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Fibre-type-specific and Mitochondrial Biomarkers of Muscle Damage after Mountain Races

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ABSTRACT

Consequences of running mountain races on muscle damage were investigated by analysing serum muscle enzymes and fibre-type-specific sarcomere proteins. We studied 10 trained amateur and 6 highly trained runners who ran a 35 km and 55 km mountain trail race (MTR), respectively. Levels of creatine kinase (CK), CK-MB isoform (CK-MB), sarcomeric mitochondrial CK (sMtCK), transaminases (AST and ALT), cardiac troponin I (cTnI) and fast (FM) and slow myosin (SM) isoforms, were assessed before, 1 h, 24 h and 48 h after the beginning of MTR. Significant SM increases were found at 24 h in the 55 km group. Levels of CK, CK-MB, AST and cTnI were significantly elevated in both groups following MTR, but in the 55 km group they tended to stabilize in at 48 h. Using pooled data, time-independent serum peaks of SM and CK-MB were significantly correlated. Moreover, concentration of sMtCK was significantly elevated at 1 and 24 h after the race in the 35 km group. Although training volume could confer protection on the mitochondria, the increase in serum CK-MB and SM in the 55 km group might be related to damage to the contractile apparatus type I fibres. Competing in long-distance MTRs might be related to deeper type I muscle fibre damage, even in highly trained individuals

Introduction

Although the physiological response to mountain trail running races has become an important topic in sports science research in recent years [24, 35, 36, 42], very little is known about the structural damage induced in the muscle fibres of athletes who participate in these strenuous events. The term 'mountain trail race' (MTR) has been generically used to refer to competitive long-distance runs ranging from 15 to over 90 km that take place in a mountain context and involve large cumulative elevation displacement (uphill and downhill) [14]. This kind of ultra-endurance race represents an exceptional model for the study of physiological responses to extreme loads and stress [35].

There is general agreement that MTRs lead to muscle damage because of the strenuous competitive conditions [36]. The mechanical stress associated with eccentric contractions during downhill running causes damage to the fibre cytoskeleton, and produces inflammation and delayed-onset muscle soreness [16, 18]. During flat running, in which the eccentric impact is lower, it has been established that the total running distance covered or the time spent running is related to the extent of muscle damage [39, 47]. Specifically, during flat ultra-endurance running, it has been report-

ed that increased serum levels of muscle enzymes such as creatine kinase (CK) indicate that active muscle suffers a significant degree of sarcolemmal damage with increased running distance [40]. Traditional biochemical indices of muscle damage, such as CK, have also been used in mountain running races, but there are often large variations among studies and between subjects. In this regard, peak CK activity has been reported to be (mean ± standard deviation) 15,775 ± 17,166; (mean) 32,956 (range: 1500–264,300); (mean) 20,484 (range: 11,714–29,253); and (mean ± standard deviation) 3719 ± 3045 IU L^{-1} after MTRs of 166 km [36], 161 km [25, 30], and 330 km [42], respectively. These differences could be explained by extrinsic factors such as a large variation in the MTR characteristics (i. e., distance and total elevation displacement), environmental factors (i. e., weather and surface conditions), or intrinsic factors such as the general fitness level and the pacing strategy used by runners, especially during downhill running when the eccentric component is exacerbated [15]. Moreover, muscle enzymes such as CK are not fibre-type-specific [17] and, due to their mainly sarcoplasmic location, their appearance in blood suggests, in most cases, a loss of sarcolemma integrity [38]. To overcome these limitations, other biomarkers related with a different cellular structure have been used in order to characterize the exercise impact on muscle fibres. Serum levels of fast and slow myosin (FM and SM, respectively) isoforms have been used as indirect biomarkers of fibre-type-specific sarcomere damage [7, 9], and sarcomeric mitochondrial CK (sMtCK) to evaluate mitochondrial integrity [8]. Specifically, significant increases in SM, suggesting sarcomere disruption in slow (type I) fibres, were found in 85 km MTR participants who completed different overall distances during the whole race [9]. Moreover, increases in serum sMtCK could be indicative of the number of myofibrils that have died by either apoptosis (due to a process triggered by large increases in sMtCK) and/or necrosis [8].

The aim of the present study was to investigate the time course response of novel biomarkers including sarcomeric mitochondrial CK (sMtCK), and sarcomere fibre-type-specific proteins, FM and SM, in different competitive scenarios: (1) amateur trained runners competing in a 35 km MTR, and (2) highly-trained amateur and sponsored runners competing in a 55 km MTR.

Materials and Methods

Participants

Ten men who ran a 35 km MTR (means ± standard deviations: age, 37.7 ± 7.4 years; weight, 73.9 ± 9.4 kg; height, 177.6 ± 3.4 cm), and 6 men who ran a 55 km MTR (mean ± standard deviation (SD): age, 34.0 ± 5.2 years; weight, 71.3 ± 8.8 kg; height, 176.2 ± 7.3 cm) volunteered for the study. The group that ran the 35 km MTR was composed of trained amateur runners with at least 3 years of mountain running training experience (mean ± SD: 7.0 ± 1.1 h per week). The 55 km MTR group was composed of highly trained amateur and sponsored runners with at least 5 years of mountain running training experience (mean ± SD: 12.6 ± 3.0 h per week). All the participants were healthy and none had suffered any muscle and/or tendon injuries in the 6 months before the study. Physical activity after the races was limited, and massages were prohibited. All the participants were asked about the type of training performed the week before the race to better understand the baseline values of serum enzymes and fibre-type-specific proteins. The study complied with the ethical standards of the International Journal of Sports Medicine [22] and all participants provided written informed consent. The study was previously approved by the Ethics Committee of the Catalan Sports Council (0099 S/690/2013).

