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Changes in cardiac and muscle biomarkers following an uphill-only marathon

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ABSTRACT

The aim of the study was to evaluate changes in cardiac troponin I levels (cTnI) and the main biomarkers of skeletal muscle damage after an uphill-only marathon, along with its relationship with athletes' physiological parameters. Twenty-two runners participated in the "Supermaratona dell'Etna" (43 km, 0–2850 m AMSL). Before and immediately after the race, body mass and hydration status were measured together with blood sampling. At the end of the race, mean cTnI increased significantly in all athletes (mean +900%), and in 52% of them the cTnI values were over the normal range. Mean creatinine and cortisol increased significantly (by 30.5% and 291.4%), while C-reactive protein levels did not change significantly. Then, an uphill-only marathon showed a significant increase in cardiac and skeletal muscle blood biomarkers of injury, and cTnI levels were not significantly correlated with age, body mass index, $\dot{V}O_2$max, training status, ultra-endurance training experience, race time and blood parameters.

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Uphill ultra-marathon; blood parameters; muscle biomarkers

Introduction

The term ultra-endurance identifies efforts greater than 4 (Laursen & Rhodes, 2001) or 6 h (Zaryski & Smith, 2005). The popularity of ultra-endurance running, including only-uphill races, as well as the number of scientific studies devoted to this sport activity are rapidly increasing in the last few years (Didier et al., 2017; Knechtle & Nikolaidis, 2017; Lazzer et al., 2014; Morin, Tomazin, Edouard, & Millet, 2011; Vernillo et al., 2014). A number of studies revealed that repeated prolonged strenuous exercise, such as marathon and ultra-marathon, may be hazardous to athletes' health. Authors reported large-artery wall stiffening, muscle and cartilage damage (Kim, Lee, & Kim, 2009), left and right ventricular dysfunction and atrial fibrillation (Mascia, Perrotta, Galanti, & Padeletti, 2013) and systemic inflammatory reaction (Wallberg, Mikael Mattsson, Enqvist, & Ekblom, 2011). As well, prolonged exercise (i.e. marathon running) performed at moderate intensity leads to an increase in muscular and cardiac biomarkers (Frassl et al., 2008; Mohlenkamp et al., 2014). Ultra-endurance running is a weight-bearing activity that

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involves both concentric and eccentric muscle contraction for several hours and skeletal muscle damage can be considered a predictable collateral damage of strenuous exercise (Millet et al., 2011). Creatine kinase (CK) and myoglobin (Mb), released in blood circulation by muscle cell, are the two main markers currently used to measure cellular muscle damage: Mb differs from CK because of a faster kinetics and of exclusive origin form muscle. For cardiac damage, the most used biochemical markers are CK (and in particular the MB cardiac isoform) and the cardiac troponin (cTn). Cardiac biomarkers increment is an intriguing and not completely clarified aspect (Shave et al., 2010) in part because the measurement of troponin I, troponin T and the high sensitivity troponin assay give results not clearly comparable. Even before the introduction of high sensitivity test for cTn concentration (Koller & Schobersberger, 2009; Regwan et al., 2010), it was clear that cTn levels were elevated after a marathon and that transitory inflammation is one of the events contributing to it (Saravia et al., 2010). However, there is not a general consensus (Lippi et al., 2012) about predictor factors of cTn post-race increase but it seems that exercise intensity (Eijsvogels, Hoogerwerf, Oudegeest-Sander, Hopman, & Thijssen, 2014) and duration (Jassal et al., 2009), young age (Nie et al., 2011; Tian, Nie, Huang, & George, 2012), few years of training (Mehta et al., 2012) and higher blood pressure (Kim et al., 2013) are correlated with higher values of post-exercise cTn. While phenomenon of exercise-induced skeletal muscle damage and cTn marker release in a large proportion of elite and non-elite athletes after flat endurance events has been considered, few studies so far have examined this phenomenon in ultra-endurance athletes during an uphill-only marathon. Uphill-only running seems less stressful at muscular level than flat race due to less eccentric component (Gottschall & Kram, 2005; Pokora, Kempa, Chrapusta, & Langfort, 2014), but potentially heavier for cardiovascular system (Kim et al., 2013). Particularly, increased blood pressure during exercise is controlled by reduction in total peripheral resistance from skeletal muscle vasodilation with increased cardiac output along with decreased afterload, preventing a greater increase in blood pressure. However, during uphill running, which is characterized by a prevalent concentric muscle contraction, total peripheral resistance did not fall adequately (Pellegrini et al., 2015) and cardiac afterload is expected to rise more than during flat terrain exercise.

