Biochemical and Muscle Mechanical Postmarathon Changes in Hot and Humid Conditions

Randall Gutiérrez-Vargas,[1,2] Saúl Martín-Rodríguez,[3] Braulio Sánchez-Ureña,[2,4] Alejandro Rodríguez-Montero,[2] Jorge Salas-Cabrera,[2] Juan C. Gutiérrez-Vargas,[1] Bostjan Simunic,[5] and Daniel Rojas-Valverde[1,2]

[1]Health and Sport Research and Diagnosis Center (CIDISAD); [2]School of Human Movement and Quality of Life, National University, Heredia, Costa Rica; [3]Canarian Physical Education Licenciates College (COLEF), Las Palmas de Gran Canaria, Gran Canaria, Spain; [4]Health and Exercise Sciences Program; and [5]Institute of Kinesiology Research, University of Priomorska, Koper, Slovenia

Abstract

Gutiérrez-Vargas, R, Martín-Rodríguez, S, Sánchez-Ureña, B, Rodríguez-Montero, A, Salas-Cabrera, J, Gutiérrez-Vargas, JC, Simunic, B, and Rojas-Valverde, D. Biochemical and muscle mechanical postmarathon changes in hot and humid conditions. *J Strength Cond Res* 34(3): 847–856, 2020—The aim of this study was to compare biochemical changes and mechanical changes in the lower-limb muscles before and after a marathon race in hot and humid conditions. Eighteen healthy runners participated in a marathon at between 28 and 34° C and 81% humidity in Costa Rica. Serum magnesium (Mg^{2+}), creatine phosphokinase (CPK), lactate dehydrogenase, and hematocrit (HCT) were measured before and after the marathon. Tensiomyography measurements from the rectus femoris (RF) and vastus medialis, muscle displacement (Dm), contraction time (Tc), and velocities of contraction to 10 and 90% of Dm (V_{10} and V_{90}) were obtained before and after the marathon. Postrace measurements showed a 544% increase in CPK ($t(17)$: -6.925, $p < 0.01$), a 16% increase in HCT ($t(17)$: -7.466, $p < 0.01$), a 29% decrease in Mg^{2+} ($t(17)$: 3.91, $p = 0.001$), a 2% decrease in body mass ($t(17)$: 4.162, $p = 0.001$), a 4% increase in Tc of the RF ($t(17)$: -2.588, $p = 0.019$), and a 12% increase in Dm of the RF ($t(17)$: -2.131, $p < 0.048$) compared with prerace measurements. No significant biochemical or mechanical differences were found between runners in terms of their finish times. These findings showed that completing a marathon in hot and humid conditions induced a significant reduction in lower-limb muscle stiffness, body mass, and Mg^{2+}, and increased neuromuscular fatigue, CPK, and HCT, because of muscle damage and dehydration. Knowledge of the effects of heat and humidity may be of value for coaches and sports medicine practitioners in developing effective hydration and recovery protocols for marathon runners in these special conditions.

Key Words creatine phosphokinase, neuromuscular fatigue, tensiomyography, exercise

Introduction

Endurance performance, such as long-distance marathon running, is impaired in conditions of high temperature and humidity, which may compromise performance and lead to the premature onset of fatigue (24). Long-distance marathon races can lead to alterations in the skeletal muscle fibers that affect their mechanical properties and performance capabilities through the repeated and prolonged use (30,33), which may deteriorate further from the effects of heat and dehydration. During exercise-induced hyperthermia, multifactorial effects on endurance performance include reduced central drive (38), perturbations in neurotransmitter systems (48), increased central nervous system temperature (37), and dehydration (44), which are all responsible for the onset of central and peripheral fatigue. Given that the optimal temperature for running a marathon is between 10 and 12° C (26), the alterations associated with exercise-induced hyperthermia may produce a decrease in sports performance and alterations at the neuromuscular and biochemical levels when a marathon is held under high thermal stress conditions.

Previous studies have shown that long-distance races are associated with a reduction in muscle function and muscle damage because of reduced serum magnesium (Mg^{2+}) levels (4,39). Because of the role of Mg^{2+} in oxygen uptake, mitochondrial activity, and ATP production (35), reduced Mg^{2+} levels could lead to reduced muscle strength, muscle cramps, and muscle fiber damage during exercise (23) and to an

Address correspondence to Daniel Rojas-Valverde, daniel.rojas.valverde@una.cr.

34(3)/847–856

Journal of Strength and Conditioning Research

increase in serum levels of markers of muscle damage, including creatine kinase, creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) (18,36,46). Also, long-distance races can be associated with dehydration and an increase in hematocrit (HCT) (21,49). Although intensive short-term exercise commonly leads to hemoconcentration, long-term exercise can induce both hemodilution (9,41) and hemoconcentration because of the effects of dehydration (7,9,41,49). Although much of the data regarding biochemical changes and mechanical changes in the lower-limb muscles before and after a marathon race has been derived from optimum environmental conditions, between 10 and 12° C, few studies have been undertaken to analyze the biochemical changes, including levels of Mg^{2+}, CPK, LDH, and HCT, associated with muscle damage and dehydration after marathon races in conditions of heat and humidity in healthy subjects (6,42).

In previous studies, neuromuscular fatigue has been evaluated by monitoring changes in static or dynamic force, or by means of electromyography (EMG) or mechanomyography (MMG). The methods of EMG and MMG often entail complex signal processing and data interpretation, and measuring several muscles with these methods can be difficult and time-consuming. Tensiomyography (TMG) is a reliable and noninvasive MMG method by which localized muscle fatigue can be easily detected in real time without postprocessing signal analysis (25). Previously published studies have shown that TMG can assess muscle fatigue using reliable parameters that include muscle displacement (Dm) and velocities of contraction to achieve 10% and 90% decrease in Dm (V_{10} and V_{90}) (16,10), whereas contraction time (Tc) in muscle fatigue may increase (14,16). Using TMG, V_{10} and V_{90} have been shown to be correlated with decreased maximal voluntary contraction of skeletal muscle ($r = 0.64–0.67$) (10). Decreased Dm, $V_{10–90}$, and increased Tc have been explained by a reduced efficiency of the excitation-contraction coupling, impairment in membrane-conducting properties, and damage to cellular structures (16,10). However, TMG markers of muscle fatigue have shown some conflicting results when assessed after a 226-km ultraendurance triathlon (13) and a 43-km uphill marathon (14), which showed an increase in Tc but also in Dm. These results have been proposed to be due to enhanced postactivation potentiation, which may counteract fatigue during endurance exercise, affecting the behavior of the muscle fibers, including reduced stiffness as a possible mechanism responsible for the increase in Dm after strenuous exercise (14). These views are supported by studies that have shown a negative correlation between the decrease in muscle mass (after disuse) and the increase in Dm, which is indicative of a lower-muscle resting tension because of muscle atrophy and to changes in the viscoelastic properties of intramuscular and tendon connective tissue (12,40). Morgan et al. (32) proposed a system that assumed that the series elastic component of the muscle-tendon complex was modeled as 2 springs connected in series, one representing the compliance of a torque-dependent component (muscle), and the other a torque-independent component (tendon). In this regard, the changes in Dm are relative to the torque-dependent component, the muscle, which is also known as "active stiffness" (22).

