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**Physiological intensity profile, exercise load and performance predictors of a 65-km mountain ultra-marathon**

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1 **Physiological intensity profile, exercise load and performance predictors of a 65-km Mountain**  
2 **Ultra-Marathon**

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26 **Abstract**

27 The aims of the study were to describe the physiological profile of a 65-km (4000-m cumulative  
28 elevation gain) running mountain ultra-marathon (MUM) and to identify predictors of MUM  
29 performance. Twenty-three amateur trail-runners performed anthropometric evaluations and an  
30 uphill graded exercise test (GXT) for  $VO_{2max}$ , ventilatory thresholds (VTs), power outputs  
31 associated with these indices (PMax, PVTs) and heart rate response (HRmax, HR@VTs). Heart rate  
32 (HR) was monitored during the race and intensity was expressed as: Zone I (<VT1), Zone II (VT1-  
33 VT2), Zone III (>VT2) for exercise load calculation (training impulse, TRIMP). Mean race  
34 intensity was  $77.1\pm4.4\%$  of HRmax distributed as:  $85.7\pm19.4\%$  Zone I,  $13.9\pm18.6\%$  Zone II,  
35  $0.4\pm0.9\%$  Zone III. Exercise load was  $766\pm110$  TRIMP units. Race time ( $11.8\pm1.6$ h) was  
36 negatively correlated with  $VO_{2max}$  ( $r=-0.66$ ,  $P<0.001$ ) and PMax ( $r=-0.73$ ,  $P<0.001$ ), resulting these  
37 variables determinant in predicting MUM performance, whereas exercise thresholds did not  
38 improve performance prediction. Anthropometric and physiological variables explained only 59%  
39 of race time variance, underlining the multi-factorial character of MUM performance. Our results  
40 support the idea that VT1 represents a boundary of tolerable intensity in this kind of events, where  
41 exercise load is extremely high. This information can be helpful in identifying optimal pacing  
42 strategies to complete such extremely demanding MUMs.

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47 **Keywords:** *mountain ultra-marathon, heart rate, exercise intensity distribution, training load,*  
48 *thresholds*

## 49 **Introduction**

50 Mountain ultra-marathons (MUMs) consist of running and walking on mountain trails over a  
51 distance longer than the traditional marathon (from 42.2 up to 350 km) with a considerable  
52 cumulative elevation gain (up to 25.000m). These events take place in mountain environments and  
53 are performed on irregular terrain, presenting positive and negative slopes. Accordingly, to face  
54 MUMs, athletes must perform prolonged concentric work against gravity force during ascents and  
55 extensive eccentric work during downhill sections (Vernillo et al., 2015). In addition, MUMs  
56 participants are exposed to multiple internal and external stressors, from exercise and environment,  
57 including possible wide fluctuations in temperature and altitude, and generally have to sustain  
58 extreme exercise loads (Millet, G. P. & Millet, 2012).

59 Because of their peculiarities some authors have suggested MUMs as an outstanding opportunity to  
60 investigate the adaptive responses of the human body to the extreme load and stress of ultra-  
61 endurance exercises (Millet, G. P. & Millet, 2012). Accordingly, recent studies have assessed the  
62 acute consequences, as well as the adaptive responses induced by MUMs. MUMs have been  
63 associated with musculoskeletal injuries and skin-related disorders (Vernillo et al., 2016b), negative  
64 energy balance (Martinez et al., 2017; Ramos-Campo et al., 2016), severe muscular damage and  
65 inflammation (Carmona et al., 2015; Saugy et al., 2013), marked neuromuscular fatigue (Easthope  
66 et al., 2010; Millet, G. Y. et al., 2011b; Saugy et al., 2013), cardiac dysfunctions and myocardial  
67 damage (Ramos-Campo et al., 2016; Vitiello et al., 2013), alterations in water diffusivity with  
68 changes of the inter-cellular space at brain level (Zanchi et al., 2017), impairment in lung functions  
69 (Vernillo et al., 2014a; Wuthrich et al., 2015) and in postural control (Degache et al., 2014). Besides  
70 the acute consequences, recent studies reported physiological adaptations that seem to occur  
71 exclusively following this specific ultra-endurance exercise. In particular specific metabolic  
72 adaptation responses, like the reduction of running and walking uphill energy cost, have been  
73 reported especially after extreme distance MUMs (Vernillo et al., 2016c; Vernillo et al., 2014b).

74 Despite the large number of investigations addressing the consequences of these extreme exercise  
75 loads, limited information is available about the sustained exercise intensity and the physiological  
76 demands faced during MUMs. The knowledge of the intensity profile and the physiological  
77 requirements of MUMs can provide essential information for optimal training, nutrition and  
78 participation, also considering the growing interest for these events, with annual numbers of races  
79 and participants that are increasing considerably (Hoffman, Ong, & Wang, 2010).

80 Only few studies reported the intensity sustained during a MUM event. In a 54-km ( $\approx 2900\text{m d+}$ )  
81 MUM the mean intensity reported was 64% of maximal heart rate (HR<sub>max</sub>) for the  $\approx 14\text{h}$  of its  
82 duration (Clemente-Suarez, 2015). Conversely, the mean intensity of 82% of HR<sub>max</sub> was reported  
83 in athletes completing a 54-km (2700 d+) MUM in  $\approx 7\text{h}$  (Ramos-Campo et al., 2016). Despite  
84 measuring two MUMs with similar characteristics, the mean exercise intensity was markedly  
85 different between the two studies, thus making the scenario not clear. Moreover, the lack of a  
86 description of participants' exercise capacities does not help the understanding of the elevated time-  
87 difference observed in MUMs, that can be related to differences in performance level as trained  
88 athletes are typically able to sustain higher exercise intensities for prolonged periods of time (Joyner  
89 & Coyle, 2008; Lucia, Pardo, Durantez, Hoyos, & Chicharro, 1998), but also the differences in  
90 athletes' motivation in competing or simply being able to complete such extremely demanding  
91 races.