Then, the first purpose of the present study was to evaluate the effects of an uphill-only marathon in cTnI and the main biomarkers (CK and Mb) of skeletal muscle damage. The second purpose was to evaluate if these skeletal and cardiac muscle blood biomarker changes were related with athletes' anthropometric parameters and physical capacities, evaluated before the race, to identify potential protective factors. We hypothesized that athletes with higher $\dot{V}O_2max$ or higher training status showed lower changes in cardiac muscle blood biomarkers.

Study design and methods

Subjects

Twenty-two healthy Italian male runners (age range 20–64 years) were enrolled in this study as participants in the "Supermaratona dell'Etna" (SME, 43 km, 0–2850 m AMSL). The experimental protocol was approved by the Ethics Committee of the University of

Udine. The participants were recruited among ultra-endurance runners, the inclusion criteria were (1) the athletes had previously run at least three races longer than 50 km; (2) their training volume in the latest 4 months was greater than 40 km · week^{-1}. Participants with a history of muscle disorder, clotting abnormality or cardiac disease, and those taking drugs that may have altered the measured variables were excluded. Before the study began, the purpose and objectives were carefully explained to each subject and written informed consent was obtained from all of them.

Study protocol

The race took place in June 2014 and the starting time was set at 8:00 am in Marina di Cottone (Catania, Italy), at sea level, the temperature and relative humidity were 28°C and 33%, respectively. At the finish line, temperature and relative humidity were 2°C and 65%, respectively. Racecourse characteristics were previously described (Giovanelli et al., 2015; Lazzer et al., 2015) even if for this year edition the finish line was set lower (2850 m AMSL) because of the bad weather conditions on the top of the mountain where the finish line was originally located.

In the week preceding the race, participants were asked to come to the laboratory to perform a graded exercise test on a treadmill (Saturn, HP Cosmos, Germany) to evaluate their maximal oxygen uptake ($\dot{V}O_2$max). During the experiment, ventilatory and gas exchange responses were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Italy). The volume and gas analysers were calibrated using a 3-L calibration syringe and calibration gas (16.00% O_2; 4.00% CO_2), respectively. During the tests, electrocardiogram was continuously recorded and displayed on line for visual monitoring, and HR was measured with a dedicated device (Polar, Finland). The tests comprised a 5-min rest period followed by running at 10 km · h^{-1} for 5 min (on a slope of 1%); the speed was then increased by 0.7 km · h^{-1} every minute until volitional exhaustion. A levelling off of oxygen uptake (defined as an increase of no more than 1 ml · kg^{-1} · min^{-1}) was observed in all subjects during the last 1 or 2 min of the exercise test indicating that $\dot{V}O_2$max had been attained. $\dot{V}O_2$max was calculated as the average oxygen uptake in a period of 20 s after reaching a plateau. Athletes were asked to refrain from any vigorous physical activity during the day preceding the test and during the preliminary testing session that they performed to familiarize with the equipment.

Before and immediately after the race, body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany), stature was measured to the nearest 0.001 m on a standardized wall-mounted height board. Body mass index (BMI) was calculated as BM (kg) · stature^{-2} (m). Hydration (Jaffrin & Morel, 2008) was measured by bioelectrical impedance technic (BIA 101, Akern, Italy) by using the software provided by the manufacturer (Bodygram, 1.31). Throughout the competition, GPS coordinates were continuously recorded via Garmin Forerunner 305 GPS (Garmin, Kansas City, MO, USA).