Previously published studies have shown that low stiffness (high Dm) allows for extreme joint motion leading to soft-tissue injury (15,50). However, contrary study findings have shown that high muscle stiffness (low Dm) is a positive factor for sports performance because higher rates of stiffness allow for maximum force and a higher rate of force development, allowing for muscle to operate closer to the end of the curve of the force-velocity relationship (27). Therefore, at this time, these results remain controversial, and despite the importance of the topic, only a limited number of previous studies have examined fatigue and stiffness using TMG in long-distance races such as marathons (13,14). Further studies are necessary to understand the neuromuscular alterations, assessed with TMG, after this type of popular competitive sporting event. To the best of our knowledge, very few studies have analyzed biochemical changes and mechanical changes in the lower-limb muscles before and after a marathon race in hot and humid conditions, in contrast to the same event performed under optimum temperature conditions of between 10 and 12° C (26).

Therefore, the primary aim of this study was to compare biochemical changes and mechanical changes in the lower-limb muscles before and after a marathon race in hot and humid conditions. The secondary aim was to examine whether the alterations in the biochemical and mechanical markers were different between fast and slow runners. Based on the review of previously published studies, the biochemical markers chosen for this study included CPK, LDH, and Mg^{2+}, and the mechanical markers chosen for TMG included Dm, Tc, and V_{10} and V_{90}.

Methods

Experimental Approach to the Problem

Prerace or basal (BS) and postrace (PR) biochemical measurements, included CPK, LDH, magnesium (Mg^{2+}), and HCT. Neuromuscular measurements from TMG, included muscle displacement (Dm), contraction time (Tc), and velocities of contraction to achieve 10% and 90% decrease in Dm (V_{10} and V_{90}). Measurements were performed in isolated rooms with an average temperature of 21.1 ± 0.36° C. Athletes received no intense stimulus or sports workload during the tapering period and at least 24 hours prior the evaluations. The marathon was of 42,195 m and was certified by the International Association of Athletics Federations and the Association of International Marathons and Distance Races. The starting time of the marathon began at 5:00 AM, in Tamarindo, Guanacaste, Costa Rica, at sea level. The altimetry range of the course was between 0 and 80 m, with a return point at half-marathon. The thermal stress index registered was

28.34 ± 3.27° C and 81% humidity from 5.00 AM to the end of the event at 11.00 AM, according to the WetBulb Globe Temperature (WBGT) (Figure 1).

Subjects

Eighteen healthy Costa Rican male ($n = 11$) and female ($n = 7$) runners were voluntarily enrolled in this study who were participants in the Tamarindo Beach Marathon, with a mean age of 35.6 ± 6.9 years (range 19–47 years), mean body mass of 67.4 ± 11.9 kg, mean height of 168.9 ± 10.4 cm, mean body fat of 20.9 ± 7.5%, mean maximal oxygen uptake ($\dot{V}O_2max$) of 52.6 ± 7 ml $O_2 \cdot (kg \cdot min^{-1})$, mean lean leg mass of 17 ± 4.2 kg, and mean finish time of 4 hours 18 ± 35.6 minutes (expressed as mean ± *SD*). The participants were grouped using the mean of the marathon's finish time in the slow group (SG) ($n = 9$) (4 women and 5 men) and the fast group (FG) ($n = 9$) (3 women and 9 men). The mean SG finish time of 4 hours 57 ± 25.6 minutes vs. the mean FG finish time of 4 hours and 2 ± 37.2 minutes was significantly different ($t = 2.259$) ($p = 0.038$).

The experimental protocol was approved by the Health and Human Movement Master Degree Review Committee of the National University, Costa Rica. All the participants were informed of the details of the experimental procedures and the associated risks and discomforts. Each subject gave written informed consent, according to the criteria of the Declaration of Helsinki, regarding biomedical research involving human subjects (18th Medical Assembly, 1964, revised in 2013 in Fortaleza). The participants were recruited among heat-acclimatized and experienced ultraendurance runners, with a mean of 9 ± 6.6 years of ultraendurance running race experience, and a mean of 43.2 ± 6.6 $km \cdot wk^{-1}$ of running training. All study participants were asked to complete a questionnaire on physical exercise activity, medical history, and lifestyle. Participants who reported any muscular or metabolic diseases or recent physical injury of the lower limbs were excluded from the study.

Procedures

Participants were asked to attend the laboratory for individual measurements and to perform a graded exercise test on a treadmill to assess their $\dot{V}O_2max$. The characterization of the participants was performed using body mass (0.1-kg sensitivity) using an Elite Series BC554, Tanita-Ironman. Height was measured using a wall-measuring rod. Body composition was measured by dual x-ray absorptiometry with an accuracy of ±3% using and enCORE 2011 (General Electric) and software version 13.6. The $\dot{V}O_2max$ was obtained using a gas analyzer (VO 2000, MedGraphics) and BreezeSuite software, and an incremental intensity protocol for the use of the treadmill that included a 3-minute warm-up, increasing rate of 1 $km \cdot h^{-1}$ increasing every 3 minutes until volitional fatigue. The gas analyzer had an accuracy of ±3% on absolute volume.