92 In this regard, a detailed analysis of MUM participants' characteristics would certainly enhance the  
93 comprehension of the determinants of MUM performance, where many factors have been shown to  
94 be involved (Millet, G. Y., Hoffman, & Morin, 2012). In addition MUMs competitions can present  
95 large withdrawal rates (Wegelin & Hoffman, 2011). Among the reasons for the considerable drop  
96 out in MUMs inadequate pacing strategies (i.e. choice of exercise intensity) must be certainly  
97 considered.

98 In the light of these observations, further investigations seem to be required to characterize the  
99 exercise intensity sustained during MUMs, as well as how athletes' efforts are distributed among  
100 the intensity spectrum for this kind of ultra-endurance exercise. Accordingly, the aim of the study  
101 was to measure the sustained intensity during a 65-km MUM, characterizing the effort on the basis  
102 of well-defined exercise intensity thresholds and quantifying the physiological load associated with  
103 the competition. The second aim was to identify predictors of MUM performance by means of  
104 multiple regression analysis between standardized laboratory testing measures (predictors) and race  
105 time (dependent variable).

## 106 **Methods**

### 107 *Participants*

108 Twenty-three recreational healthy trail-runners (age  $40.2 \pm 7.3$  yr), 17 males and 6 females, were  
109 recruited for the study through advertisements on the official website of the race. None of the  
110 participants involved had clinical evidence of cardiovascular, neuromuscular, or articular diseases.  
111 Information about subjects' training history was collected through a questionnaire (Vernillo et al.,  
112 2016b). Participants had  $7 \pm 7$  yrs of training experience in running and  $3 \pm 3$  yrs of experience in  
113 MUMs. Usually they ran  $7 \pm 3$  h/week covering  $55 \pm 31$  km weekly. They participated in the  
114 competition with the aim to complete it in the best time possible. Before data collection, all  
115 participants were properly informed about the experimental protocol and gave their written  
116 informed consent for the measures. The experimental protocol was approved by the Ethics  
117 Committee of the University the investigators belong to.

### 118 *Experimental Protocol*

119 The study was conducted in two phases consisting of preliminary laboratory testing and during-race  
120 monitoring. This study examined the HR response during a 65-km MUM in relation with HR-based  
121 intensity markers: maximal heart rate (HR<sub>max</sub>), heart rate at the first and at the second ventilatory



122 threshold (HR@VT1, HR@VT2). All participants visited our laboratories within the two weeks  
123 before the competition for the preliminary testing session. Athletes performed a measure of  
124 anthropometric characteristics and an uphill running graded exercise test (GXT) to identify  
125 physiological parameters, including  $\text{VO}_{2\text{max}}$  and ventilatory thresholds (VT1, VT2), as well as the  
126 HR response. Athletes were asked to refrain from caffeine, alcohol and heavy exercise on the day  
127 before the tests. All tests were conducted under controlled conditions ( $20 \pm 1^\circ\text{C}$ , 40-60% relative  
128 humidity).

### 129 *Anthropometric characteristics*

130 Body mass (BM), was measured to the nearest 0.1 kg with a digital weighing scale (Seca, Hamburg,  
131 Germany). Height was measured to the nearest 0.001 m with a wall-mounted stadiometer (Gima,  
132 Milan, Italy). Body composition was performed with plicometry method by an experienced  
133 investigator. Skin-fold data were obtained using a skin-fold calliper (Gima, Milan, Italy) and  
134 recorded to the nearest 0.2 mm. Measurements were taken twice, and a mean of the two measures  
135 was used for body fat calculation. To calculate values of fat mass (FM) and free-fat mass (FFM),  
136 the percentage of body fat (%BF) was estimate according to estimated equations (Jackson &  
137 Pollock, 1978; Jackson, Pollock, & Ward, 1979).

### 138 *Graded exercise test*

139 An uphill graded exercise test (GXT), by means of power increments (combined increases of speed  
140 and inclination), was conducted on a motorized treadmill (Rodby Innovation AB, Vänge, Sweden).  
141 Mechanical power expressed (W/kg) was calculated as  $[\text{Power} = g \cdot v \cdot \sin(\alpha)]$ , where  $g$  was the  
142 gravitational acceleration ( $\text{m/s}^2$ ),  $v$  the belt speed (m/s) and  $\alpha$  the angle of treadmill inclination.  
143 Before the test, each athlete performed a 10 min warm-up at a constant power of 0.5 W/kg. The test  
144 started at a workload of 0.5 W/kg with increments of 0.5 W/kg (0.3 W/kg for females) every 3 min  
145 until the volitional exhaustion. Cardio-respiratory measures were collected continuously with

146 breath-by-breath method using an automated open-circuit gas analysis system (Quark PFT Ergo,  
147 Cosmed Srl, Rome, Italy). HR was recorded continuously during the test by a HR monitor  
148 incorporated into the gas analysis system. Careful calibrations of flow sensors and gas analyzers  
149 were performed before each measurement according to the manufacturer's instructions.

#### 150 *Competition measurements*

151 The competition was a 65-km MUM, the second edition of Vigolana Trail® (Vigolo Vattaro, TN,  
152 Italy) and was held in the first week of June. It involved 4000 m of cumulative elevation gain. The  
153 starting point and the finish line were at 725 m altitude. Overall, the race was performed at medium  
154 altitude, with an altitude range between 725 and 2100 m. The race started at 6.30 am with a  
155 temperature of 20 °C. The recorded temperatures (minimum-maximum) were 20-33 °C. Maximal  
156 allowed time for the 65 km MUM was 15.5 hours and the winner completed it in 7.1 hours. 154  
157 participants of 188 starters finished the race (82%) with a mean time of  $11.3 \pm 1.7$  h.