Mountain trail races

Both races, the 35 km and 55 km MTR, formed part of the Volta a la Cerdanya Ultrafons® 2013 mountain endurance races, and had similar total elevation gains: 2089 and 2259 m, respectively. Average negative slopes were 8.9 % for the 35 km MTR and 8.0 % for the 55 km MTR (see the mountain trail profiles in ▶ **Fig. 1**). Environmental conditions for the 35 km MTR were 8.9 °C and 92 % relative humidity (RH) at the start of the race and 13.9 °C and 59 % RH at the end of the race. Environmental conditions for the 55 km MTR were 10.4 °C and 79 % RH at the start of the race, and 10.8 °C and 83 % RH at the end of the race.

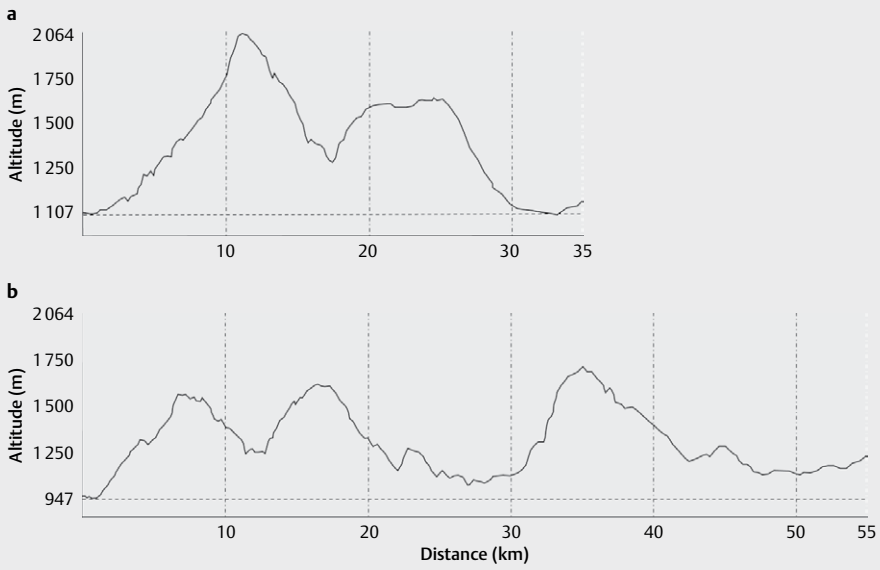
Blood sampling

Blood samples of 5 mL were drawn from an antecubital vein by standard venipuncture one day before the competition (pre), 1 h after (post), and 24 and 48 h after the beginning of the races. The samples were allowed to clot in a tube (SST II Advance, Becton Dickinson Vacutainer Systems, Oxford, UK) for 30 min and then centrifuged at 3000 × g for 10 min. 3 aliquots of serum were obtained and stored at –80 °C until analysis.

Biochemical assays

Automated analyses of CK, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed in an Advia 2400 (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) following the method of the International Federation of Clinical Chemistry's Committee primary reference procedures for the measurement of catalytic activity concentrations of enzymes [45]. Creatine kinase MB isoform (CK-MB) and cardiac troponin I (cTnI) analyses were performed using a Dimension Clinical Chemistry System (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), with an analytical measurement range of 0.5–300 ng · mL^{-1}, and 0.017–40 ng · mL^{-1}, respectively. The serum concentration of sMtCK was measured using a commercial ELISA kit SEC386Hu (Cloud Clone Corp., Houston, TX, USA) according to the manufacturer's protocol.

The concentration of myosin isoforms, FM and SM, was measured using the enzyme-linked immunosorbent assay (ELISA sandwich) described elsewhere [7]. Briefly, 2 plates (Corning 96-well EIA/RIA, Sigma Aldrich, Poole, UK) were coated overnight at 4 °C with capture monoclonal antibodies (all Sigma Aldrich, Poole, UK), anti-myosin (skeletal, fast) clone My-32 and anti-myosin (skeletal, slow) clone NOQ7.5.4D, for FM and SM assessment, respectively. Monoclonal anti-myosin (skeletal, fast) clone My-32 localizes an epitope on the MHC 2, and monoclonal anti-myosin (skeletal, slow) clone NOQ7.5.4D recognizes an epitope located on the heavy meromyosin portion of ß/slow MHC. The plates were then washed 3 times (phosphate buffered saline, pH 7.4, 10 mM) and blocked with block buffer (Super Blocking Buffer, Thermo Fisher Scientific



▶ **Fig. 1** General view of race profiles. Altitudinal profile and distance scale in km of the 35 km mountain trail race (35 km MTR) **a**; and the 55 km mountain trail race (55 km MTR) **b**.

