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# Mountain Ultramarathon and Sarcomere Disruptions of Slow Fibers

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***Objective:*** To investigate changes after a mountain ultramarathon (MUM) in the serum concentration of fast (FM) and slow (SM) myosin isoforms, which are fiber-type-specific sarcomere proteins. The changes were compared against creatine kinase (CK), a widely used fiber-sarcolemma-damage biomarker, and cardiac troponin I (cTnI), a widely used cardiac biomarker. ***Methods:*** Observational comparison of response in a single group of 8 endurance-trained amateur athletes. Time-related changes in serum levels of CK, cTnI, SM, and FM from competitors were analyzed before, 1 h after the MUM, and 24 and 48 h after the start of the MUM by 1-way ANOVA for repeated measures or Friedman and Wilcoxon tests. Pearson correlation coefficient was employed to examine associations between variables. ***Results:*** While SM was significantly ($P$ = .009) increased in serum 24 h after the beginning of the MUM, FM and cTnI did not change significantly. Serum CK activity peak was observed 1 h after the MUM ($P$ = .002). Moreover, serum peaks of CK and SM were highly correlated ($r$ = .884, $P$ = .004). ***Conclusions:*** Since there is evidence of muscle damage after prolonged mountain running, the increase in SM serum concentration after a MUM could be indirect evidence of slow-(type I) fiber-specific sarcomere disruptions.

***Keywords:*** eccentric contraction, creatine kinase, muscle myosin isoforms

Mountain ultramarathons (MUM) are competitive events consisting of walking and running on mountain trails over a great cumulative elevation gain and over a longer distance than the athletic marathon (>42.195 km). Distance and cumulative elevation gain are the main determinants of MUM difficulty. Long-distance trail competitions have risen in popularity over the last few years.[1] However, the acute physiological responses to extreme endurance events still remain unclear. It is known that MUM competitions are strenuous and generally include negative slopes, so long distances are run downhill. It has been stated that strenuous exercise can result in muscle damage,[2] which is particularly exacerbated if eccentric contractions are performed (for a review see Proske and Allen[3]). Downhill running increases the eccentric component because the peak flexion angles are significantly greater, and it is a much stronger stimulus for damage than level or uphill running.[4] Therefore, it seems reasonable to relate most of the muscle damage to the negative-slope phases of the trail. MUM is a great opportunity for field-specific assessments of a physiologically stressful competitive event that induces muscle damage.[5,6]

Direct evaluation of muscle damage involves histological examination of muscle tissue by biopsy. However, in a sports context, the analysis of exercise-induced muscle damage is essentially based on proxy markers such as measurements of enzyme activity in blood, especially the activity of creatine kinase (CK). Previous studies evaluated the muscle damage induced by MUMs[5,6] and revealed large increases in total CK concentrations. However, CK is not a specific biomarker of skeletal muscle.[7] Koller et al[8] used slow (type I) myosin heavy-chain (MHC) fragments, and Melin et al[9] used beta MHC as muscle-fiber-specific damage biomarkers. Those groups found increases in this protein in plasma after mountain-running events. Although slow (type I) MHC fragments and beta MHC are common to skeletal and cardiac muscle, the damage was mainly related to slow (type I) fibers of skeletal muscle. However, the results found by Koller et al[8] and Melin et al[9] were highly unspecific, since plasma levels of MHC fragments were not compared with any cardiac-specific biomarker. Since it has been stated that strenuous exercise could induce a significant release of cardiac proteins such as troponin into the bloodstream,[10] it seems reasonable to assume that proteins found in cardiac and skeletal muscle, such as MHC fragments and beta MHC, could also be released from myocardium to blood.

Recently, myosin isoforms have been proposed as fiber-type-specific biomarkers of muscle damage that would represent indirect evidence of sarcomere disruptions.[11] However, Carmona et al[11] observed selective release of fast myosin isoforms (FM) after high-intensity knee-extensor exercise, but no changes in slow-myosin-isoform (SM) serum concentration were reported. SM is found in both cardiac and skeletal muscle, and FM is characteristic of fast skeletal muscle. Limb skeletal muscles are composed of slow (type I) and fast (type II) fibers,[12] but adult skeletal muscles shows plasticity and can undergo conversion between different fiber types in response to exercise.[13] Endurance athletes tend to have a predominance of slow (type I) fibers.[14,15] For these reasons, we hypothesized that serum increases in myosin isoforms, especially in SM, in endurance-trained participants after a MUM could indicate not only the extent but also the type of fiber affected. Furthermore, in the current study, the lack of specificity of SM was minimized by analyzing the changes in serum concentration of cardiac troponin I (cTnI), a widely used myocardial-specific biomarker. It has been shown that cTnI is released after prolonged exercise. However, in contrast to myocardial infarction, in which cTnI is usually over 0.6 ng/mL and remains stable in blood for at least 5 days, cTnI release after prolonged exercise does not achieve such high serum levels and returns to