158 During the race, HR was continuously monitored using portable HR monitors (Polar RS800 SD,  
159 Polar Electro, Kempele, Finland) averaged at 5 s intervals. Racing  $\text{VO}_2$  was estimated for every  
160 subject from the HR responses, according to the equations for the linear relationship between  
161 oxygen uptake and HR obtained during the GXT. Due to technical problems related to difficulties  
162 of such long-distance events not all participants were successfully monitored during the whole race.  
163 The main reason was the discomfort caused by the thoracic belt for HR recording. Thus only 12 (8  
164 males) out of 23 participants' HR profiles were available for the analysis. The characteristics of this  
165 sub-group were not significantly different from the whole group of the study, all  $p$  values were  
166  $>0.05$ .

#### 167 *Data Analysis*

168 The maximal power output (PowerMax), achieved at athlete's exhaustion, was determined  
169 according to the equation:  $\text{PowerMax (W/kg)} = \text{power output last stage completed (W/kg)} + [t$

170 (s)/step duration (s) \* step increment (W/kg)], where  $t$  is the time of the uncompleted stage  
171 (Kuipers, Verstappen, Keizer, Geurten, & Van Kranenburg, 1985).  $VO_{2max}$  was defined as the  
172 highest values of a 20-s average (Robergs, Dwyer, & Astorino, 2010). Other breath-by-breath data  
173 were averaged over 10s for further analysis of other physiological parameters that have been shown  
174 to be important determinants of performance in endurance exercise (Lucià, Hoyos, Paèrez, &  
175 Chicharro, 2000). The first and the second ventilatory thresholds (VT1 and VT2) were determined  
176 from visual inspection by two independent operators according to methods described in detail  
177 elsewhere (Ahmaidi et al., 1993; Wasserman, Hansen, Sue, Stringer, & Whipp, 1999). Therefore, it  
178 was possible to establish the specific heart rate (HR@VT1 and HR@VT2) and power values  
179 associated with these intensities. Exercise intensity distribution during the race was calculated using  
180 HR profile and expressed into three zones: Zone I (<VT1) low intensity, Zone II (VT1-VT2)  
181 moderate intensity, Zone III (>VT2) high intensity. Total exercise load was calculated by means of  
182 the time spent in the three zones multiplied by arbitrary weighting factors, according to Lucia's  
183 training impulse method (Lucia's TRIMP) (Lucia, Hoyos, Santalla, Earnest, & Chicharro, 2003).  
184 Accordingly, 1 min in Zone I was given a score of 1 TRIMP unit, 1 min in Zone II was given a  
185 score of 2 TRIMP units, and 1 min in Zone III was given a score of 3 TRIMP units. The total  
186 TRIMP score was obtained by combining the results of the three zones.

### 187 *Statistical Analysis*

188 All test data are presented as means  $\pm$  standard deviations (SD). All the data were tested for their  
189 normal distribution (Shapiro–Wilk test). The relationships between performance and subjects  
190 characteristics were analyzed using Pearson's correlation. To assess the relationship between  
191 performance and laboratory variables we conducted a forward stepwise hierarchical multiple  
192 regression analysis. We used performance time as dependent variable, and subjects' characteristics  
193 as independent factors. Independent factors entered in four steps into the regression model in the  
194 following order:

- 195 1. Anthropometry (Age, BMI and Body Fat)
- 196 2. Anthropometry + maximal values (PowerMax and VO<sub>2max</sub>)
- 197 3. Anthropometry + maximal values + values@VT2 (Power@VT2 and VO<sub>2</sub>@VT2)
- 198 4. Anthropometry + maximal values + values@VT2 + values@VT1 (Power@VT1 and
- 199 VO<sub>2</sub>@VT1)

200 All statistical analysis was completed using a statistical software (SPSS Inc, Chicago, Illinois,

201 USA). The level of statistical significance was set at  $p < 0.05$ .

## 202 **Results**

203 Descriptive statistics of preliminary laboratory testing were reported in Table 1. Mean race time for

204 participants in the study was  $11.8 \pm 1.6$  h (range 8.2-14.3 h),  $11.5 \pm 1.7$  h (range 8.2-14.3 h) in HR

205 monitored sub-group. Athletes performed the race at a mean intensity of  $140.3 \pm 8.6$  bpm,  $77.1 \pm$

206  $4.4\%$  of HRmax equal to  $89.1 \pm 6.1\%$  of HR@VT1. Mean estimated VO<sub>2</sub> was  $63.2 \pm 9.1\%$  of

207 VO<sub>2max</sub>.

208 \*\*\*\*\*Table1 about here\*\*\*\*\*

209 Representative example of HR response was reported in Figure 1.

210 \*\*\*\*\*Figure1 about here\*\*\*\*\*

211 HR distribution during the race was reported in Fig2a. During the race the exercise intensity

212 distribution was:  $85.7\% \pm 19.4\%$  Zone I,  $13.9\% \pm 18.6\%$  Zone II,  $0.4\% \pm 0.9\%$  Zone III (Fig2b).

213 Total exercise load was  $766 \pm 110$  TRIMP units. Correlations between laboratory variables and

214 performance time were reported in Table2.

215 \*\*\*\*\*Figure2 about here\*\*\*\*\*

216 \*\*\*\*\*Table2 about here\*\*\*\*\*

217 Race time was negatively correlated with maximal physiological parameters,  $VO_{2max}$  ( $r=-0.66$ ,  
218  $P<0.001$ ) and PowerMax ( $r=-0.73$ ,  $P<0.001$ ), resulting these variables determinant in predicting  
219 MUM performance. In contrast, despite the strong relationships observed with race time,  
220 Power@VT2 ( $r= -0.70$ ,  $P<0.001$ ) and Power@VT1 ( $r= -0.71$ ,  $P<0.001$ ), sub-maximal parameters  
221 associated with exercise thresholds did not improve performance prediction.