Blood sampling

Before and immediately after the race, blood samples were taken using a tourniquet with a sterile technique from an antecubital vein. Approximately 20 ml of blood was

collected in two Vacutainers ® tubes (Belliver Industrial Estate, Plymouth, UK): an EDTA anticoagulated sample for full blood count and a clotted sample for biochemistry. To reduce errors from postural changes in plasma volume, all subjects were sampled in the supine position for 5 min before and after the race.

Full blood count analyses were performed at the Department of Diagnostics Laboratory (Catania, Italy), where all samples were processed within four hours from venipuncture using a Coulter UniCel analyzer (Beckman Coulter, Brea, CA, USA). Samples for the remaining investigations were centrifuged (at 4000 $\times g$ at 4°C) and serum was transferred into plastic tubes and kept at −70°C until further analysis were performed. Then, biochemistry (Mb, CK, creatinine, cTnI and the others) analyses were done in the Laboratory of the S. Maria della Misericordia Hospital, Udine. Roche™ Elecsys and Roche™ 917 analyzers were respectively used for Mb (Elecsys) and CK and creatinine determinations, while cTnI was measured with AccuTnI method (Beckman Coulter). Among the different cTn tests available on the market we chose the AccuTnI assay, not an high sensitive one but very reliable for our thawed samples and routinely used for cardiac damage evaluation in our University laboratory. This test is characterized by a 99th percentile reference limit, an optimal imprecision (i.e. concentration corresponding to 10% CV), a limit of detection and a limit of blank of 0.034, 0.049, 0.010 and 0.010 ng · ml^{-1}, respectively (Zaninotto et al., 2009). The other biochemistry assays were performed using a Cobas 600 analyzer (Roche Diagnostics Italy) measuring, as the previous citied assays, all pre and post competition samples in the same session.

Statistical analyses

All results are expressed as mean and standard deviation (SD). Normal distribution of the data was tested using the Kolmogorov–Smirnov test. Sphericity (homogeneity of covariance) was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of the *F*-ratios was adjusted according to the Greenhouse–Geisser procedure. The coefficient of variation (CV = SD·mean^{-1}) was calculated for blood parameters. The pre- and post-race results were compared using a paired *T*-test with PASW Statistic 18 (SPSS Inc., Chicago, IL, USA). Spearman correlation coefficients were used to assess the effects of age, BMI, $\dot{V}O_2$max, ultra-endurance training experience, training status, race time, CK, Mb, C-reactive protein, white blood cells (WBCs), granulocytes, platelets (PLTs), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cortisol and creatinine on cTnI levels post-race. A *p*-value of 0.05 was also accepted as the level of statistical significance.

Results

Baseline characteristics

Physical characteristics of the athletes measured before the race and race time are reported in Table 1. The present study considered a group of 22 athletes, ranging from moderately to well-trained individuals. Race time of the winner of the SME was 3:50:38, while the average time of the subjects of the present study was 5:50:29 ± 0:54:52 (ranking 3rd–159th).

Table 1. Physical characteristics of athletes (n = 22) measured before the race.

Age (years)	46.1 ± 10.8	[20–64]
Body mass (kg)	70.0 ± 7.1	[54.5–91.0]
Stature (m)	1.73 ± 0.07	[1.59–1.88]
BMI ($kg \cdot m^{-2}$)	23.5 ± 2.3	[20.5–28.1]
$\dot{V}O_2max$ ($ml \cdot min^{-1} \cdot kg^{-1}$))	49.8 ± 6.4	[40.0–66.0]
Training status ($km \cdot week^{-1}$)	65.3 ± 21.2	[40–140]
Endurance training experience (years)	12.3 ± 9.1	[2–40]
Ultra-endurance training experience (years)	5.2±5.4	[0–15]
Race time (h:mm:ss)	05:50:29 ± 00:54:52	[04:00:11–07:29:41]

All values are mean ± standard deviation (SD). Range in square brackets.
BMI: body mass index; $\dot{V}O_2max$: maximal oxygen uptake.