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baseline within 24 to 48 hours.[16] To the best of our knowledge, this is the first field study to use a combination of myosin isoforms and cTnI to assess indirectly the muscle damage induced by a MUM.

The aim of this study was to investigate changes in serum concentration of myosin isoforms after a MUM. SM and FM were compared with CK, a widely used biomarker of exercise-induced muscle damage. The lack of specificity of SM was countered with the measurement of serum cTnI concentration. Since there is evidence of muscle damage after prolonged mountain running,[5,6] we hypothesized that a specific increase in SM serum concentration after a MUM would be indirect evidence of slow (type I) -fiber sarcomere damage in endurance-trained mountain runners.

## Methods

### Participants

We initially recruited 17 endurance runners, 14 men and 3 women. However, due to bad weather conditions during the competition, only 8 subjects decided to complete the study: 7 men and 1 woman (mean ± SD; men, n = 7, 39.8 ± 3.3 y, 178.7 ± 5.2 cm, 76.9 ± 7.9 kg; women, n = 1, 39.1 y, 173.0 cm, 67.0 kg). All of the participants were experienced white nonprofessional athletes (mean training regimen, 450.0 ± 210.31 min/wk of endurance training) who were specifically trained for MUM. All were healthy and had incurred no muscle injuries in the 6 months before the study. To avoid bias, no instructions were given about the type of training performed the week before the competition, but athletes were asked about it to better interpret the baseline serum levels of enzymes and contractile proteins. Physical activity after the race was limited and massages were prohibited. The study conformed to the Declaration of Helsinki for medical research, participants provided written informed consent, and the research was approved by the ethics committee of the Catalan Sports Council (Government of Catalonia).

### Design

The study design used observational comparison of response in a single group of endurance-trained amateur athletes.

### Methodology

The participants ran in the "Cavalls del Vent" MUM in 2012, an official competition organized by Salomon Nature Trails. It was a circular route with an official length of 84.84 km (~85 km) and a total cumulative elevation gain of 12,180 m. The start of the race was at 755 m above sea level and the maximum summit achieved during the trail was 2520 m (Figure 1). Each runner's average speed was calculated according to the total distance run divided by his or her official time.

\<<<<<<<<<<**FIGURE 1**>>>>>>>>>>>>\

Four blood samples were obtained: 1 day before the competition (pre), less than 1 hour after finishing the competition (post), and, because a significant degree of damage can occur during the race, 24 and 48 hours after the beginning of the MUM. A 5-mL blood sample was drawn from an antecubital vein. Blood was allowed to clot for 30 minutes in a tube (SST II Advance, Becton Dickinson Vacutainer Systems, UK) before being centrifuged at 3000 *g* for 10 minutes at 4°C. Three 200-µL aliquots of serum were stored at –80°C until analysis.

CK determinations were performed in an Advia 2400 automatic device (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA), and cTnI determinations were made in a Dimension Clinical Chemistry System automatic device (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) with an analytical measurement range of 0.017 to 40 ng/mL. To obtain muscle myosin-isoform concentrations in serum, we developed an enzyme-linked immunosorbent assay (ELISA-sandwich), which is described elsewhere.[11] Briefly, a calibration curve was obtained by a serial dilution from 0 to 250 ng of pure myosin from porcine muscle M0273, and the ELISA was completed by using monoclonal antimyosin (skeletal, fast) clone My-32, monoclonal antimyosin (skeletal, slow) clone NOQ7.5.4D, antimyosin polyclonal antibody M7523, and mouse anti-IGG linked to peroxidase A6154 (all Sigma Aldrich, Poole, UK). Intra-assay coefficients of variation were below 8% for FM and below 7.5% for SM. The linearity of the FM assay results was 80%, and it was 90% for the SM assay.