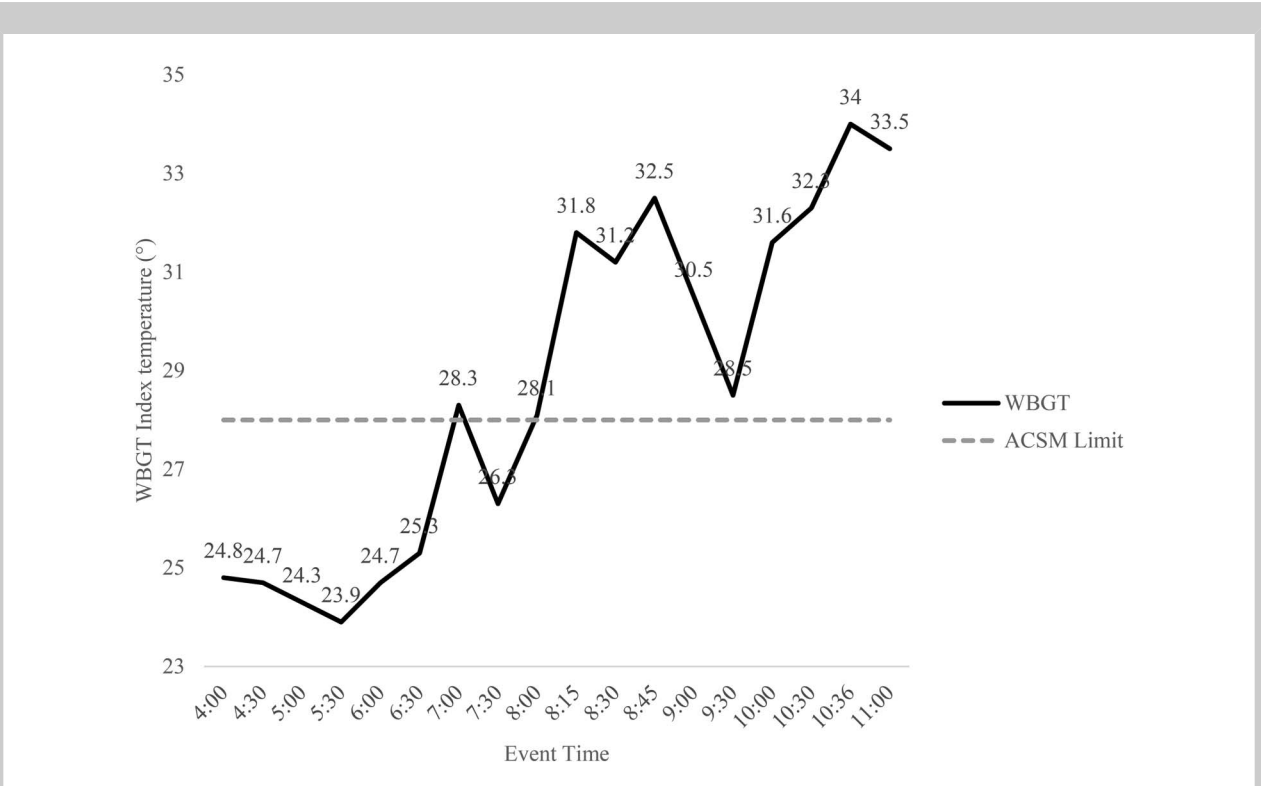

Figure 1. Variations of the WetBulb Globe Temperature (WBGT) during the marathon race. ACSM = American College of Sports Medicine.

Table 1. Premarathon test and postmarathon test results for biochemical blood markers, including creatine phosphokinase (CPK), lactate dehydrogenase (LDH), magnesium (Mg^{2+}), the HCT, and body mass.

Variable	Pre	Post	*T*	*p*	*d*	Δ%
CPK (IU/L)	188.8 ± 127.2	1,215.5 ± 694	−6.925	<0.01*	−1.63, large	544
LDH (IU/L)	389.5 ± 104.3	435.3 ± 112.6	−1.512	0.149	−0.36, small	12
Mg^{2+} (mg/dl)	0.9 ± 0.1	0.7 ± 0.01	3.91	0.001*	0.92, large	−29
Hematocrit (%)	41.7 ± 3.1	48.5 ± 4.2	7.466	<0.01*	−1.76, large	16
Body mass (kg)	67.4 ± 11.9	66.4 ± 11.5	4.162	0.001*	0.98, large	−2

*$p < 0.01$.

Running Kinematics, Heart Rate, and Thermal Variables During the Race. To measure time-motion variables, including average speed ($km \cdot h^{-1}$) and impact (*g*-forces) during the race, an 18-satellite global positioning system (GPS) (SP PRO X II GPSports) was used for tracking the runners, working at a sampling frequency of 15 Hz, and used to analyze the total marathon distance (0–40 km). The manufacturer software V2.5.4 firmware AMS Team (GPSports, Canberra, Australia) was used for data analysis. The devices were coupled with a cervical-mounted 100-Hz triaxial accelerometer that allowed for the estimation of body impact, which recorded all accelerations and decelerations above $\times 2g$ forces during running. Impacts were recorded as the total impact during a lap of 0–40 km.

Eighteen heart rate (HR) monitors were used to analyze total HR response in beats per minute ($b \cdot min^{-1}$). Global positioning system and HR data were analyzed in 4 laps of the race: lap 1, 0–10 km; lap 2, 11–20 km; lap 3, 21–30 km; and lap 4, 31–40 km. The GPS and HR devices were attached to the participants and activated 15 minutes before the beginning of the race, according to the manufacturer's guidelines. For the assessment of the WBGT thermal stress index, a heat stress monitor (QuestTemp 36, 3M) was used. The equipment was calibrated according to the manufacturer's instructions and placed on a tripod 50 m from the finish line and 30 minutes before the start of the event. Measurements were taken through the whole event.

Serum Assessment. One hour before the race, 9 ml of blood was drawn from an antecubital vein from all study participants directly into vacutainer tubes (Becton Dickinson, USA) containing EDTA for serum separation and immediately stored on ice for basal blood sample assessment. Postrace, 9-ml blood samples were obtained in the medical area within 10 minutes of completion of the race. After all participants had their BS and PR measurements, further studies were immediately performed by a researcher in a controlled room. An enzymatic method was used for the measurement of serum levels of CPK (IU/L), Mg^{2+} ($mg \cdot dl^{-1}$), and LDH (IU/L) using a semiautomatic biochemical analyzer (RT-1904C, Rayto). All procedures were performed under the relevant protocols for the handling and disposal of biological materials, according to the manufacturer's instructions for the equipment and reagents used.

Hematocrit. An analogical microhematocrit centrifuge was used with 24 capillary tubes (KHT-400, GEMMY Industrial Corp., Taipei, Taiwan) minutes before and after the marathon. Blood samples were stored in heparinized capillary tubes, with the ends of the capillaries sealed with modeling clay.

Neuromuscular Assessment. Tensiomyography was used to assess neuromuscular alterations of the rectus femoris (RF) knee extensor and hip flexor, and vastus medialis (VM) knee extensor muscle, in both legs. These muscles were selected because of their fundamental role in specific running kinematics, control, and stabilization (31). Both BS and PR measurements were taken 30 minutes before and after the race.