Inc., Rockford, IL, USA) before being incubated (60 min at 37 °C). After a washing step, samples (10 µL) were added to the plates in triplicate, and a calibration curve of 6-point serial dilution, from 0–250 ng, of commercial pure myosin of porcine muscle M0273 was obtained. To complete the ELISA, anti-myosin polyclonal antibody M7523 was used as the primary antibody, and mouse ant-IGG linked to peroxidase A6154 as the secondary antibody. Finally, myosin concentrations ($\mu g \cdot L^{-1}$) were obtained by the interpolation of the calibration curve ($r^2 > 0.95$). Intra-assay coefficients of variation were 10.0 and 5.5 % for FM and SM, respectively. The linearity of the FM assay was 80 %, and it was 90 % for the SM assay.

Statistical analysis

Data were tested for approximation to a normal distribution using the Shapiro-Wilk test. One-way repeated-measures ANOVA was performed in both distance groups separately (35 km MTR and 55 km MTR) to identify the effect of time (pre, post, and 24 and 48 h following the start of the race) on serum biomarkers of muscle damage (CK, CK-MB, sMtCK, AST, ALT, cTnI, FM and SM). When significant effects were found, a post hoc test was performed by applying a paired t-test with a Bonferroni correction to locate significant differences from baseline and peak values. The time course of changes in skewed data, such as cTnI serum concentration (35 km MTR group), was evaluated using Friedman and Wilcoxon non-parametric tests. Pearson's correlation coefficient was used to assess relationships between variables of interest by using log-transformed pooled data from both distance groups (35 km MTR and 55 km MTR). The differences between group training and performance characteristics were analysed using the unpaired t-test or Mann-Whitney test (depending on the variable distribution). Data are presented as mean ± standard error of the mean unless otherwise stated. The level of significance was set at $P < 0.05$. The statistical analysis was conducted using SPSS version 23.0 (SPSS Statistics, IBM Corp., Armonk, NY, USA).

Results

Training and performance

The training volume (training hours per week) of the 55 km MTR group was 80 % ($P = 0.04$) higher than that of the 35 km MTR group. No differences were seen in training experience measured as years of mountain running training. As expected, the official running time was significantly ($P = 0.04$) higher for the group that ran the 55 km MTR, but no differences were found in the average running velocity ($km \cdot h^{-1}$) (▶ **Table 1**).

▶ **Table 1** Training, status and performance characteristics of participants.

	Distance group	
Variables	**35 km MTR**	**55 km MTR**
Training		
Years of mountain running training	6.1 ± 1.3	8.1 ± 1.2
Training hours ($h \cdot wk^{-1}$)	7.0 ± 1.1	12.6 ± 3.0 *
Status		
Amateur (n)	10	2
Sponsored (n)	0	4
Performance		
Official running time (h:min:sec)	3:50:50 ± 0:08:38	6:52:38 ± 0:34:22 *
Average running velocity ($km \cdot h^{-1}$)	9.22 ± 0.33	8.29 ± 0.69

Values are means ± SEM. * Significantly different from the 35 km MTR group value at $P < 0.05$.

Serum muscle enzymes

The serum CK activity in the 35 km MTR group increased significantly at every time point analysed after the race, reaching a peak of 832 ± 267 U L^{-1} (P = 0.003) 24 h after the start of the MTR. The serum CK activity in the 55 km MTR group increased significantly over time and also peaked (1238 ± 201 U L^{-1}; P < 0.001) at 24 h after the start of the MTR. However, a sharp, significant decrease was observed 48 h after the start of the race in the 55 km MTR group (▶ **Fig. 2**). Pooled CK peak activity, regardless of the time to peak, was similar for both groups (slightly above 1000 IU L^{-1}) (▶ **Table 2**).