222 \*\*\*\*\*Figure3 about here\*\*\*\*\*

223 Results from multiple regression analysis were reported in Table 3.

224 \*\*\*\*\*Table3 about here\*\*\*\*\*

## 225 **Discussion**

### 226 *MUM exercise intensity*

227 Despite the high number of recent investigations performed on MUMs, limited information is  
228 available about the sustained exercise intensity and the physiological demands of these events. Most  
229 of the knowledge available on ultra-marathons is based on flat running performance, where  
230 intensities have been reported to be 60%-70% of  $VO_{2max}$  in 6-h events (Davies & Thompson, 1979),  
231 decreasing to 40%-50% of  $VO_{2max}$  in 24-h events (Millet, G. Y. et al., 2011a). Only few studies,  
232 based on HR monitoring, reported the intensity sustained during MUMs. The mean intensities of  
233 64% of HRmax and 82% of HRmax were respectively reported for participants completing a 54-km  
234 MUM in  $\approx 14$ h (Clemente-Suarez, 2015) and  $\approx 7$ h (Ramos-Campo et al., 2016).

235 In our study the intensity observed,  $\approx 77\%$  of HRmax, equal to an estimated intensity of  $\approx 63\%$  of  
236  $VO_{2max}$ , was comparable to other ultra-endurance events of similar duration ( $\approx 10-11$ h) (Barrero,  
237 Chaverri, Erola, Iglesias, & Rodriguez, 2014; Laursen et al., 2005). In ultra-endurance triathlons  
238 mean intensities observed were 78% (Barrero et al., 2014) and 83% (Laursen et al., 2005) of  
239 HRmax during cycling and 77% HRmax during running (Barrero et al., 2014; Laursen et al., 2005).

240 Differently, for events of longer duration lower HR values have been usually observed together  
241 with a decrease of intensity with time (Gimenez, Kerhervè, Messonnier, Fèasson, & Millet, 2013;  
242 Neumayr, Pfister, Mitterbauer, Maurer, & Hoertnagl, 2004). Gimenez and colleagues (2013)  
243 observed a decrease from 72% to 62% of HRmax between the first to the last 6 h of a 24-h treadmill  
244 running, with mean intensity sustained of 68% of HRmax (Gimenez et al., 2013). Accordingly, our  
245 results obtained during a MUM event seem to be in line with other studies on ultra-endurance  
246 exercise.

247 To the best of our knowledge this is the first investigation analyzing the exercise intensity  
248 distribution during a MUM, characterizing the effort by means of well-defined exercise thresholds  
249 (VTs). In previous mentioned investigations (Clemente-Suarez, 2015; Ramos-Campo et al., 2016)  
250 MUM exercise intensity was found to be below the onset of blood lactate accumulation (OBLA),  
251 however no evaluation tests were conducted in order to characterize athletes' effort continuously  
252 during the competition. According to exercise intensity distribution found in this investigation most  
253 of the race was spent in Zone I, below HR@VT1 (Fig2b). In line with our findings, previous  
254 authors have suggested that the intensity associated with VT1 cannot be maintained throughout an  
255 ultra-endurance event (Laursen et al., 2005), showing that in the running phase of ultra-endurance  
256 triathlons athletes performed below HR@VT1 (Barrero et al., 2014; Laursen et al., 2005).  
257 Accordingly, in ultra-endurance exercise the existence of an ultra-endurance threshold lower than  
258 VT1 and 80% of HRmax has been previously proposed (Laursen et al., 2005; O'Toole, Douglas, &  
259 Hiller, 1998). In our study the mean exercise intensity maintained was slightly below  
260 90%HR@VT1. It has been suggested that exercise intensities marginally below VT1 allow a better  
261 balance of substrates oxidation, promoting higher fat to carbohydrate utilization, sparing  
262 carbohydrate reserves, delaying muscle and liver glycogen depletion, and maintaining blood  
263 glucose concentration (Barrero et al., 2014; Laursen et al., 2005; Laursen & Rhodes, 2001). This  
264 strategy has been recommended to help ultra-endurance athletes in reducing fatigue and improving  
265 performance (Laursen & Rhodes, 2001). Moreover, during ultra-endurance events athletes present

266 large energy expenditures and require constant energy refuelling (Jeukendrup, 2011; Kreider, 1991).  
267 Particularly, despite nutritional strategies adopted by the athletes, MUMs competitions, are  
268 associated with large energy deficits (Martinez et al., 2017; Ramos-Campo et al., 2016). Thus, the  
269 adoption of an optimal exercise intensity, together with an adequate nutritional intake (Jeukendrup,  
270 2011; Martinez et al., 2017), probably represent the best solution to delay the onset of fatigue and  
271 compete in MUMs. Accordingly, an intensity slightly lower than VT1 could represents a boundary  
272 of sustainable intensity for runners in >10h MUMs, since athletes sustaining a large part of the race  
273 in Zone I could manage their energy reserves, avoid nutrient-related fatigue and optimize  
274 competitive result. This information observed in runners that successfully completed a 65-km  
275 MUM can be helpful for athletes and coaches in order to better plan the trainings and the  
276 participation in this kind of events. In particular our findings can help athletes' pacing strategy  
277 during MUMs competitions, providing a reference threshold for athletes who aim to complete such  
278 extreme races.

#### 279 *MUM exercise load*

280 The three zones approach defining exercise intensity by means of the HR at the two ventilatory  
281 thresholds has been extensively used to calculate the exercise load of trainings and competitions  
282 (TRIMP), as well as the optimal training intensity distribution, both in endurance and ultra-  
283 endurance athletes (Muñoz, Cejuela, Seiler, Larumbe, & Esteve-Lanao, 2014; Seiler & Kjerland,  
284 2006; Stöggl & Sperlich, 2015). HR-based TRIMP score in literature showed training loads of  
285  $\approx 1000$ -1500 TRIMP units/week in professional cycling (Lucia et al., 2003) ,  $\approx 1000$  units/week in  
286 ultra-endurance tri-athletes (Muñoz et al., 2014),  $\approx 800$  units/week elite runners (Billat et al., 2003),  
287  $\approx 400$  units/week sub-elite runners (Esteve-Lanao, San Juan, Earnest, Foster, & Lucia, 2005) and  
288  $\approx 800$  units/week in elite junior Nordic skiers (Seiler & Kjerland, 2006). Moreover, taking into  
289 account competition loads, values of  $\approx 2000$  TRIMP units/week during professional road cycling  
290 competition (Lucia, Hoyos, Carvajal, & Chicharro, 1999),  $\approx 1000$  units during Ironman triathlon

291 (Muñoz et al., 2014) and  $\approx 800$  units during a 24-h cycling race were reported (Bescos et al., 2012).  
292 In the light of the above, the  $\sim 750$  TRIMP units observed in this study can be considered extremely  
293 high, especially for amateur athletes, as such values are often reached by endurance athletes during  
294 an entire week of training.