During TMG assessment, participants were asked to remain relaxed. For RF and VM assessment, the participants were placed in a supine position, a cushioned pad used to fix the knee at 30° of flexion. The TMG measurements were taken by 1 experienced researcher. The participants were asked to remain in a resting position for 5 minutes. After the rest period, the skin of the leg was shaved, and the area was cleaned with ethyl alcohol pads. Two 5 × 5-cm adhesive electrodes were used during TMG (TheraTrode, TheraSigma, CA, USA) with an interelectrode distance of 3 cm and placed in the respective muscle. The measurement point was set at the area of maximal muscle mass of each muscle, which was established visually and on palpation of the muscle during a voluntary contraction. The electrodes were connected to a TMG-S2 stimulator (EMF-Furlan & Co. d.o.o., Ljubljana, Slovenia) that triggered a quadrangular, monophasic, 1-millisecond pulse duration, and amplitude between 0.1 and 100 mA.

A digital displacement transducer (GK 40; Panoptik d.o.o., Ljubljana, Slovenia) was positioned perpendicular to the previously established measurement points of the

Table 2. Premarathon test and postmarathon test results for body mass and biochemical blood markers by speed group, including creatine phosphokinase (CPK), lactate dehydrogenase (LDH), magnesium (Mg^{2+}), the hematocrit (HCT), and body mass.

	Slow group		Fast group				
Variable	Pre	Post	Pre	Post	*F*	*p*	ω_p^2, rating
CPK (IU/L)	217.4 ± 172.9	1,336.2 ± 815.4	160.2 ± 51.6	1,094.8 ± 571	0.372	0.551	−0.02, small
LDH (IU/L)	409.5 ± 135.2	461.2 ± 146.1	369.5 ± 62.8	409.4 ± 63.8	0.036	0.852	−0.03, small
Magnesium^{2+} (mg·dl^{-1})	0.85 ± 0.08	0.76 ± 0.09	0.99 ± 0.17	0.74 ± 0.1	3.65	0.074	0.07, small
Hematocrit (%)	41 ± 2.6	48.1 ± 5.5	42.4 ± 3.6	49 ± 2.7	0.667	0.085	−0.01, small
Body mass (kg)	65.6 ± 10.6	64.6 ± 9.5	69.2 ± 13.5	68.2 ± 13.5	0.053	0.821	−0.03, small

muscle belly, with an initial constant spring, as previously described (10). The measurement protocol began with triggering a 40-mA electrical stimulus to induce muscle contraction. The electrical stimulus was increased by 20 mA until the maximal Dm was obtained. Each electrical stimulus was separated by a 10-second rest to avoid fatigue or post-tetanic activation (11). The maximal TMG response was selected and saved for further analysis. From TMG measurements, the following parameters were obtained: Tc (in ms), Dm (in mm), and the muscle contraction velocities from the onset of electrical stimulation until it reached 10% (V_{10}) and 90% (V_{90}) of Dm, expressed in mm·s^{-1}, obtained by the formula developed in 2015 by de Paula Simola et al. (10). All TMG variables used had previously demonstrated a high intraclass correlation coefficient (0.86–0.98), as described in previous studies (10,11).

Statistical Analyses

Descriptive statistics were implemented through the mean (M) and their respective ±*SD*. The normality of the data of each of the variables was evaluated using the Shapiro-Wilk test and the Levene test for homogeneity of variance, Box's M test and Mauchly's sphericity test for the homogeneity of the covariance matrices of the dependent variables. Data from CPK, Mg^{2+}, LDH, and TMG measurements were analyzed using a repeated-measures *t*-test to compare prerace and PR data, and subjected to a speed group analysis 2 variable-mixed general linear model (GLM) with a statistical significance level set at $p \leq 0.05$.

The degree of differences from the independent-samples *t*-test was analyzed using Cohen's *d*, which was determined by calculating the mean difference between the 2 groups, and dividing the result by the pooled *SD*, categorized as follows: $d = 0.2$ was small, $d = 0.5$ was moderate,

Table 3. Premarathon test and postmarathon test results for the rectus femoris (RF) and vastus medialis (VM) tensiomyography (TMG) values.*

		Whole group					
Muscle	TMG	Pre	Post	*t*	*p*	*d*, rating	Δ%
Rectus femoris	Tc (ms)	27.9 ± 3	28.9 ± 2.6	−2.588	0.019†	−0.6, moderate	4
	Dm (mm)	8.7 ± 2.2	9.7 ± 2.1	−2.131	0.048†	−0.5, moderate	12
	V_{10} (mm·s^{-1})	37.3 ± 9.1	40.6 ± 8.3	−1.699	0.108	−0.4, small	9
	V_{90} (mm·s^{-1})	153.4 ± 36.1	165.68 ± 33.04	−1.615	0.125	−0.38, small	8
Vastus medialis	Tc (ms)	23.3 ± 1.7	23.6 ± 1.9	0.788	0.441	0.19, small	1
	Dm (mm)	7.4 ± 1.2	7.7 ± 1.8	−0.644	0.528	0.15, small	4
	V_{10} (mm·s^{-1})	32.1 ± 11.2	35.9 ± 7.3	−1.314	0.206	−0.3, small	12
	V_{90} (mm·s^{-1})	138.1 ± 42.5	153.6 ± 31	−1.348	0.195	−0.32, small	11

*Tc = contraction time; Dm = muscle displacement, V_{10} = velocity of contraction to 10% of Dm; V_{90} = velocity of contraction to 90% of Dm.
†$p < 0.05$.

Table 4. Premarathon test and postmarathon test results for rectus femoris (RF) and vastus medialis (VM) tensiomyography (TMG) by speed group.*

		Slow group		Fast group				
Muscle	TMG	Pre	Post	Pre	Post	F	p	ω_p^2, rating
Rectus femoris	Tc (ms)	26.6 ± 2	28.3 ± 2.5	29.1 ± 3.3	29.6 ± 2.8	2.5	0.113	0.04, small
	Dm (mm)	8.2 ± 2.1	8.8 ± 2.1	9.2 ± 2.2	10.7 ± 1.7	1.021	0.327	0, small
	V_{10} (mm·s^{-1})	35.6 ± 10	36.6 ± 8.9	39 ± 8.3	44.6 ± 5.45	1.459	0.245	0.01, small
	V_{90} (mm·s^{-1})	148.4 ± 37.9	151.2 ± 33.9	158.5 ± 35.8	180.2 ± 26.4	1.6	0.224	0.02, small
Vastus medialis	Tc (ms)	23.8 ± 1.2	24 ± 0.9	22.8 ± 1.6	23.1 ± 2.5	0	0.985	−0.03, small
	Dm (mm)	7.3 ± 1.4	7.1 ± 1.5	7.6 ± 1	8.3 ± 1.9	1.796	0.199	0.02, small
	V_{10} (mm·s^{-1})	31.4 ± 11.8	33.74 ± 7.1	32.7 ± 11.2	38.7 ± 7.1	0.536	0.457	−0.01, small
	V_{90} (mm·s^{-1})	134.1 ± 45.1	140 ± 29.1	142.2 ± 42.2	167.2 ± 28	0.677	0.423	−0.01, small