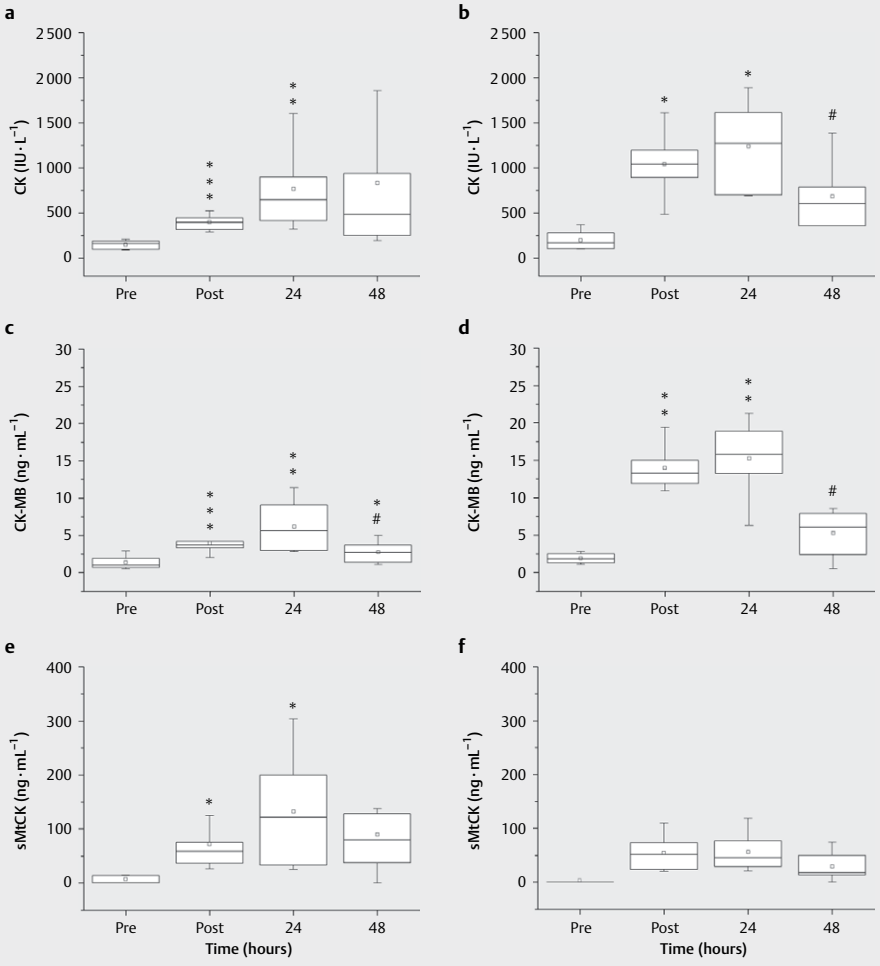
The serum concentration of CK-MB was significantly elevated in both groups following the race, until a peak at 24 h (6.15 ± 1.2 ng mL^{-1}; P < 0.001, and 15.2 ± 2.1 ng mL^{-1}; P < 0.001, for the 35 km MTR and 55 km MTR groups, respectively). At 48 h after the beginning of the races, a significant decrease was observed in both groups of competitors (▶ **Fig. 2**, ▶ **Table 2**).

The serum concentration of sMtCK was only significantly elevated 1 h post-race (72 ± 17 ng mL^{-1}; P = 0.031) and 24 h following the start of the race (132 ± 32 ng mL^{-1}; P = 0.022) in the 35 km group (▶ **Fig. 2**). High sMtCK time-independent peak concentrations were also observed in the 35 km group (▶ **Table 2**).

Serum AST levels were significantly increased in both groups at every time point analysed following the race. Serum peaks were observed at 24 h (49 ± 4 U L^{-1}, P < 0.001 and 63 ± 7 U L^{-1}, P < 0.001 for the 35 km MTR and 55 km MTR groups, respectively). No significant time-related changes were seen in ALT serum activity (▶ **Fig. 3**; ▶ **Table 2**).

Serum muscle myosin isoforms

Although the serum SM concentration of the 35 km MTR group did not differ from baseline values at any point, the serum SM concentration of the 55 km MTR group was significantly increased 24 h following the race (4043 ± 921 µg L^{-1}; P = 0.042). Accordingly, high time-related and time-independent peak values of serum SM were observed in the group that ran the 55 km MTR (▶ **Fig. 4**, ▶ **Table 2**). Furthermore, in both groups (pooled data), time-independent serum peaks of SM and CK-MB were significantly correlated (r = 0.751, P < 0.001) (▶ **Fig. 5**). No significant changes from base-



▶ **Fig. 2** Creatine kinase. Changes in enzyme activities or concentrations of creatine kinase (CK), creatine kinase MB isoform (CK-MB), and sarcomeric mitochondrial creatine kinase (sMtCK) in 35 km MTR participants **a**, **c**, **e**; and 55 km MTR participants **b**, **d**, **f**. Boxes represent the interquartile range (IQR), whiskers represent 1.5X the IQR values, horizontal lines represent medians, and squares represent means. *, ** and *** Significantly different from baseline at *P* < 0.05, *P* < 0.01, and *P* < 0.001, respectively. #Significantly different from peak values at P < 0.01.

▶ **Table 2** Individual peak values of measured biomarkers.