#### 295 *MUM performance*

296 The 65-km MUM performance was highly correlated with athletes'  $VO_{2max}$  and peak power output  
297 reached in the graded exercise test (Fig. 3). By including the oxygen consumption and mechanical  
298 power exerted at the ventilatory thresholds, despite being highly correlated with MUM  
299 performance, the prediction of race time did not improve (see the results of steps 3 and 4 of  
300 hierarchical regression analysis reported in Table 3). Considering the submaximal intensities  
301 sustained in MUMs, it was plausible that the oxygen consumptions associated with sub-maximal  
302 indices ( $VO_2@VTs$ ) represented parameters able to predict the performance. Particularly, for  
303 endurance exercise, submaximal indices (e.g. power output or speed exerted at the ventilatory  
304 thresholds) seem to be more reflective of athletes' performance capability (Impellizzeri, Marcora,  
305 Rampinini, Mognoni, & Sassi, 2005; Lucia et al., 1998), as well as better descriptive of training  
306 status especially in an homogenous group of athletes (e.g. similar  $VO_{2max}$ ) (Joyner & Coyle, 2008).  
307 Nevertheless for ultra-endurance exercises values associated with these intensity markers seem to  
308 be not so determinant (Millet, G. Y. et al., 2011a), resulting maximal values the best performance  
309 predictors (Barrero et al., 2014). In line with existing ultra-endurance literature our analysis,  
310 conducted in a heterogeneous group of athletes, further showed the importance of maximal values  
311 over those associated with exercise thresholds in ultra-endurance exercise, as previously reported  
312 for ultra-distance running (Millet, G. Y. et al., 2011a; Millet, G. Y. et al., 2012) and ultra-endurance  
313 triathlon (Barrero et al., 2014). In particular,  $VO_{2max}$  is still associated with performance also in  
314 ultra-endurance events up to 24-h in duration (Lazzer et al., 2012; Millet, G. Y. et al., 2011a). The  
315 importance of a high  $VO_{2max}$  has been also explained by a favorable metabolic condition, connected



316 with an advantageous substrates utilization, during low intensities observed in ultra-endurance  
317 exercises (Millet, G. Y. et al., 2011a). In this regard, high values of  $VO_{2max}$  could represent also a  
318 beneficial aspect for the sub-maximal intensities and long duration of a MUM.

319 In the present study the power outputs exerted in graded exercise test, calculated at the level of  
320 ventilatory thresholds and  $VO_{2max}$ , were better correlated with performance ( $r$  coefficients ranged  
321 from -0.73 to -0.71) than the measure of oxygen consumptions at the same intensities ( $r$  coefficients  
322 ranged from -0.66 to -0.56, see Table 2). Differently from the measure of oxygen consumptions, the  
323 measurement of external power output takes into account the efficiency of converting metabolic  
324 power in mechanical power (Ettema & Loràs, 2009), representing one of the main determinants of  
325 endurance performance (Joyner & Coyle, 2008). Thus, the power output that an athlete can produce,  
326 determined by an uphill GXT, may represent an important factor, determining the ascent rate and  
327 consequently performance time in uphill sections of a MUM.

328 The variables derived from anthropometry and a GXT were found to explain only the 59% of MUM  
329 performance variance. In this regard, in ultra-distance running events other factors, associated with  
330 the extreme character of the races, as the resistance to muscle damage and mental abilities, can play  
331 an important role in determining the final result (Millet, G. Y. et al., 2012). In addition an  
332 extensively investigated variable in ultra-distance running that was not evaluated in this study is  
333 energy cost of locomotion (Lazzer et al., 2012; Millet, G. Y. et al., 2011a; Vernillo et al., 2016c;  
334 Vernillo et al., 2015; Vernillo et al., 2014b). The role of energy cost in determining ultra-running  
335 performance is still a topic of discussion (Millet, G. Y. et al., 2012). Previous authors have shown  
336 that mean energy cost of level running together with  $VO_{2max}$  and its fractional utilization can  
337 explain the 87% of performance in multi-day running (Lazzer et al., 2012). In addition, as acute  
338 consequence of MUM participation, changes in energy cost in different running conditions have  
339 been reported (Vernillo et al., 2016c; Vernillo et al., 2015; Vernillo et al., 2014b), with variations  
340 that have been shown to be related to MUM performance (Vernillo et al., 2015). For instance,

341 Vernillo and colleagues (Vernillo et al., 2015) reported a positive correlations between race time  
342 and the energy cost variation in level and uphill running, after a previous edition of this MUM (65-  
343 km). In this study we did not measure the energy cost in different running conditions, and its  
344 variation after the race, this may explain why anthropometric and physiological characteristics  
345 measured with a GXT accounted only for the 59% of MUM performance variance. Accordingly,  
346 these results and the factors above mentioned can further underline the multi-factorial character of  
347 MUM performance (Millet, G. P. & Millet, 2012).