*Tc = contraction time; Dm = muscle displacement, V_{10} = velocity of contraction to 10% of Dm; V_{90} = velocity of contraction to 90% of Dm.

and $d = 0.8$ was a large effect size. The magnitudes of the differences for GLM were analyzed using the omega-squared formula (ω_p^2) and qualitatively categorized as follow: $\omega_p^2 = 0.01$ as a small effect, $\omega_p^2 = 0.06$ as a moderate effect, and $\omega_p^2 = 0.15$ as a large effect size (Cohen, 1977). A 2-step multiple linear regression was performed using Tc and Dm as independent variables; CPK, Mg^{2+}, LDH and HCT as predictors The Pearson correlation coefficient was used to correlate the relationships between the analyzed variables. Changes were presented in percentage of change (Δ%). Analysis of data was performed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM, Chicago, IL, USA).

Results

There was a significantly increased CPK (544%), HCT (16%), and a significantly reduced magnesium (Mg^{2+}) (−29%), and body mass (−2%) at the end of the marathon (PR), compared with the beginning of the marathon (before race), in the 18 Costa Rican male ($n = 11$) and female ($n = 7$) runners (Table 1).

No significant differences in biochemical markers and body mass by speed group (time to complete the marathon) were found (Table 2).

From the TMG data analysis, there was a significantly increased muscle displacement (Dm) (12%) and contraction time (Tc) (4%) of the RF postrace compared with prerace (Table 3).

Table 5. Mean heart rate (HR), mean speed, and total impact.

Variable	Lap	Slow group	Fast group	t	p	d, rating
Impact (g force)	0–10k	1,302.7 ± 1,542	3,022.4 ± 2,967.7	−1.543	0.142	−0.36, small
	11–20k	1,496.7 ± 1829.4	3,415.4 ± 3,192.5	−1.564	0.137	−0.37, small
	21–30k	989.2 ± 1,535.3	3,787.5 ± 3,144.5	−2.399	0.029*	−0.57, small
	31–40k	556.7 ± 756.5	4,261.5 ± 3,090.4	−3.49	0.003†	−0.82, small
	0–40k	4,345.2 ± 5,035	14,486.8 ± 11,788.7	−2.373	0.030†	−0.56, small
Heart rate (b·min^{-1})	0–10k	162.6 ± 12.4	158 ± 4.9	1.046	0.311	0.25, small
	11–20k	164.2 ± 14.3	162.8 ± 4.2	0.298	0.770	0.07, small
	21–30k	159.9 ± 18.8	165.8 ± 5.1	0.899	0.382	0.09, small
	31–40k	152.5 ± 17.8	163 ± 9	−1.581	0.133	0.03, small
	0–40k	159.8 ± 14.8	162.4 ± 4.9	−0.493	0.629	−0.12, small
Speed (km·h^{-1})	0–10k	10.6 ± 0.9	12 ± 1.3	−2.640	0.018*	−0.62; small
	11–20k	10.7 ± 0.9	12.2 ± 1.3	−2.997	<0.01†	−0.71; small
	21–30k	9 ± 1	11.7 ± 1.5	4.559	<0.01†	−1.07; small
	31–40k	7.8 ± 0.9	10.4 ± 1.5	−4.547	<0.01†	−1.07; small
	0–40k	9.5 ± 0.5	11.6 ± 1.3	−4.000	<0.01†	−1.09; small

*$p < 0.05$.
†$p < 0.01$.

There were no significant differences in the RF or VM by speed group postrace compared with prerace (Table 4).

There were significant differences by speed group in impacts suffered between 21 and 40 km during the second half of the marathon. An inverse correlation was found between $\dot{V}O_2$max and race time ($r = -0.629$, $p = 0.005$). Also, a linear correlation between the Δ% of Tc in RF and CPK ($r = 0.482$, $p = 0.043$) was shown. There was a linear correlation between the speed and total impacts ($r = 0.56$, $p = 0.016$) and between $\dot{V}O_2$max and total impacts ($r = 0.74$, $p < 0.01$). Also, Δ% of Tc in RF was explained by Δ% CPK of 23.2% ($\beta = 0.482$, $p = 0.043$) (Table 5).

Discussion

This study examined the mechanical and biochemical changes in the lower-limb muscles before and after a marathon race in hot and humid conditions in 18 healthy subjects. Tensiomyography measurements were used in this study. The hypothesis that drove this study was that a marathon in hot and humid conditions could lead to mechanical changes, including increased muscle displacement (Dm), decreased contraction time (Tc), and decreased velocities of contraction to 10 and 90% of Dm (V_{10} and V_{90}). However, the findings of the study showed that Tc and Dm significantly increased in the RF muscle of the whole group and the speed group after the race, whereas for the prerace and PR V_{10} and V_{90}, a small effect was observed. The same small effect was observed in all TMG parameters of the VM.

Also, the findings of this study showed, contrary to the original study hypothesis, that the fastest runners would show smaller alterations in both biochemical and mechanical markers when compared with the slowest, the results showed no significant differences between both speed groups. However, supported by previously published studies (4,19), there were also significant differences in the serum levels of biochemical markers in the prerace group compared with the PR group, including a significant increase in CPK, but not in LDH (small effect), and a significant decrease in serum magnesium (Mg^{2+}) levels in all groups, regardless of speed group. These findings are supported by previously published studies, which have shown both alterations in the neuromuscular and biochemical profile after marathon races conducted in optimal environmental conditions (4,14,19). Results derived from body mass measurement showed a reduction in PR participants. There was also a significant increase in HCT at the end of the marathon, which indicated dehydration, which is commonly reported in this type of event and with similar environmental conditions of heat and humidity (6,42).

Exercise hyperthermia is known to produce dehydration and alterations in biochemical markers, including CPK, LDH, and Mg^{2+} (6,23,42). Some previously published studies have reported a 5–10% increase in HCT after marathons conducted in environments with temperatures ranging between 10 and 15° C (7,18,49), which indicates a loss in plasma volume. This increase in HCT can be exacerbated in hot-humid conditions, as there is a close relationship between the loss of electrolytes through sweating and the increase in body temperature (29). In this study, a 16.3% increase in HCT was found (Table 1), together with a significant loss in body mass loss (−2%) (Table 1). These results differ from those found by previous authors (7,49), who showed that and increase in HCT of between 5 and 10%, which could be explained by the high thermal stress that produces an increase in fluid and electrolyte loss in the form of sweat (49).