	Participant	Peak CK Activity ($IU \cdot L^{-1}$) [time of peak][a]	Peak CK-MB Concentration ($ng \cdot mL^{-1}$) [time of peak][a]	Peak sMtCK Concentration ($ng \cdot mL^{-1}$) [time of peak][a]	Peak AST Activity ($IU \cdot L^{-1}$) [time of peak][b]	Peak cTnI Concentration ($ng \cdot mL^{-1}$) [time of peak][c]	Peak SM Concentration (% Change from Baseline) [time of peak][d]	Peak FM Concentration (% Change from Baseline) [time of peak][d]
35 km MTR	1	2755[48 h]	5.3[24 h]	107[48 h]	90[48 h]	0.037[Post]	0	7[Post]
	2	411[24 h]	3.9[Post]	125[24 h]	35[24 h]	0.192[Post]	15[Post]	47[24 h]
	3	588[24 h]	2.8[24 h]	199[24 h]	40[24 h]	0.070[Post]	17[Post]	73[Post]
	4	588[24 h]	6.4[24 h]	110[24 h]	48[24 h]	0.067[Post]	6[48 h]	16[24 h]
	5	900[24 h]	11.4[24 h]	138[48 h]	66[24 h]	0.107[Post]	4[48 h]	19[Post]
	6	1855[48 h]	9.1[24 h]	278[24 h]	64[48 h]	0.096[Post]	20[24 h]	0
	7	702[24 h]	9.3[24 h]	303[24 h]	50[24 h]	0.089[Post]	30[48 h]	9[24 h]
	8	1599[24 h]	5.9[24 h]	140[24 h]	64[24 h]	0.028[Post]	31[48 h]	0
	9	322[24 h]	5.3[24 h]	27[Post]	43[Post]	0.037[Post]	1[48 h]	11[Post]
	10	388[24 h]	3.3[Post]	67[Post]	40[Post]	0.117[Post]	0	0
55 km MTR	11	1425[24 h]	13.2[24 h]	74[48 h]	86[24 h]	0.113[Post]	28[24 h]	36[48 h]
	12	687[24 h]	21.3[24 h]	50[48 h]	54[24 h]	0.038[Post]	80[48 h]	8[Post]
	13	1612[24 h]	17.2[24 h]	77[24 h]	83[24 h]	0.098[Post]	109[24 h]	36[Post]
	14	1119[24 h]	18.9[24 h]	74[Post]	51[24 h]	0.505[Post]	156[24 h]	69[48 h]
	15	1889[24 h]	19.4[Post]	118[24 h]	62[24 h]	0.089[Post]	130[48 h]	34[Post]
	16	940[48 h]	10.9[Post]	29[24 h]	41[Post]	0.017	75[24 h]	100[48 h]
35 km MTR (mean value)		1011	6.3	149	54	0.084	12	18
55 km MTR (mean value)		1279	16.8	70	63	0.143	96	47

Individual peak values [time of peak] for main variables. Time-independent mean values of each group are shown. Creatine kinase (CK), creatine kinase-MB isoform (CK-MB), sarcomeric mitochondrial creatine kinase (sMtCK), aspartate aminotransferase (AST), cardiac troponin I (cTnI), slow myosin (SM), fast myosin (FM); [a] ▶ **Fig 2**; [b] ▶ **Fig 3;** [c] ▶ **Fig 6;** [d] ▶ **Fig 4**

line values were seen in average serum FM concentration over time in either of the groups. However, participants 2 and 3 in the 35 km MTR, and 14 and 16 in the 55 km MTR showed high relative serum peaks at different time points (▶ **Table 2**).

Serum cardiac troponin I

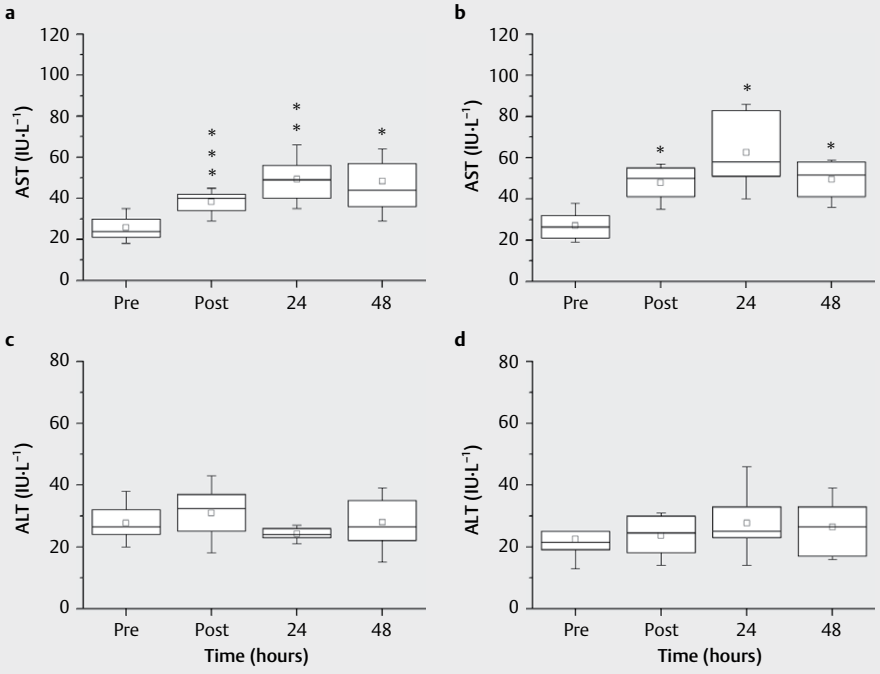
Significant cTnI increases over the clinically normal range ($0.050\ ng \cdot mL^{-1}$) were seen in both groups 1 h after finishing the race, returning to baseline values 24 h after the start of the race (▶ **Fig. 6**). For individual and group mean peak values, ▶ **Table 2**.