#### 348 *Limitations*

349 Some issues should be considered when interpreting the present results. The long distance, the  
350 alternation of high elevation gain and loss of the MUM may have favoured the use of conservative  
351 pacing strategies, decreasing the risk of premature exhaustion. In addition, several factors might  
352 have influenced the HR response during the MUM. The effect of altitude (Bartsch & Gibbs, 2007)  
353 as well as subjects' hydration status (Lambert, Mbambo, & Gibson, 1998) could have indeed caused  
354 increases in HR. Furthermore, reductions in HR have been observed after ultra-endurance exercise  
355 (Lucas et al., 2008; Mattsson et al., 2010) due to plasma volume expansion (Robach et al., 2014)  
356 and the desensitization of the heart's adrenergic receptors (Hart et al., 2006; Welsh et al., 2005).  
357 The downhill sections of the MUM, generating more exercise-induced muscle damage and fatigue-  
358 related outcomes (Giandolini et al., 2016), may have played a direct role on the physiological load  
359 not considered in the study. If the athletes stayed for most of the time at an intensity  $< \text{HR@VT1}$   
360 during downhill sections the physiological stress may have been quite blind by the intrinsic features  
361 of the downhill locomotion (Giandolini et al., 2016; Minetti, Moia, Roi, Susta, & Ferretti, 2002;  
362 Vernillo et al., 2016a). Nevertheless, prolonged eccentric loads can lead to an increase of the  
363 oxygen consumption, mainly related to the exercise-induced muscle damage (Giandolini et al.,  
364 2016; Vernillo et al., 2016a), and thus in the physiological strain. In this regard GPS data could be

365 helpful to contextualize the different contribution of uphill and downhill sections and, thus, the  
366 physiological load of MUMs (Kerhervè, Millet, & Solomon, 2015).

## 367 **Conclusions**

368 Mean exercise intensity during the 65-km MUM was  $\approx 77\%$  of HR<sub>max</sub> and most of the race time  
369 was spent at intensity below HR@VT1. This finding supports the idea that the first ventilatory  
370 threshold represents a boundary of tolerable intensity for amateur runners in a MUM longer than  
371 10h, where the exercise load was found to be extremely high (>750 TRIMP units). The results can  
372 be helpful for athletes and coaches in order to better plan the training strategies and the participation  
373 in this kind of events. In particular our findings can help athletes' pacing strategy during MUMs  
374 competitions, providing a reference threshold for athletes who aim to complete such extreme races.

375 In addition, the study showed that parameters associated with VO<sub>2max</sub> were determinant in  
376 predicting MUM performance, whereas exercise thresholds did not improve performance prediction  
377 in this heterogeneous group of athletes, which is in line with previous research in ultra-endurance  
378 events. However, the variables derived from anthropometry and a graded exercise test explained  
379 only 59% of race time variance, further underlining the multi-factorial character of MUM  
380 performance.

## 381 **Disclosure of interest**

382 The authors report no conflicts of interest.

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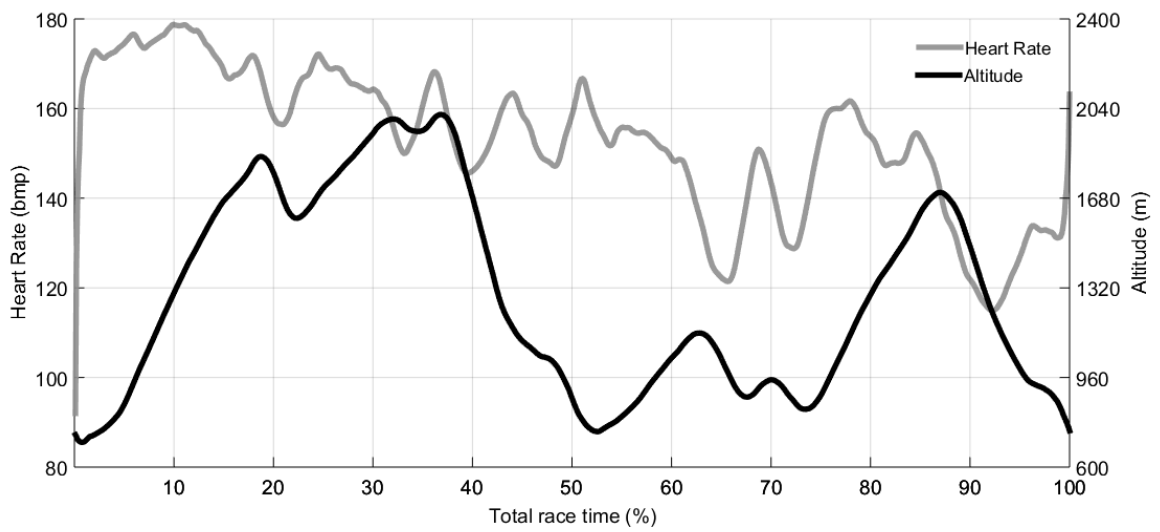
573 **Figures captions**

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576 **Figure 1.** Heart rate response (bpm) and change in altitude (m) during the MUM expressed as % of  
577 total race time in a representative participant.