In addition, marathon running in hot-humid conditions is known to produce increased muscle damage because of the high rates of excessive muscular contractions (2). The findings of this study are supported by those of previously published studies of long-distance races, which have shown increased levels of PR CPK and LHD (up to 420% and up to 50%, respectively) (18,36,46), and reduced levels of Mg^{2+} (by up to 12%) (21,49). However, in this study, the PR increase in CPK increased by 543.8% (Table 1), and the decrease in Mg^{2+} was −28.6% (Table 1), which are greater than those reported in studies conducted in optimal temperature environments (21,46,49). Also, the findings of this study showed a nonsignificant increase of 11.8% in LDH, which was lower than reported previous studies with similar race distances (18). The findings of this study indicate that the greater the thermal stress, the greater the biochemical alterations in long-distance races when compared with the previously published literature.

The TMG results in this study were similar with those observed in previous studies, which showed an overall deterioration in the neural response (increased Tc) and a loss in muscle stiffness (increased Dm) (13,14). In this regard, some investigations have indicated that increases in Dm amplitude are due to velocity-related decreases in muscle stiffness, which allows for greater muscle fiber oscillations (12,40). A recently published study (1) has shown that decreases in stiffness resulting from quadriceps overuse during long-distance running may have been responsible for the development of inflammation and muscle swelling. In this previously published study (1), prolonged low-intensity exercises induced changes in quadriceps muscle stiffness, as observed in this study through increase found in Dm. In terms of muscle damage, this study observed a high PR increase of CPK (+543.8%) (Table 1), but there was no significant correlation between increased CPK and Dm in the RF or in the VM.

However, in this study, a significant relationship was found between increased CPK and Tc in the RF. Increased Dm or loss of muscle stiffness was found only in the RF (12%), but a similar, if nonsignificant, trend was also found in the VM (4%). Differences in these findings could not be explained by differences in muscle composition, as anatomical studies in men have demonstrated that both muscles, the RF and VM, have similar fiber composition of 46.9% type I

and 53.1 type II fibers in the RF, and. 43.7% type I and 56.3% type II fibers in the VM (17).

It is possible that there are different patterns of muscle utilization in the RF and VM during running (34). Sloniger et al (45). found a 25% increase in RF muscle volume activation during running compared with the VM muscles, with no differences in activation intensity, which was lowest for RF and VM muscles among 13 muscles studied. This previous finding indicates a higher proportion of slow-twitch motor unit utilization of the RF during running, resulting in a longer Tc and higher Dm because of postactivation potentiation of fast-twitch motor units. As a result, the VM only participates in the running mechanics during the swing phase, to extend the knee, whereas the RF participates both in the stance and swing phase to provide mechanical support and impulse (take-off) roles during rear foot contact, and to synergize with the flexion of muscles of the hip joint. The above findings might explain the increased contribution of the RF compared with the VM because of their different roles on running mechanics, which would also explain the differences in muscle deterioration, higher levels of fatigue, and lower stiffness. Although it is known that long-distance races induce additional quadriceps muscle contractions that generate large amounts of mechanical strain leading to muscle damage (18) and neuromuscular functional impairment (30), the results of this study might indicate that not all quadriceps muscles tolerate the extent of strain in the same way, as the muscular properties of the RF were significantly more affected.

In terms of the changes in muscle contraction velocity (V_{10}–V_{90}), the ratio between Dm, Tc, and Td (delay time), the results of this study showed a consistent, but nonsignificant, increase PR in RF V_{10} (+8.8%), RF V_{90} (+8%), VM V_{10} (+11.8%), and VM V_{90} (+11.2%), supporting the Tc and Dm alterations. However, because of intrasubject variations in *SD* and low study number (N), these findings could not be statistically confirmed. The higher values of V_{10} and V_{90} indicated that the ability of both muscles, the RF and VM, to contract rapidly was diminished because of the great muscular effort that this type of long-distance event demands, which is a finding previously reported by García-Manso (13). It has previously been demonstrated that the following fatigue caused by extensive excessive muscular activity, as occurs in a marathon, the degree of muscle stiffening is notably reduced (13), which would result in reduced velocities of contraction, or increased V_{10} and V_{90}. To explain this finding, some previous investigators (3,20) have suggested that the excess of laxity in the myotendinous tissues, because of decreased stiffness, causes difficulties in the return of the elastic energy, which limits performance by decreasing the force-generation capacity of the muscles, thus reducing contraction velocity, increasing V_{10} and V_{90}, and increasing Dm. In the 1990s, Metzger and Moss (28) showed that during reduced pH, mammalian fast-twitch and slow-twitch skinned skeletal muscle fibers resulted in reduction of muscle tension (35 ± 7%) and stiffness (12 ± 4%).

Although pH was not analyzed in this study, it has been previously confirmed by multiple studies that pH decreases after a marathon, and so, it can be suggested that reduced pH due to fatigue decreases the force-generation capacity, passive muscle tension, and stiffness (28). The suggested mechanism by which muscle tension and stiffness decrease was previously suggested to be due to low pH, which reduces the apparent number of cross-bridge attachments during maximal Ca^{2+} activation in fast-twitch but not slow-twitch fibers that account for the greater pH (+)-induced depression of maximum force in fast-twitch fibers (28). Also, the decrease of the force-generation capacity after long-distance races has been commonly quantified though the countermovement jump test, which has shown a significant reduction in PR values when compared with BS values (14,43).

However, another factor that might have favored the deterioration of muscle contractile properties could have been the high thermal stress experienced by subjects during the race in this study. Previous study findings support this view, as when the muscle temperature rises, the muscle contraction force, the rate of force development, and muscle conduction velocity, or electromechanical delay components, have been shown to become significantly reduced (5). Furthermore, the number of impacts, or *g*-forces, produced during the race did not correlate with any TMG parameters, although the FG showed a greater total number of impacts (14,486.8 ± 11,788.7) compared with the SG (4,345.2 ± 5,035) correlating a higher speed with greater impact. The latter might be because most impacts produced during the race were classified as low impact, of between 2 and ×3*g* (8). The TMG results in this study show some differences with those of previously published studies, where other authors have shown relationships between maximum voluntary isometric contraction (MVIC) and the behavior of TMG parameters, including a correlation between a decrease in MVIC with a decrease in Dm, $V_{10–90,}$ and increase in Tc (16,10).