Discussion

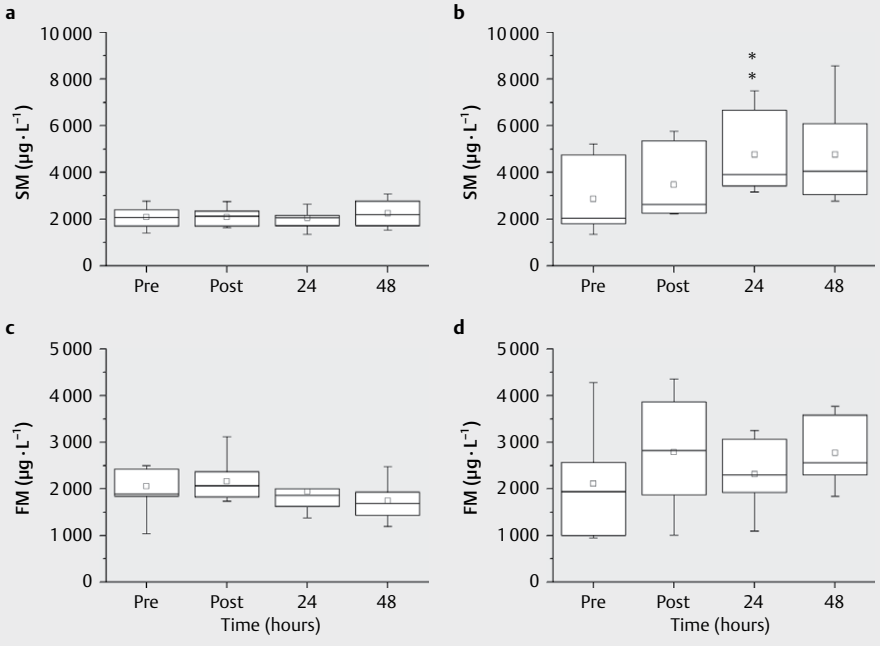
The aim of the present study was to investigate the temporal response of muscle enzymes and sarcomere fibre-type-specific proteins in a trained group of amateur runners performing a 35 km MTR, and a group of highly trained amateur and/or sponsored competitors participating in a 55 km MTR. The main finding of the present study was that only the 55 km MTR caused SM release, which signifies sarcomere damage of slow (type I) fibres. This may suggest that total downhill distance running volume is related to the degree of muscle damage, even when the race is undertaken by highly trained athletes. However, the serum enzyme time course suggested enhanced clearance rates in the 55 km MTR. Another relevant finding was that in both groups (pooled data), time-independent serum peaks of SM and CK-MB were significantly correlated. Finally, selective sMtCK increases in the 35 km MTR group, but not in the 55 km group, indicate that training volume might confer protection on mitochondria. So, it seems that sMtCK could be a promising novel biomarker of muscle damage that is sensitive to training volume.

Slow (type I) sarcomere damage and downhill running distance

The release of sarcomere proteins, such as myosin, from muscle fibres to the bloodstream has been reported previously as indirect evidence of sarcomere damage [7, 9]. In the present study, only the 55 km MTR induced an increase in SM in serum that suggests the presence of slow (type I) fibre sarcomere damage. In contrast, the fibre damage inflicted by the 35 km MTR was mainly located at the membrane level, because no significant increases in sarcomere fibre-type-specific proteins were found and only moderate levels of muscle enzyme efflux to the bloodstream were observed (i. e., ~1000 $IU\ L^{-1}$ of CK), which indicates increased sarcolemmal permeability (for a review see [28]). Notably, although there were significant differences in the training volume of the groups (the number of hours spent training was 80 % higher in the group that ran the 55 km MTR), no protective effect on muscle fibre sarcomeres



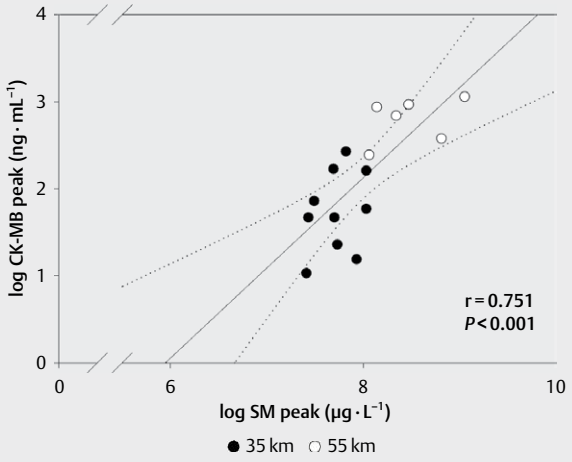
▶ **Fig. 3** Transaminases. Changes in enzyme activities of aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in 35 km MTR participants **a**, **c**; and 55 km MTR participants **b**, **d**. Boxes represent the interquartile range (IQR), whiskers represent 1.5X the IQR values, horizontal lines represent medians, and squares represent means. *, ** and *** Significantly different from baseline at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.



▶ **Fig. 4** Myosin isoforms. Changes in serum concentration of slow myosin (SM) and fast myosin (FM) in 35 km MTR participants **a**, **c**; and 55 km MTR participants **b**, **d**. Boxes represent the interquartile range (IQR), whiskers represent 1.5X the IQR values, horizontal lines represent medians, and squares represent means. **Significantly different from baseline at $P<0.01$.

seemed to be provided by increased training volume because only the 55 km MTR group showed significant SM serum increases.