### Statistical Analyses

The normality of each variable was tested using the Shapiro-Wilk test. As SM and CK were asymmetrically distributed, these variables were log-transformed before analysis. One-way repeated-measures ANOVA was used to identify the effect of time on CK activity and SM and FM serum levels. When any significant main effects were found, pairwise *t*-test comparisons with a Bonferroni correction were used. The time course of changes in cTnI serum concentration was evaluated with the use of Friedman and Wilcoxon nonparametric tests. Effect sizes (ES) (Cohen *d*) were calculated to determine the practical difference between baseline values and serum peaks of enzymes and proteins. ES values of above 0.8, 0.8 to 0.5, 0.5 to 0.2, and lower than 0.2 were considered large, moderate, small, and trivial, respectively. Pearson correlation coefficient was employed to evaluate the association between the variables of interest. Data are presented as mean ± standard error of the mean unless otherwise stated. The level of significance was set at $P < .01$. All statistical analyses were conducted using SPSS version 20.0 statistical-analysis software (SPSS Statistics, IBM Corp, Armonk, NY).

## Results

The week before the competition, participants reported the use of similar training strategies, based on a decrease in training volume (km/wk) and an increase in intensity (running average velocity). At the competition, only 1 participant finished the MUM (~85 km). The rest of the participants left the competition at different points along the trail due to bad weather conditions: temperature, range 0.9°C to 13.1°C; rain, range 0 to 6.1 mm/h; humidity, range 90% to 97%; and wind speed (east-southeast), range 1.1 to 5 m/s. The total distance (km) covered and official time (h:min:s) of each participant who decided to carry on with the study protocol were determined by the last official control point passed just before leaving the MUM. Individual average speed (km/h) was calculated according to these results (Table 1). With respect to biochemical markers, the average serum CK activity at baseline was in the clinically normal range (35–175 U/L) and rose significantly from 132 ± 22 U/L (pre) to a peak of 2052 ± 860 U/L (ES = 3.02) less than 1 hour after finishing the MUM. Average CK serum activity remained significantly elevated 24 hours (1345 ± 651 U/L) (ES = 2.59) after the beginning of the competition, but a clear decreasing trend was observed (Figure 2). The only woman who participated in the study until the end and also completed the whole long-distance trail (participant number 7) had the highest values of CK in serum in all samples (Table 1). Almost all participants were in the clinically normal range of cTnI (<0.017–0.050 ng/mL), or slightly above, in all analyzed samples (Table 1). No significant increase was seen in average cTnI serum concentrations 1 hour after the MUM (from 0.018 ± 0.001 ng/mL to

0.067 ± 0.028 ng/mL), and values returned to baseline 1 day after the trail (Figure 2). However, the average values of cTnI were highly biased, because participant number 7 showed an almost 10-fold increase in cTnI serum concentration 1 hour after the competition, which remained around 4-fold elevated 24 and 48 hours after the start of the MUM (Table 1). A nonsignificant slight increase in average FM serum concentration was observed 1 hour after the competition (from 1508 ± 222 μg/L to 1731 ± 204 μg/L), which remained stable until 24 hours after the beginning of the competition (1744 ± 250 μg/L) and returned to baseline values at 48 hours (1520 ± 318 μg/L) (Figure 1). Average FM serum values of all time points analyzed were in the previously established normal range (> 1000 μg/L).[11] Finally, no changes in SM were found until 24 hours after the start of the MUM, when SM serum concentration rose significantly from 1443 ± 390 μg/L to 3743 ± 1110 μg/L (ES = 1.34). SM serum activity remained nonsignificantly elevated 48 hours after the initiation of the competition (2828 ± 762 μg/L). Average baseline SM serum levels were in the normal range (> 2000 μg/L).[11] (Figure 2). The SM serum peak at 24 hours after the start of the MUM was also highly correlated with the CK serum peak found 1 hour after the competition ($r$ = .884; $P$ = .004) (Figure 3). Finally, SM was not correlated with cTnI.

\<<<<<<<<<**TABLE 1**>>>>>>>>>>>>>\

\<<<<<<<<<**FIGURE 2**>>>>>>>>>>>>>\

\<<<<<<<<<**FIGURE 3**>>>>>>>>>>>>>\

## Discussion

To our knowledge, this is the first study to assess the utility of the myosin isoforms SM and FM as serum biochemical markers of fiber-specific muscle damage induced by a MUM in experienced endurance runners. SM and FM were compared against CK, a widely used biomarker of exercise-induced muscle damage, and cTnI, a specific biomarker of cardiac damage. The novel finding of the current study was that only SM serum levels were significantly raised, while FM serum levels remained almost unaltered after the MUM competition. Another remarkable finding was that CK and SM serum peaks were highly correlated.