Although the Tc results were consistent with those found by previous authors, our Dm and $V_{10–90}$ were not (13,14). In a previously reported study (14), Tc was shown to be significantly decreased in the vastus lateralis (−27.35 ± 18.0%) after an uphill marathon in high altitude, with a 3,063-m elevation gain, and 1810-m above mean sea level. We suggest that the difference in Tc findings compared with those previously reported (14), might be due to the differences between both races, an uphill high-altitude race compared with a flat course at sea level. In this regard, a recently published review has indicated that there are substantial biomechanical and physiological differences between uphill running and running on the horizontal, which alters muscle contraction patterns with increasing muscle fatigue (47).

This study has several limitations. First, the study did not use a gold standard method to analyze passive muscle stiffness or to corroborate the reduced stiffness of each

analyzed muscle but used TMG. However, the relationships between Dm and stiffness have been observed by this method in previous studies (12,14,40). Second, force measurements used to determine maximal power of the lower limbs were not used to confirm that fatigue had occurred. However, it is known that this type of event generates large degrees of fatigue, which decrease the maximal power of the lower limbs (14,43). Finally, we did not conduct a cross-over study to evaluate the different behaviors of the neuromuscular and biochemical alterations in a cooler environment because the race took place in Costa Rica, which has a tropical climate with temperatures ranging from 27 to 36° C throughout the year.

Further research is required to determine how TMG may best be applied to assess and monitor the acute effect of this type of popular events in similar environmental conditions on the neuromuscular system and to clarify how TMG data should be interpreted and how they relate to potential indicators of fatigue and stiffness by comparing this method with established or gold standard methods. In conclusion, the findings of this study have shown that running a marathon in high heat and humidity environment led to increased PR mechanical and biochemical changes compared with those previously reported in thermoneutral conditions. It may be necessary to intensify nutritional, hydration, and physical recovery protocols and to consider the environmental factors after running endurance races under these adverse circumstances.

Practical Applications

The results of this study showed that running a marathon in hot and humid conditions induced a significant reduction in lower-limb stiffness as well as high rates of neuromuscular fatigue, accompanied by several changes in biochemical markers, including CPK, magnesium (Mg^{2+}), and HCT, which are products of muscle damage and dehydration during the marathon. Given these findings, medical and sports medicine practitioners should increase and personalized fluid intake before, during, and after the marathon in the form of water or isotonic beverages to restore electrolytes such as Mg^{2+} and reduce dehydration associated with an increased HCT, from the effects of heat and sweat loss. The personalized protocols must be carefully prepared by coaches and athletes considering adverse environmental conditions such as those of this study during resistance events in hot and humid conditions, to improve the performance and decrease the impact on the health of the participants.

Acknowledgments

The authors have no conflicts of interest to disclose.

References

1. Andonian, P, Viallon, M, Le Goff, C, de Bourguignon, C, Tourel, C, Morel, J, et al. Shear-wave elastography assessments of quadriceps stiffness changes prior to, during and after prolonged exercise: A longitudinal study during an extreme mountain ultra-marathon. *PloS One* 11: e0161855, 2016.
2. Appell, HJ, Soares, JM, and Duarte, JA. Exercise, muscle damage and fatigue. *Sports Med* 13: 108–115, 1992.
3. Avela, J and Komi, PV. Reduced stretch reflex sensitivity and muscle stiffness after long-lasting stretch-shortening cycle exercise in humans. *Eur J Appl Physiol Occup Physiol* 78: 403–410, 1998.
4. Buchman, AL, Keen, C, Commisso, J, Killip, D, Ou, CN, Rognerud, CL, et al. The effect of a marathon run on plasma and urine mineral and metal concentrations. *J Am Coll Nutr* 17: 124–127, 1998.
5. Ce, E, Rampichini, S, Agnello, L, Limonta, E, Veicsteinas, A, and Esposito, F. Effects of temperature and fatigue on the electromechanical delay components. *Muscle Nerve* 47: 566–576, 2013.
6. Costa, RJ, Teixeira, A, Rama, L, Swancott, AJ, Hardy, LD, Lee, B, et al. Water and sodium intake habits and status of ultra-endurance runners during a multi-stage ultra-marathon conducted in a hot ambient environment: An observational field-based study. *Nutr J* 12: 13, 2013.
7. Costill, DL and Fink, WJ. Plasma volume changes following exercise and thermal dehydration. *J Appl Physiol* 37: 521–525, 1974.
8. Cummins, C, Orr, R, O'Connor, H, and West, C. Global positioning systems (GPS) and microtechnology sensors in team sports: A systematic review. *Sports Med* 43: 1025–1042, 2013.
9. Davidson, RJ, Robertson, JD, Galea, G, and Maughan, RJ. Hematological changes associated with marathon running. *Int J Sports Med* 8: 19–25, 1987.
10. de Paula Simola, RA, Harms, N, Raeder, C, Kellmann, M, Meyer, T, Pfeiffer, M, et al. Assessment of neuromuscular function after different strength training protocols using tensiomyography. *J Strength Cond Res* 29: 1339–1348, 2015.
11. de Paula Simola, RA, Raeder, C, Wiewelhove, T, Kellmann, M, Meyer, T, Pfeiffer, M, et al. Muscle mechanical properties of strength and endurance athletes and changes after one week of intensive training. *J Electromyogr Kinesiol* 30: 73–80, 2016.
12. Evetovich, TK, Housh, TJ, Stout Johnson, GO, Smith, DB, and Ebersole, KT. Mechanomyographic responses to concentric isokinetic muscle contractions. *Eur J Appl Physiol Ocupp Physiol* 75: 166–169, 1997.
13. García-Manso, JM, Rodríguez-Ruiz, D, Rodríguez-Matoso, D, de Saa, Y, Sarmiento, S, and Quiroga, M. Assessment of muscle fatigue after ultra-endurance triathlon using tensiomyography (TMG). *J Sport Sci* 29: 619–625, 2011.
14. Giovanelli, N, Taboga, P, Rejc, E, Simunic, B, Antonutto, G, and Lazzer, S. Effects of an uphill marathon on running mechanics and lower-limb muscle fatigue. *Int J Sports Physiol Perform* 11: 522–529, 2016.
15. Granata, KP, Padua, DA, and Wilson, SE. Gender differences in active musculoskeletal stiffness. Part II. Quantification of leg stiffness during functional hopping tasks. *J Electromyogr Kinesiol* 12: 127–135, 2002.
16. Hunter, AM, Galloway, SD, Smith, IJ, Tallent, J, Ditroilo, M, Fairweather, MM, et al. Assessment of eccentric exercise-induced muscle damage of the elbow flexors by tensiomyography. *J Electromyogr Kinesiol* 22: 334–341, 2012.
17. Johnson, MA, Polgar, J, Weightman, D, and Appleton, D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci* 18: 111–129, 1973.
18. Kim, HJ, Lee, YH, and Kim, CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *Eur J Appl Physiol* 99: 443–447, 2007.
19. Kobayashi, Y, Takeuchi, T, Hosoi, T, Yoshizaki, H, and Loeppky, JA. Effect of a marathon run on serum lipoproteins, creatine kinase, and lactate dehydrogenase in recreational runners. *Res Q Exerc Sport* 76: 450–455, 2005.