Race effects on BM and hydration

After the race, BM decreased by mean −2.7 ± 1.1 kg (range 0.0 to −3.5 kg, −3.8%, $p < 0.001$). As well, total water decreased by mean −2.5 ± 2.2 L (range 0.6 to −6.5 L, −5.8%, $p < 0.001$) and extracellular water decreased by mean −2.0 ± 1.4 L (range 0.2 to −5.3 L, −10.2%, $p < 0.001$), while intracellular water did not change significantly (Table 2).

Race effects on whole blood parameters

Mean haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration slightly changed ($p < 0.05$) at the end of the race but remained within their normal ranges (Table 3). Mean PLT count showed a significant increase (+19.3%, $p < 0.001$) at the end of the race remaining within normal range. Mean WBC count increased significantly (+195.5%, $p < 0.001$), mainly due to a marked increase in neutrophil count (+369.4%, $p < 0.001$) and monocytes (+150.0%, $p < 0.001$) whereas lymphocyte count decreased significantly (−21.7%, $p < 0.001$) as eosinophils (−87%, $p < 0.001$) at the end of the race (Table 3).

Race effects on blood biochemistry

Mean [Cl], total protein and serum albumin slightly changed at the end of the race but remaining within normal range, while K^+ increased significantly (+23.0%). Total protein, [Na], [Cl] and [K] showed relatively small inter individual variability (CV: 6.3%, 1.6%, 2.9% and 11.0%, respectively) at the end of the race.

Mean activity of AST, LDH and CK increased significantly at the end of the race by 42.0%, 37.4% and 191.5%, respectively ($p < 0.001$, Table 4). AST values were within normal range at the end of the race, while LDH and CK increased more than normal

Table 2. Body mass and hydration determined before and immediately after the race.

	Before the race	After the race	Difference %	p
Body mass (kg)	70.0 ± 7.1	67.3 ± 6.8	−3.8	0.001
Total water (L)	43.2 ± 4.0	40.7 ± 3.8	−5.8	0.001
Extracellular water (L)	19.6 ± 2.3	17.6 ± 2.2	−10.2	0.001
Intracellular water (L)	23.7 ± 2.6	23.1 ± 2.5	−2.5	0.222

All values are mean ± standard deviation (SD).
p: significance by paired T-test.

Table 3. Blood parameters determined before and immediately after the race.

	Normal range	Before the race	After the race	Difference %	*p*
Haemoglobin ($g \cdot dL^{-1}$)	13.0–17.5	14.8 ± 0.8	14.8 ± 0.9	0.0	0.666
Red blood cell count ($10^9\ L^{-1}$)	4.5–5.9	4.9 ± 0.3	4.9 ± 0.3	0.0	0.351
HCT (%)	40–54	44.1 ± 1.9	43.5 ± 1.9	−1.4	0.048
MCV (fi)	82.0–98.0	90.82 ± 3.97	88.93 ± 3.90	−2.1	0.001
MCH (pg)	27.0–30.0	30.41 ± 1.22	30.29 ± 1.30	−0.4	0.039
MCHC (%)	34.0–36.0	33.50 ± 0.88	34.07 ± 1.03	1.7	0.001
Platelet count ($10^9\ L^{-1}$)	150–400	229.7 ± 41.5	274.1 ± 60.8	19.3	0.001
White blood cell count ($10^9\ L^{-1}$)	4.0–11.0	6.7 ± 1.4	19.8 ± 3.2	195.5	0.001
Neutrophils ($10^9\ L^{-1}$)	2.5–7.5	3.6 ± 0.8	16.9 ± 3.0	369.4	0.001
Monophils ($10^9\ L^{-1}$)	0.2–0.8	0.4 ± 0.1	1.0 ± 0.3	150.0	0.001
Lymphocyte ($10^9\ L^{-1}$)	1.5–3.5	2.3 ± 0.7	1.8 ± 0.4	−21.7	0.001
Eosinophils ($10^9\ L^{-1}$)	0.04–0.40	0.23 ± 0.19	0.03 ± 0.05	−87.0	0.001
Basophils ($10^9\ L^{-1}$)	0.01–0.10	0.04 ± 0.03	0.04 ± 0.02	0.0	0.957