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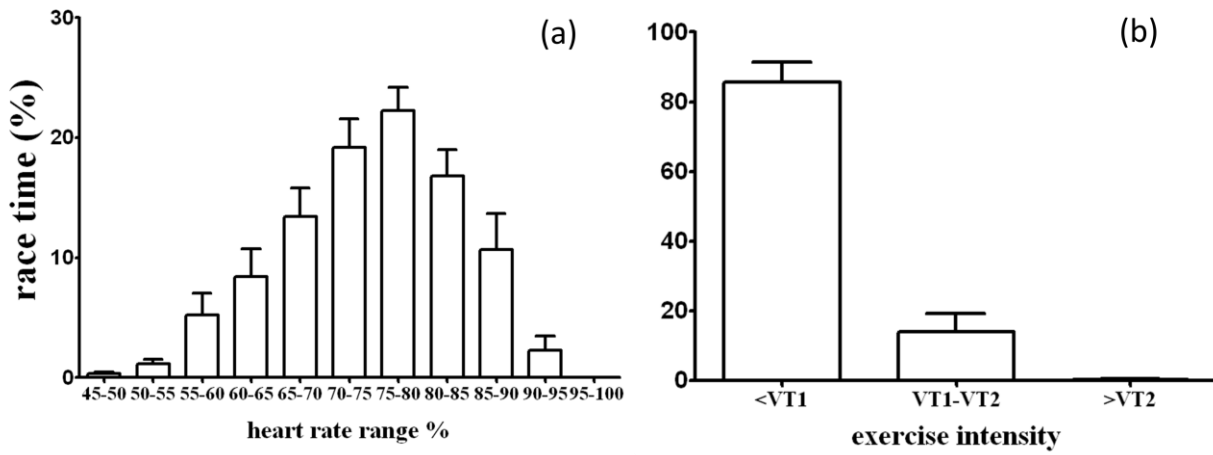
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584 **Figure 2. (a)** Heart rate distribution during the race. Time spent at different ranges of maximal heart  
585 rate expressed as % of total race time. **(b).** Exercise intensity distribution during the race. Time  
586 spent in Zone 1 (<VT1), Zone 2(VT1-VT2), Zone 3 (>VT2) expressed as % of total race time.  
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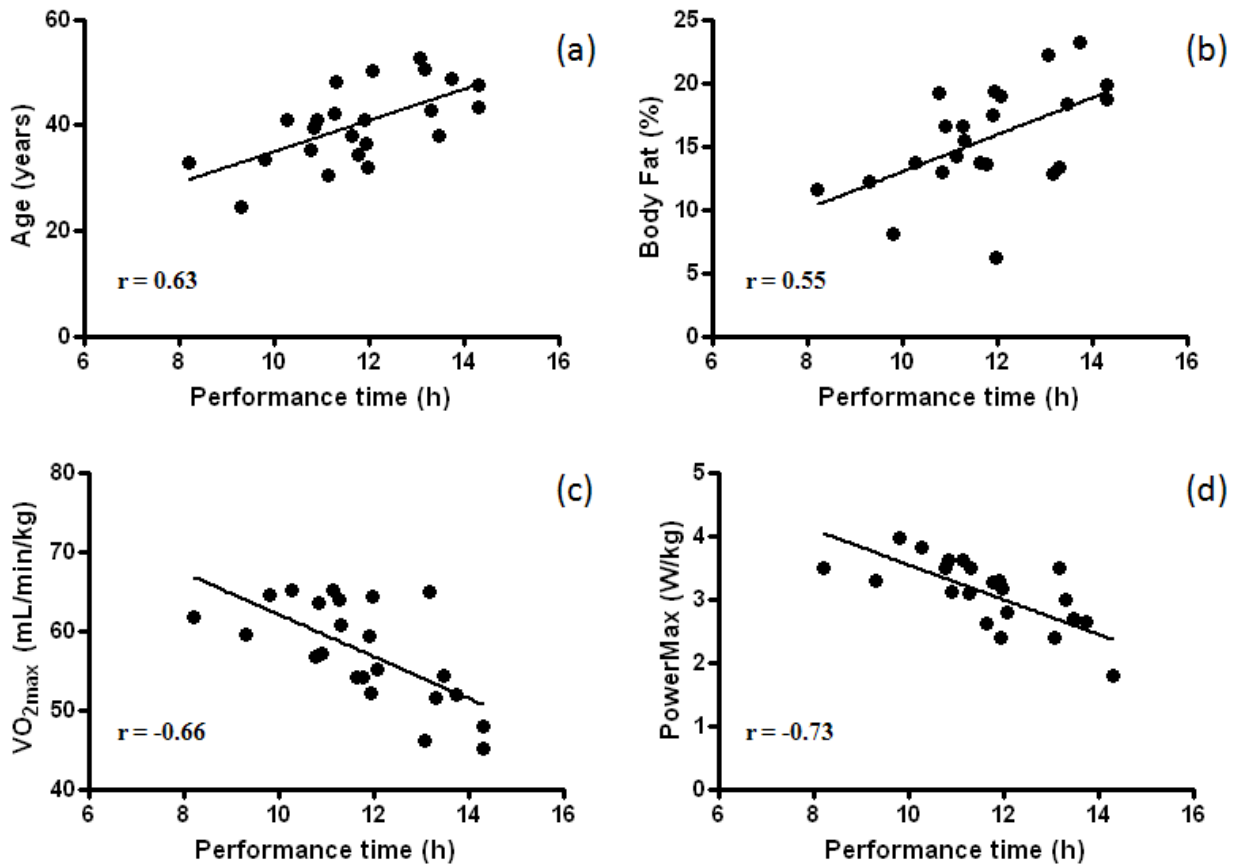


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602 **Figure 3.** Correlations with performance time in MUM (a) Age (years) (b) Body Fat (%) (c)

603  $VO_{2max}$  (mL/min/kg) (d) Maximal power in uphill graded exercise test

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617 **Table 1.** Characteristics of the participants resulting from preliminary laboratory testing session.

<b>Characteristics of the subjects</b>								
	<b>Whole group (n=23)</b>				<b>Subgroup HR monitored (n=12)</b>			
	<b>mean</b>	<b>±</b>	<b>s.d</b>	<b>range</b>	<b>mean</b>	<b>±</b>	<b>s.d</b>	<b>range</b>
Age (years)	40.2	±	7.3	24.4 - 52.7	38.6	±	6.1	30.4 - 48.9
<b>Anthropometry</b>								
Body mass (kg)	69.2	±	11.8	47.0 - 86.1	65.8	±	12.1	47.0 - 83.5
Height (cm)	173	±	8	157 - 187	171	±	9	157 - 181
BMI (kg/m <sup>2</sup> )	22.9	±	2.5	18.8 - 27.3	22.2	±	2.7	18.8 - 27.3
Fat-free mass (kg)	58.4	±	9.9	39.6 - 73.0	55.8	±	10.5	39.6 - 68.8
Fat mass (kg)	10.8	±	3.8	3.9 - 19.4	10.0	±	4.1	3.9 - 19.4
Body fat (%)	15.6	±	4.2	6.2 - 23.3	15.1	±	5.0	6.2 - 23.3
<b>Graded exercise test</b>								
VO <sub>2max</sub> (ml/min/kg)	57.4	±	6.3	45.2 - 65.1	58.4	±	6.2	48.0 - 65.1
VO <sub>2</sub> @VT2 (ml/min/kg)	51.9	±	5.5	40.3 - 59.5	52.9	±	5.0	45.5 - 59.5
VO <sub>2</sub> @VT1 (ml/min/kg)	45.3	±	5.1	33.0 - 52.1	46.3	±	4.5	36.8 - 52.1
HRmax (bpm)	181	±	8	166 - 196	182	±	8	166 - 196
HR @VT2 (bpm)	169	±	10	150 - 186	171	±	10	154 - 186
HR @VT1 (bpm)	155	±	11	128 - 175	158	±	11	136 - 175
PowerMax (W/kg)	3.1	±	0.6	1.8 - 4.0	3.1	±	0.6	1.8 - 4.0
Power@VT2 (W/kg)	2.3	±	0.5	1.4 - 3.0	2.4	±	0.4	1.6 - 3.0
Power@VT1 (W/kg)	1.7	±	0.4	1.0 - 2.2	1.7	±	0.3	1.0 - 2.2
<b>Performance</b>								
Total race time (h)	11.8	±	1.6	8.2 - 14.3	11.5	±	1.7	8.2 - 14.3