The appearance of SM in serum is the result of the degradation of sarcomere proteins due to the activation of Ca^{2+}-dependent proteases such as Calpain [20]. Since Ca^{2+} accumulation in the human vastus lateralis after 20 km of flat running has been shown in humans [39], significant Ca^{2+} accumulation in lower limb muscles might be expected following a MTR. Progressive intercellular Ca^{2+} accumulation during mountain running can be explained by increased sarcolemmal permeability [34]. Moreover, the eccentric component during downhill running [15] may lead to stretch-induced Ca^{2+} entry through the ion channels [2], contributing to an increase in the intracellular Ca^{2+} accumulation. Therefore, as the accumulation of Ca^{2+} is dependent on the time of exposure to exercise [39, 40], and downhill running distance may substantially contribute to increasing this accumulation by stretch-induced Ca^{2+} entry, it seems reasonable to assume that the greater the total downhill running distance of an MTR, the greater the damage to myofibre structure. This suggestion is also reinforced by the fact that both the 35 km and 55 km MTR had similar average negative slopes (8.9 and 8.0, respectively), so the greatest difference between races was the total negative distance covered in the 55 km MTR. Therefore, it can be concluded that although other intrinsic factors that were not measured during the races, such as pace fluctuations [13] or foot strike pattern variability [18], may substantially influence the degree of muscle damage, in the present study the downhill running distance was a relevant factor inducing slow (type I) fibre sarcomere damage.



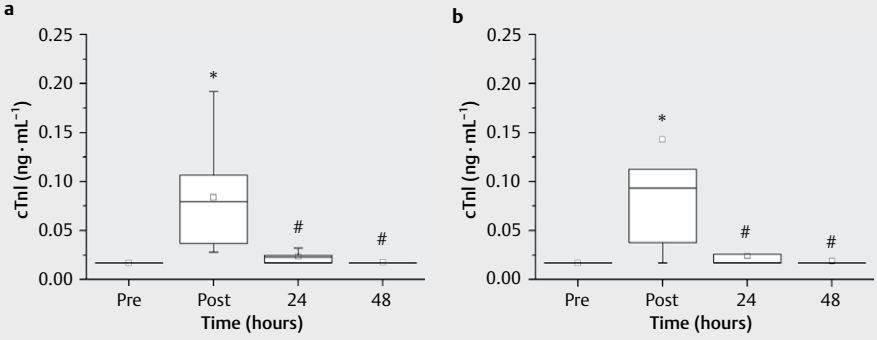
▶ **Fig. 5** Associations between creatine kinase MB isoform and slow myosin time-independent serum peak values. Pearson correlation coefficient (r) between time-independent peak serum values of slow myosin (SM) and creatine kinase MB isoform (CK-MB) from pooled data (35 km and 55 km participants). Dotted lines represent 95 % confidence intervals (0.553–0.889).

Muscle enzyme clearance

The activities of CK and CK-MB enzymes from the 55 km MTR group tended to stabilize in the blood at 48 h, showing sharp decreases from peak values. Although not all the mechanisms involved in CK clearance from the blood are known [4], it is commonly accepted that the reticuloendothelial system is responsible for this function and that the clearance time depends, among other factors, on the subject's training status [6, 12]. Therefore, since the 55 km MTR participants were highly trained amateur and sponsored runners, the possibility that they presented an adaptive mechanism leading to enhanced clearance rates cannot be ruled out.

Muscle enzymes and slow myosin association

Another notable finding is that, in all participants, the time-independent serum peaks of CK-MB and SM were strongly correlated, which indicates a close relationship between the magnitudes of these biochemical markers. Although fast (type II) and slow (type I) fibres present similar CK activity [3], competitive distance runners tend to have higher percentages of slow (type I) fibres [21, 43], and it is reasonable to assume that it is slow (type I) fibres that are predominantly recruited and damaged during an MTR [9]. Therefore, CK serum activity could be mostly related to the contractile activity of slow (type I) fibres. The correlation between CK-MB and SM could be explained by the fact that slow (type I) fibres have higher levels of activity of these enzymes [48]. CK-MB consists of the subunits M and B [6]. The M-subunit of CK is in the M-region (also called the M-band) of the sarcomere [27]. The M-region is the centre of many metabolic processes, mechanosensing and intracellular proteolysis (proteasome) [1, 26]. All these processes sustain fibre homeostasis, myofi-



▶ **Fig. 6** Cardiac troponin I. Changes in serum cardiac troponin I (cTnI) in 35 km MTR participants **a**; and 55 km MTR participants **b**. Clinically normal range (< 0.050 ng mL⁻¹). Boxes represent the interquartile range (IQR), whiskers represent 1.5X the IQR values, horizontal lines represent medians, and squares represent means. *Significantly different from baseline at $P < 0.05$. #Significantly different from peak values at $P < 0.05$.

brillar organization, and contractile activity by maintaining sarcomere integrity to meet energy demands during contraction and to adapt to various biochemical and biomechanical stimuli [27]. Thus, large increases in serum CK-MB could be related to the disruption of sarcomere integrity. Moreover, since it has been shown that slow (type I) fibres present higher levels of CK-MB than fast (type II) fibres [48], a significant increase in the serum concentration of CK-MB could be more precisely associated with slow (type I) fibre damage.