20. Kokkonen, J, Nelson, A, and Cornwell, A. Acute muscle stretching inhibits maximal strength performance. *Res Q Exerc Sport* 69: 411–415, 1998.
21. Kratz, A, Wood, MJ, Siegel, AJ, Hiers, JR, and Van Cott, EM. Effects of marathon running on platelet activation markers: Direct evidence for *in vivo* platelet activation. *Am J Clin Pathol* 125: 296–300, 2006 pp. 20–24.
22. Kubo, K. Active muscle stiffness in the human medial gastrocnemius muscle. In vivo. *J Appl Physiol* 117: 1020–1026, 2014.
23. Landis, K. *The Effect of Creatine and Magnesium Supplementation on Delayed Muscle Soreness.* Master's thesis, Western Washington University, Bellingham, 2013. pp. 20–24.
24. Marino, FE. Anticipatory regulation and avoidance of catastrophe during exercise-induced hyperthermia. *Comp Biochem Physiol B Biochem Mol Biol* 139: 561–569, 2004.
25. Martín-Rodríguez, S, Loturco, I, Hunter, AM, Rodríguez-Ruiz, D, and Munquia-Izquierdo, D. Reliability and measurement error of tensiomyography to assess mechanical muscle function: A systematic review. *J Strength Cond Res* 31: 3524–3536, 2017.
26. Maughan, RJ. Distance running in hot environments: A thermal challenge to the elite runner. *Scand J Med Sci Sports* 20: 95–102, 2010.
27. Mayfield, D, Launkonis, B, Creeswell, A, and Lichtwark, G. Additional in-series compliance reduces muscle force summation and alters the time course of force relaxation during fixed-end contractions. *J Exper Biol* 219: 3587–3596, 2016.
28. Metzger, JM and Moss, RL. Effects of tension and stiffness due to reduced pH in mammalian fast- and slow-twitch skinned skeletal muscle fibres. *J Physiol* 428: 737–750, 1990.
29. Meyer, F, Bar-Or, O, MacDougall, D, and Heigenhauser, GJ. Sweat electrolyte loss during exercise in the heat: Effects of gender and maturation. *Med Sci Sports Exerc* 24: 776–781, 1992.
30. Millet, GY, Lepers, R, Maffiuletti, NA, Babault, N, Martin, V, and Lattier, G. Alteration of neuromuscular function after an ultra-marathon. *J Appl Physiol* 92: 486–492, 2002.
31. Montgomery, WH, Pink, M, and Perry, J. Electromyographic analysis of hip and knee musculature during running. *Am J Sports Med* 22: 272–278, 1994.
32. Morgan, DL. Separation of active and passive components of short-range stiffness of muscle. *Am J Physiol* 232: C45–C49, 1977.
33. Nicol, C, Komi, PV, and Marconnet, P. Fatigue effects of marathon running on neuromuscular performance II: Changes in force, integrated electromyographic activity and endurance capacity. *Scand J Med Sci Sports* 1: 18–24, 2007.
34. Nicola, TL and Jewison, DJ. The anatomy and biomechanics of running. *Clin Sports Med* 31: 187–201, 2012.
35. Nielsen, FH and Lukaski, HC. Update on the relationship between magnesium and exercise. *Magnesium Res* 19: 180–189, 2006.
36. Nuviala, RJ, Roda, L, Lapieza, MG, Boned, B, and Giner, A. Serum enzymes activities at rest and after a marathon race. *J Sports Med Phys Fitness* 32: 180–186, 1992.
37. Nybo, L. Brain temperature and exercise performance. *Exp Physiol* 97: 333–339, 2012.
38. Nybo, L and Nielsen, B. Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol* 91: 1055–1060, 2001.
39. Olha, AE, Klissouras, V, Sullivan, JD, and Skoryna, SC. Effect of exercise on concentration of elements in the serum. *J Sports Med Phys Fitness* 22: 414–425, 1982.
40. Pisot, R, Narici, MV, Simunic, B, De Boer, M, Seynnes, O, Jurdana, M, et al. Whole muscle contractile parameters and thickness loss during 35-day bed rest. *Eur J Appl Physiol* 104: 409–414, 2008.
41. Pivarnik, JM, Leeds, EM, and Wilkerson, JE. Effects of endurance exercise on metabolic water production and plasma volume. *J Appl Physiol Respir Environ Exerc Physiol* 56: 613–618, 1984.
42. Rama, LM, Minuzzi, LG, Carvalho, HM, Costa, RJ, and Texeira, AM. Changes of hematological markers during a multi-stage ultra-marathon competition in the heat. *Int J Sports Med* 37: 104–111, 2016.
43. Rousanoglou, EN, Noutsos, K. Pappas, A, Bogdanis, G, Vagenas, G, Baylos, IA, et al. Alterations of vertical jump mechanics after a half-marathon mountain running race. *J Sports Sci Med* 15: 277–286, 2016.
44. Sawka, MN, Montain, SJ, and Latzka, WA. Hydration effects on thermoregulation and performance in the heat. *Comp Biochem Physiol Mol Integr Physiol* 128: 679–690, 2001.
45. Sloniger, M, Cureton, K, Prior, B, and Evans, E. Lower extremity muscle activation during horizontal and uphill running. *J Appl Physiol* 83: 2073–2079, 1997.
46. Tojima, M, Noma, K, and Torii, S. Changes in serum creatine kinase, leg muscle tightness, and delayed onset muscle soreness after a full marathon race. *J Sports Med Phys Fitness* 56: 782–788, 2016.
47. Vernillo, G, Giandolini, M, Edwards, WB, Morin, JB, Samozino, P, Horvais, N, et al. Biomechanics and physiology of uphill and downhill running. *Sports Med* 47: 615–629, 2017.
48. Watson, P, Hasegawa, H, Roelands, B, Piacentini, MF, Looverie, R, and Meeusen, R. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *J Physiol* 565: 873–883, 2005.
49. Whiting, PH, Maughan, RJ, and Miller, JD. Dehydration and serum biochemical changes in marathon runners. *Eur J Appl Physiol Occup Physiol* 52: 183–187, 1984.
50. Williams, DS, McClay, IS, and Hamil, J. Arch structure and injury patterns in runners. *Clin Biomech* 16: 341–347, 2001.