difference in plasma volume due to dehydration may be of minor importance to preload analysis than the running performance since the reduction of body mass was similar between groups. Body mass losses of 1.9–5.0% appear to be needed to sustain body water balance (i.e. euhydration) through glycogen oxidation and by the generation of water due to mitochondrial substrate oxidation during ultra-endurance races of ~25–30 h (Hoffman et al., 2017). In addition, normal hemoglobin and hematocrit levels found in both groups (Table 1) support that plasma volume was not significantly different between the EXP and NOV groups.

We suggest that the NT-proBNP response found in this study could be associated with cortisol levels. Glucocorticoids are known to acutely increase blood pressure. Dexamethasone treatment on healthy male subjects increased systolic blood pressure and NT-proBNP levels (Brotman et al., 2005). In this vein, a significant correlation between cortisol and NT-proBNP was observed (Fig. 4). Thus, the increased NT-proBNP and cortisol levels showed in EXP compared to NOV and the correlation of the NT-proBNP with cortisol may be related with a greater pituitary-adrenocortical response to sustain the cardiac debt required for greater running performance during prolonged endurance exercise.

5.4. Methodological considerations

This present study is a naturalistic observation. We described the environmental characteristics of the race and examined the outcomes in EXP and NOV runners arbitrary classified based in self-declared training volume and competitive experience. Environmental conditions on cardiac biomarkers should be further investigated in controlled laboratory tests for proper manipulation of independent variables (e.g. temperature, humidity, oxygen pressure, and slope). However, this kind of approach is of a great challenge considering UM distances and durations, thus, we still know very little about the potential for greater harm in more extreme conditions. Moreover, we did not measure aerobic power (i.e. VO_2 max) to estimate the mean relative intensity performed by EXP and NOV runners or parameters of heart function to evaluate the cardiac load. Therefore, conclusions about intensity-dependent blood biomarkers levels and differences of training status between EXP and NOV influencing the biomarkers responses are limited.

6. Conclusion

This study does not show clear evidence of cardiac risk when comparing cardiac biomarkers levels with clinical cut-off values in agreement with other studies on cardiac biomarkers responses measured after ultramarathons (Kim et al., 2014; Scott et al., 2009). The absence of complaints and clinical symptoms after the 24UM also supports a physiological response within the homeostatic limit. Prolonged heart overload, however, varies according to running speed that may be also associated with a greater pituitary-adrenocortical system response in more experienced runners. In perspective, the impact of extreme environmental conditions and repeated UM competitions on cardiac function and blood biomarkers of runners with distinct performance levels should be studied in additional systematic naturalistic observations.

Declarations

Author contribution statement

Rodrigo Hohl: Analyzed and interpreted the data; Wrote the paper.
Fernando Nazário de Rezende: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Guillaume Y Millet: Wrote the paper.
Gustavo Ribeiro da Mota: Analyzed and interpreted the data.
Moacir Marocolo: Conceived and designed the experiments.

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Additional information

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