In the absence of myocardial infarction, muscle injury, or disease, large and time-sustained increases in blood CK activity have been widely accepted as a biomarker of muscle damage.[17] As expected, the MUM induced large increases in CK serum levels, up to an almost 16-fold rise 1 hour after the competition. Significant CK serum increases in healthy and well-trained participants have been previously documented after MUM competitions.[5] Large variability in serum CK concentrations among participants is common and has been previously described in experienced ultramarathoners after 24 hours of treadmill running[18] and after a 166-km MUM.[6] The reason for this variability still remains unclear, but it has been suggested that susceptibility is mainly related to genotype characteristics.[19] The CK serum peak 1 hour after the MUM and its recovery over the following 48 hours were also consistent with previous studies.[6] However, CK levels provide a gross indication of muscle-fiber damage, because they cannot identify the magnitude of damage[20] or the type of fibers affected.[7]

Although direct evidence of muscle damage is histological, force-generating capacity is considered a reliable and valid marker of muscle damage.[21] It has moreover been demonstrated that running a MUM induces large decreases in the knee-extensor force-generating capacity (a 35% decrease after a 166-km MUM), which are related to fatigue and muscle damage.[6] Furthermore, different studies have recently proposed sarcomere proteins such as troponin as proxy fiber-type-specific biomarkers of muscle damage.[7,22] According to the observed time course, molecular mass, and fiber compartment in which these proteins are located, their elevation in serum suggests more severe damage, signifying sarcomere damage.[22] As fiber-type-specific sarcomere proteins, SM and FM could allow for indirect diagnosis of sarcomere damage and the type of fiber affected.[11] Because MUM running has been demonstrated to induce muscle damage,[6] the significant increases in SM serum concentration seen 24 hours after the start of the MUM suggested selective slow (type I) fiber damage, and due to its elevated molecular weight (493 kDa) and its fiber intrasarcomeric compartmentalization, increased serum SM could indicate sarcomere disruptions of those fibers. Moreover, since cTnI showed a nonsignificant serum increase 1 hour after the competition and returned to almost baseline values 24 hours after the beginning of the MUM, SM serum increases in healthy individuals can be mainly related to skeletal-muscle slow-fiber damage, rather than myocardial damage. Furthermore, there was no statistical relationship between SM and cTnI. However, further research is needed on cTnI and the distance of ultraendurance events, because the only participant who completed the whole MUM (participant number 7) showed the highest increases in cTnI in every sample analyzed after the competition.

No changes in FM serum concentration were seen after the MUM, which indicates that the fast fibers suffered no damage or, at least, less damage than the slow fibers. Certainly, it has been proved by histology that fast fibers are most susceptible to eccentric contractions,[23] and downhill running, which is a mainly eccentric activity, induces muscle damage.[4] However, since endurance runners have greater levels of slow (type I) fibers,[14,15] it is reasonable to assume that slow (type I) fibers are predominantly recruited and damaged during a MUM. However, the hypothesis that a MUM also induces fast (type II) -fiber damage cannot be completely ruled out because CK is non–fiber-type-specific. Moreover, baseline FM serum levels were over the previously described normal range,[11] a phenomenon that can be attributed to the type of training (higher intensity and less volume) performed by the runners the week before the race, but this is only speculation. Future studies in this area should carefully analyze the training strategies used the week before the competition.

Due to its mainly sarcoplasmic location, CK is thought to indicate increased membrane permeability after membrane disruptions at early time points[24] and the peroxidation of membrane lipids caused by an increase in reactive oxygen species and the activation of ion ($Na^+$ and $Ca^{2+}$) channels for several days after exercise.[25] The peak value for CK serum was found 1 hour after the MUM. This could be explained by the long duration of the competition, causing a significant degree of membrane damage during the run.[26] It has been demonstrated that the resealing of artificially produced membrane disruptions occurs in less than a minute,[27] but CK efflux may occur during the run due to a continuous process of membrane disruption followed by rapid resealing. The CK serum peak post-MUM and its recovery kinetics over the following 2 days were consistent with previous studies.[6] Nevertheless, the average 1-hour post-MUM CK serum peak was lower than that found after longer distance races.[28] In this respect, and according to Waskiewicz et al,[28] since the volume of exercise increases the metabolic demands for intracellular $Ca^{2+}$,[26] and it is associated with muscle-damage indices, it seems reasonable to assume that augmented membrane permeability is related to the distance covered, as can be seen in Table 1. While participants 2 and 3, who completed 26 km, showed mild increases in serum CK activity post-MUM, participant 7, the only one who completed the whole MUM (~85 km), presented the greatest increases in CK serum activity after the competition. In contrast,