All values are mean ± standard deviation (SD).
HCT: haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.
p: significance by paired *T*-test.

Table 4. Blood biochemistry determined before and immediately after the race.

	Normal range	Before the race	After the race	Difference %	*p*
Sodium ($mmol \cdot L^{-1}l$)	135–145	143.5 ± 1.4	142.1 ± 2.3	−1.0	0.106
Potassium ($mmol \cdot L^{-1}$)	3.50–5.10	4.86 ± 0.64	5.98 ± 0.66	23.0	0.001
Chloride ($mmol \cdot L^{-1}$)	96–109	101.3 ± 2.3	98.8 ± 2.9	−2.5	0.005
Total protein ($g \cdot L^{-1}$)	66–81	68.9 ± 4.9	73.1 ± 4.6	6.1	0.001
Serum albumin ($g \cdot L^{-1}$)	35–50	46.4 ± 2.3	48.9 ± 2.2	5.4	0.001
Aspartate aminotransferase ($U \cdot L^{-1}$)	5–40	24.5 ± 5.0	34.8 ± 8.7	42.0	0.001
Lactate dehydrogenase ($U \cdot L^{-1}$)	240–480	376.3 ± 70.6	517.1 ± 84.8	37.4	0.001
Creatine kinase ($U \cdot L^{-1}$)	39–190	111.6 ± 67.2	325.3 ±176.6	191.5	0.001
Troponin I ($ng \cdot ml^{-1}$)	0–0.045	0.01 ± 0.00	0.10 ± 0.12	900.0	0.015
Myoglobin ($ng \cdot ml^{-1}$)	16–116	43.3 ± 13.9	475.9 ± 255.3	999.1	0.001
Creatinine ($mg \cdot L^{-1}$)	0.40–1.30	0.95 ± 0.11	1.24 ± 0.16	30.5	0.001
C-reactive protein ($mg \cdot L^{-1}$)	0.00–5.00	0.95 ± 0.85	1.35 ± 1.39	42.1	0.211
Cortisol ($nmol \cdot L^{-1}$)	150–650	321.1 ± 69.0	1256.7 ± 326.4	291.4	0.001

All values are mean ± standard deviation (SD).
p: significance by paired *T*-test.

maximal values (+8% and +71% respectively, $p < 0.001$). AST and LDH showed relatively small inter-individual variability (CV: 25.0% and 16.4%), while CK showed a large inter-individual variability at the end of the race (CV: 54.3%).

Marked running-related increases were also found in serum cardiac and inflammatory biomarkers (Table 4). Mean cTnI increased significantly in all athletes by mean +900.0%, ($p < 0.001$) and showed a large inter-individual variability at the end of the race (CV: 120.0%). In 52% of the athletes, the cTnI values after the race were over the normal range. Mean Mb increased significantly at the end of the race by mean 999.1% ($p < 0.001$) with a large inter-individual variability (CV: 53.6%). Mean creatinine increased significantly (+30.5%, $p < 0.001$) with a relatively small inter-individual variability (CV: 12.9%). Finally, cortisol increased significantly (+291.4%, $p < 0.001$) with a small inter individual variability (CV: 26.0%).