The various features of CK-MB, such as the M-subunit location on the sarcomere [26, 27] and its higher levels in slow (type I) fibres [48] together with the significant high correlations found between CK-MB and SM, suggest that an increase in this isoenzyme over the clinically normal range ($4\ ng \cdot mL^{-1}$) may indicate not only an increase in membrane permeability, but also deeper damage related to the contractile apparatus (M-region of the sarcomere) of slow (type I) fibres.

A novel biomarker: sarcomeric mitochondrial creatine kinase

To the best of our knowledge, this is the first study to test sMtCK as a biomarker of muscle damage in MTR participants. Interestingly, significant increases in serum sMtCK were found only in the 35 km MTR group. Serum increases of sMtCK are likely to be indicative of mitochondrial swelling, disruption and decline in muscle respiratory capacity [10]. Although the total elevation gain of the 2 races was similar, the actual altitudinal profiles differed between the races (2064 and 1750 m peak altitudes for 35 and 55 km MTR, respectively). Taken together with the fact that the 35 km MTR group showed a moderately faster average velocity, participants in the 35 km race might have completed the race with a higher intensity of effort, which could have led to higher hypoxia. Hypoxia induces metabolic stress that triggers the Ca^{2+}-mediated phospholipolytic pathways, resulting in a loss of membrane integrity that leads to muscle enzyme efflux to the bloodstream [19, 32].

In contrast, in the 55 km MTR group, the serum sMtCK values did not increase significantly after the race. It has been demonstrated in rats that prolonged exercise induces mitochondrial damage but that training status has a protective effect on the mitochondria against exertion [10] by a specific reduction in mitochondrial Ca^{2+} uptake [5] and an increase in antioxidant capacity [46]. The 55 km MTR group had a significantly higher training volume, which could lead to enhanced mitochondrial training protection by reducing the specific mitochondrial Ca^{2+} uptake and thus preserving mitochondria integrity. Moreover, the higher training volume presented by the 55 km group may produce the so-called repeated bout effect, which is defined as a protective adaptation occurring after a single bout of unaccustomed eccentric exercise that induces muscle damage [31]. Although the adaptive mechanisms underlining the repeated bout effect remain unclear, recent well-supported theories suggest that eccentric exercise induces changes to connective tissue structures, such as extracellular matrix remodelling [23], and improves mitochondrial Ca^{2+} homeostasis, thereby stabilizing the mitochondrial respiratory function [41].

Therefore, although further research is needed involving runners with substantially different training volumes as well as different training status (sponsored vs. amateur) during a single MTR, our results show that sMtCK is a novel muscle damage biomarker that may be sensitive to training volume [8].

Cardiac troponin I

Prolonged endurance exercise has been related to cTnI release in healthy individuals [37]. In the present study, the group that ran the 55 km MTR showed larger increases in cTnI than the 35 km MTR group, which suggests that run length, and consequently exercise duration, could be closely related to cTnI release. However, the average values in the 55 km MTR group were biased by the value recorded for participant 14, which was $0.505\ ng\ mL^{-1}$ following the race (▶ **Table 2**). The mechanisms of prolonged exercise-induced cTnI release are not yet fully understood, but some hypotheses have been developed [11, 29]. In any case, it seems likely that prolonged exercise-induced cTnI release is a benign process [44], especially when serum levels return to baseline within 24–48 h [33], as in the present study.

Fast (type II) fibre damage?

Although no significant time-related changes were seen in the average serum FM concentration, fast (type II) fibre damage cannot be completely ruled out, especially because some individuals (participants 2 and 3 from the 35 km MTR, and 14 and 16 from the 55 km MTR) showed large FM increases following the races (▶ **Table 2**). Because the biomarkers used in the present study offer an accurate method for assessing fibre-type-specific muscle damage [7, 9], and due to large interindividual variability in the response to exercise, it seems reasonable to recommend an individualized analysis of results in clinical practice.

Limitations

In this study, the low number of participants from the 55 km MTR might be regarded as a limitation with regard to the interpretation of the results. However, it should be taken into account that this group comprised highly trained amateur and sponsored athletes. The study design is another limitation for making inferences between biochemical responses among groups. An observational design based on a comparison of response in 2 groups (i. e., high and low trained runners) competing in the same MTR would broaden our understanding of the time course of serum biochemical markers and would allow us to make inferences related to training status.

Summary and Conclusions

In summary, this is the first study to indicate that mountain running distance might be related to fibre-type-specific biochemical indices of muscle damage. SM serum concentrations measured in the 55 km MTR competitors suggest that physicians, trainers and runners should be conscious that competing in long-distance mountain running events might be related to deeper slow (type I) muscle fibre damage, even in highly trained individuals. Moreover, increases in serum CK-MB might be related to deeper damage to the contractile apparatus (M-region of the sarcomere) of slow (type I) fibres. Regarding muscle enzymes, our results suggest that training volume might lead to enhanced clearance rates. Finally, selective increases in sMtCK in the 35 km MTR group, but not the 55 km group, indicate that training volume might confer protection on the mitochondria. It seems that sMtCK could be a promising novel biomarker of muscle damage that is sensitive to training status.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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