exercise intensity, expressed as average speed (km/h) at which the participants ran the MUM, showed no trend, since participants left the competition at different points along the route (see Table 1). Further research with a larger sample is therefore needed in this area to clarify the relationship between biochemical markers of muscle damage and both distance (km) and average speed (km/h).

Myosin isoforms have a different serum time course than CK.[11] Sarcomere-protein turnover is longer than that of sarcoplasm proteins,[29] so the SM serum peak 1 day after the MUM can be explained by the increased activity of calpain 2 days after exercise.[30] Calpain is a $Ca^{+2}$-dependent protease. The long duration of the MUM may have led to large increases in intracellular $Ca^{+2}$ during the run, which would accelerate calpain degradation and lead to the significant increases in SM serum 1 day after the MUM. Calpain removes myosin from the filamentous structure of the sarcomere.[31] At this time, increased membrane permeability due to the activation of stretch-activated ion channels[25] could lead to a release of large proteins into the interstitium. Once in the interstitial space, proteins are mainly transported via the lymphatic system into the bloodstream, because the capillary membranes in skeletal muscle are almost impermeable to proteins.[7–32] The muscle-fiber compartment in which SM is located and its comparatively slow degradation process could explain the delayed increases in serum SM.

Finally, CK and SM serum peaks occurred with 1-day difference but were strongly correlated. Membrane damage could accompany sarcomere disruptions, due to the tight connection between myofibrils, cytoskeleton, and membranes.[33] Therefore, it seems that membrane damage could be related to subsequent fiber sarcomere disruption of slow fibers.

## Practical Implications

The current study shows that SM could provide indirect information about fiber-type-specific sarcomere damage 1 day after a MUM, and since the serum peak of myosin isoforms is not reached until 1 day after a MUM, they could be used in diagnoses that are not made immediately after the competition. Although fiber specificity cannot be determined by CK, its serum activity 1 hour after a MUM seems to be related to the subsequent SM serum response. MUM trainers and runners should be aware that the total distance covered could be related to muscle damage, and sharp SM serum increases suggest that a longer recovery may be needed. Further research regarding MUM distance covered, training and performance variables, and damage degree inflicted to skeletal-muscle slow (type I) fibers is needed.

## Conclusions

In summary, since there is evidence of muscle damage after prolonged mountain running, an increase in SM serum concentration after a MUM could be indirect evidence of selective slow (type I) -fiber-specific sarcomere disruptions.

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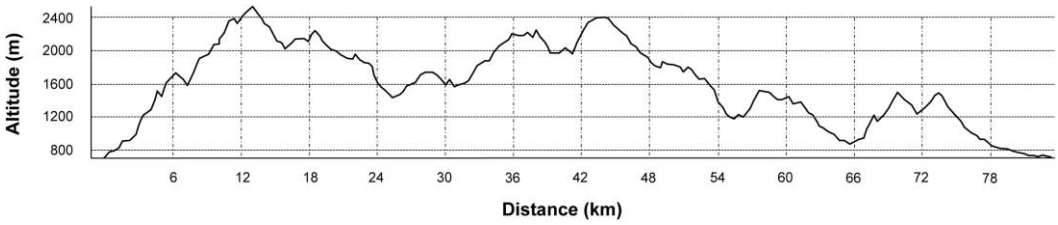


Figure 1 — Altitude profile of the entire mountain ultramarathon and the distance scale.