In addition, cTnI levels were not significantly correlated with age, BMI, $\dot{V}O_2max$, training status, ultra-endurance training experience, race time, and blood parameters measured before or after the race (Table 5).

Table 5. Correlation of iroponin I measured immediately after the race and athletic characteristics of subjects measured before and immediately after the race.

	Before the race		After the race	
	r	*p*	*r*	*p*
Age (years)	0.07	0.754		
BMI ($kg \cdot m^{-2}$)	0.14	0.561		
$\dot{V}O_2max$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	−0.03	0.296		
Training status ($km \cdot week^{-1}$)	0.16	0.525		
Ultra-endurance training experience (years)	−0.29	0.154		
Race time (h:mm:ss)			0.24	0.305
Creatine kinase ($U \cdot L^{-1}$)	−0.33	0.158	0.05	0.835
Myoglobin ($ng \cdot ml^{-1}$)	−0.14	0.354	0.18	0.658
C-reactive protein ($mg \cdot L^{-1}$)	−0.34	0.185	−0.23	0.338
White blood cell count ($10^9 \cdot L^{-1}$)	−0.06	0.235	0.25	0.292
Platelet count ($10^9 \cdot L^{-1}$)	0.11	0.2145	0.18	0.398
Cortisol ($nmol \cdot L^{-1}$)	0.46	0.365	0.05	0.856
Aspartate aminotransferase ($U \cdot L^{-1}$)	0.38	0.678	0.34	0.679
Lactate dehydrogenase ($U \cdot L^{-1}$)	0.21	0.321	0.16	0.495
Creatinine ($mg \cdot L^{-1}$)	0.16	0.267	0.27	0.257

BMI: body mass index; $\dot{V}O_2max$: maximal oxygen uptake.

Discussion

The main results of the present study show that an uphill-only marathon induced (1) a significant increase in cardiac and skeletal muscle blood biomarkers of injury and (2) these changes were not correlated with athletes' haematological, biochemical and anthropometric parameters evaluated before or after the race.

It is well known that intense and/or prolonged exercise causes an injury on muscle skeletal cells with cellular membrane breakdown and the release of intracellular substances such as CK and Mb into circulation. Considering the major effect of eccentric contraction on muscle damage, we would have expected a minor effect of uphill running on muscle damage because of lesser ground force and a predominance of isometric and concentric over eccentric contraction. But from our data it seems that effort intensity and length is more important than its kind on skeletal muscle damage. It could be that the major work, compared to flat marathon, for climbing the volcano steep paths causes an energy deficit in muscle fibres that, possibly added to the higher ischemic stress due to longer concentric contraction, is responsible for a muscle damage not different from what has been reported in flat marathon.

On the side of cardiac muscle, the uphill-only running is accompanied by a prevalent concentric muscle contraction that could cause, compared with concentric–eccentric muscle contraction of flat marathon (Gottschall & Kram, 2005), an higher cardiac afterload and an higher vascular resistance, somehow resembling a situation of higher than normal pressure that has been seen correlated with higher cTnI release (Kim et al., 2013). As well, in this study, the positive ascent from the sea level to ~3000 m without acclimatization, and the extreme variability of climate situation as temperature and humidity, could influence negatively cardiovascular system (Rennie, 1989). If cTnI release into circulation secondary to exercise is a marker of real cardiac damage, our data suggest that uphill running could be very stressful at cardiac level, regardless of athlete's age, training and performance status.