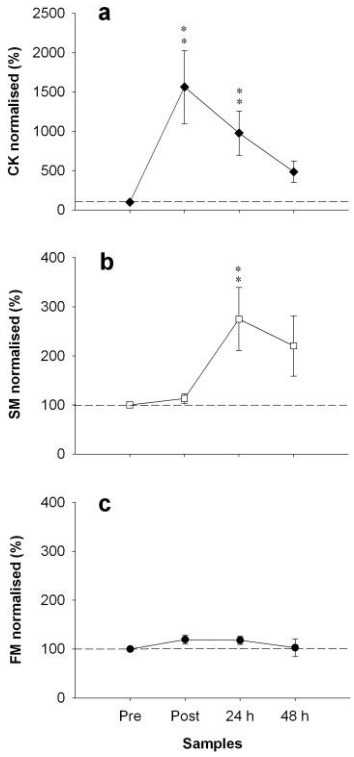


Figure 2 — Changes in (a) serum concentration of creatine kinase (CK) (n = 8), (b) slow myosin (SM) (n = 8), and (c) skeletal-muscle fast myosin (FM) (n = 7) 1 day before the competition (pre), less than 1 hour after finishing the competition (post), and 1 and 2 days after the mountain ultramarathon. Data are normalized (mean ± standard error of the mean) to pre-mountain-ultramarathon values (100%). **Significantly different from preexercise value at $P < .01$.

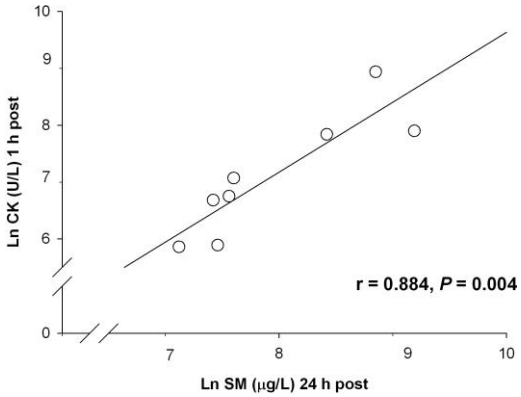


Figure 3 — Association between serum creatine kinase (CK) (natural log) peak activity 1 hour after finishing the long-distance trail competition (post) and slow myosin (SM) (natural log) peak concentration 24 hours after the start of the mountain ultramarathon (n = 8). *r,* Pearson correlation coefficient.

Table 1 Concentrations of Serum Creatine Kinase, Slow Myosin, and Cardiac Troponin I 1 Day Before the Competition (Pre), Less Than 1 Hour After Finishing the Competition (Post), and 24 and 48 Hours After the Beginning of the Mountain Ultramarathon for Each Participant

| | | | | | Creatine Kinase (U/L) | | | | Slow Myosin (µg/L) | | | | Cardiac Troponin I (ng/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Participant | Gender | km | Time (h:min:s) | Av speed (km/h) | Pre | Post | 24 h | 48 h | Pre | Post | 24 h | 48 h | Pre | Post | 24 h | 48 h |
| 1 | M | 41 | 09:39:18 | 4.25 | 70 | 793 | 933 | 611 | 856 | 703 | 1674 | 1333 | 0.017 | 0.029 | 0.017 | 0.017 |
| 2 | M | 26 | 06:47:33 | 3.83 | 108 | 363 | 356 | 214 | 811 | 920 | 1741 | 1832 | 0.017 | 0.022 | 0.017 | 0.017 |
| 3 | M | 26 | 06:47:16 | 3.83 | 156 | 350 | 196 | 126 | 628 | 505 | 1233 | 963 | 0.017 | 0.040 | 0.017 | 0,017 |
| 4 | M | 41 | 09:10:12 | 4.47 | 74 | 2691 | 1118 | 468 | 3097 | 3159 | 9812 | 5543 | 0.018 | 0.034 | 0.017 | 0.018 |
| 5 | M | 53 | 10:26:24 | 5.08 | 173 | 856 | 553 | 259 | 842 | 1225 | 1928 | 1162 | 0.017 | 0.029 | 0.017 | 0.017 |
| 6 | M | 53 | 12:09:28 | 4.36 | 179 | 2542 | 1156 | 531 | 3338 | 3205 | 4553 | 3979 | 0.017 | 0.079 | 0.017 | 0.017 |
| 7 | F | 85 | 11:48:48 | 7.26 | 233 | 7643 | 5819 | 2722 | 988 | 1404 | 6999 | 6361 | 0.027 | 0.260 | 0.115 | 0.101 |
| 8 | M | 53 | 12:09:32 | 4.36 | 59 | 1175 | 626 | 288 | 984 | 1400 | 2007 | 1454 | 0.017 | 0.043 | 0.017 | 0.017 |

Abbreviations: M, male; F, female; km, kilometers of mountain ultramarathon completed; Av, average.