CK post-race values showed an increase by 191.5%, while AST and Mb values increased respectively by 42.0% and 999.1%, supporting the idea that an uphill-only exercise is not less stressful for muscle compared with analogous flat race, where CK and Mb values increased respectively by 229% and 1500% (Jassal et al., 2009), despite the reduced eccentric component and lighter ground forces (Gottschall & Kram, 2005). Even if C-reactive protein increment was minimal, due to his slow kinetics, the inflammatory response was strong showing high WBCs (+195%) and neutrophils (+369%) count as it was observed in flat marathon (about +170% and 280%, respectively (Del Coso et al., 2013; Jassal et al., 2009; Smith, Garbutt, Lopes, & Pedoe, 2004). In particular, the cortisol response was massive (+291%) and reasonably responsible for the reduction in lymphocytes and eosinophils count after the race. The renal function was slightly impaired considering the 30.5% of creatinine increment, not enough to influence biomarkers measurement even considering an 8% decrement of plasma volume. As in other kind of endurance races (Smith et al., 2004), in the present study, potassium increased after exercise (+23%) mainly as marker of cell damage considering the small influence of endurance exercise on acid-base status. As expected, athletes suffered from mild dehydration despite abundant water supplies along the race. The water loss was mainly extracellular and so presumably not responsible for cell impairment and serum biomarkers increment, as observed previously (Cutrufello, Dixon, & Zavorsky, 2016; Valentino, Stuempfle, Kern, & Hoffman, 2016). Specifically, [Na] and [Cl] small variations, together with mild decrease of plasma volume as indicated by albumin and total protein concentration, suggest that the athletes experienced an isotonic dehydration with correct electrolytic replacement during the race.

Moreover, changes in cardiac biomarkers observed after this uphill-only marathon were not correlated with athletes' race time and anthropometric parameters evaluated before or after the race. Particularly, in the present study, no correlation between cTnI increase and time to finish the race was observed, in contrast to previous authors (Eijsvogels et al., 2015; Jassal et al., 2009), who showed a positive correlation between elevations of cardiac injuries biomarkers and endurance time. However, other authors reported cTnT increase, an assay different from our cTnI test, in an healthy individual following a 10-km race (Tolkin, Goldstein, & Rott, 2009), and Saravia et al. (2010) concluded that faster runners suffer from significantly stronger cTnT release after a marathon. As well, exercise intensity could be related to the magnitude of cTn elevation (Eijsvogels et al., 2014; Legaz-Arrese et al., 2011; Shave et al., 2010), and other authors (Kim et al., 2014; Salvagno et al., 2014) showed that cTn increases was smaller during ultra-marathon exercises compared with shorter efforts, probably due to the lower intensity at which they are performed.

Even if, some authors found young age (Eijsvogels et al., 2015; Jassal et al., 2009; Tian et al., 2012) and less training experience (Mehta et al., 2012) linked to a higher cTn response, our athletes did not show this pattern of correlation although there was great variability in age and experience among them. Not either inflammation biomarkers, as WBCs and C-reactive protein, showed in our athletes a significant correlation with cTn increase in contrast with previous studies (Saravia et al., 2010; Sawka et al., 2007; Scherr et al., 2011), in which inflammation and cTn seem strictly linked. Considering the discrepancies of past work, our study suggest that other influencing factors, if any, are secondary and that if the effort is intense and long, as in this kind of competition, there

is a significant increment of biochemical markers of muscle and cardiac damage regardless of age, performance, training status, etc.

We acknowledge that our study presents some limitations. First, we were not able to monitor the running intensity during the race because we did not want to force the athletes to use the heart rate belt if they were not used to. Second, this is only a descriptive study, in which we could not follow up the athletes after the race. For this reason the cTnI kinetic were not monitored during the post-race phase when the cTnI release could be higher. Last we used a non-high sensitive assay for cTn due to laboratory policy considering, however, that the lowest sensibility could have only influenced the results reducing the amount of cardiac damage marker increment, not adding but reducing evidence in favour of a peculiar effect of uphill only running.

In conclusion, an uphill-only marathon showed a significant increase in cardiac and skeletal muscle blood biomarkers of injury. Moreover, most athletes showed cTnI increment above threshold of normality without correlation with other predictive parameters, which suggests that if the exercise is strenuous, there is no protective factor (i.e. good training, experience and adaptation through years of training) versus cardiac (i.e. cTn I increase) damage.

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Disclosure statement

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