VO<sub>2max</sub> : maximal oxygen consumption; VO<sub>2</sub> @VTs: oxygen consumption at ventilatory thresholds;  
 HRmax: maximal heart rate; HR @ VTs: heart rate at ventilatory thresholds; PowerMax: maximal  
 mechanical power output; Power @VTs: power output at the ventilatory thresholds

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620 **Table 2.** Relationship between participants' anthropometric and physiological characteristics and  
 621 MUM performance (race time).

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**Performance Correlation Analysis**

(n=23)				
	<b>r</b>	<b>90% CI</b>		<b>p</b>
Age (years)	0.63	0.44	0.77	<b>&lt;0.001</b>
<b>Anthropometry</b>				
BMI (kg/m <sup>2</sup> )	0.07	-0.27	0.40	0.384
Fat-free mass (kg)	-0.26	-0.56	0.08	0.112
Fat mass (kg)	0.40	0.12	0.64	0.028
Body fat (%)	0.55	0.29	0.76	<b>0.004</b>
<b>Graded exercise test</b>				
VO <sub>2max</sub> (ml/min/kg)	-0.66	-0.83	-0.44	<b>&lt;0.001</b>
VO <sub>2</sub> @VT2 (ml/min/kg)	-0.65	-0.74	-0.35	<b>&lt;0.001</b>
VO <sub>2</sub> @VT1 (ml/min/kg)	-0.56	-0.83	-0.44	<b>0.003</b>
PowerMax (W/kg)	-0.73	-0.87	-0.56	<b>&lt;0.001</b>
Power@VT2 (W/kg)	-0.70	-0.87	-0.46	<b>&lt;0.001</b>
Power@VT1 (W/kg)	-0.71	-0.90	-0.45	<b>&lt;0.001</b>

VO<sub>2max</sub> : maximal oxygen consumption; VO<sub>2</sub> @VTs: oxygen consumption at ventilatory thresholds; HRmax: maximal heart rate; HR @ VTs: heart rate at ventilatory thresholds; PowerMax: maximal power output; Power @ VTs: power output at ventilatory thresholds

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633 **Table 3.** Model Summary resulting from forward stepwise hierarchical multiple regression analysis.

Model	Coefficients		90% CI for B		Standardized Coefficients	Sig.	Partial R	R	R <sup>2</sup>	Adjusted R <sup>2</sup>	R <sup>2</sup> Change	Sig. Change
	B	Lower	Upper									
1 (Constant)	7.844	3.323	12.365			0.007						
Age	0.103	0.026	0.180		0.481	0.032	0.470	0.682	0.465	0.381	0.465	0.007
BMI	-0.088	-0.279	0.103		-0.142	0.435	-0.180					
Body Fat	0.116	-0.023	0.255		0.311	0.166	0.313					
2 (Constant)	13.961	7.437	20.486			0.002						
Age	0.097	0.033	0.160		0.451	0.016	0.542					
BMI	0.025	-0.150	0.200		0.040	0.808	0.060	0.827	0.684	0.591	0.219	0.011
Body Fat	-0.057	-0.216	0.103		-0.151	0.545	-0.148					
VO <sub>2max</sub>	-0.016	-0.155	0.123		-0.065	0.843	-0.049					
PowerMax	-1.583	-2.898	-0.268		-0.593	0.052	-0.453					
3 (Constant)	14.479	7.449	21.509			0.003						
Age	0.096	0.029	0.163		0.448	0.024	0.543					
BMI	0.007	-0.185	0.199		0.011	0.950	0.016					
Body Fat	-0.046	-0.217	0.124		-0.124	0.640	-0.122	0.834	0.696	0.554	0.012	0.743
VO <sub>2max</sub>	0.135	-0.258	0.529		0.547	0.555	0.154					
PowerMax	-2.591	-5.449	0.267		-0.971	0.133	-0.380					
VO <sub>2@VT2</sub>	-0.170	-0.577	0.236		-0.595	0.474	-0.186					
Power@VT2	1.279	-2.027	4.585		0.379	0.508	0.172					
4 (Constant)	15.641	7.974	23.309			0.003						
Age	0.099	0.032	0.166		0.464	0.021	0.589					
BMI	0.052	-0.141	0.245		0.084	0.640	0.132					
Body Fat	-0.124	-0.315	0.068		-0.331	0.273	-0.303					
VO <sub>2max</sub>	0.156	-0.243	0.554		0.630	0.501	0.189	0.869	0.756	0.587	0.060	0.242
PowerMax	-3.714	-6.726	-0.701		-1.391	0.048	-0.518					
VO <sub>2@VT2</sub>	-0.446	-1.105	0.213		-1.559	0.252	-0.316					
Power@VT2	5.020	0.072	9.968		1.487	0.096	0.446					
VO <sub>2@VT1</sub>	0.268	-0.088	0.625		0.865	0.206	0.347					
Power@VT1	-3.272	-6.885	0.341		-0.744	0.133	-0.406					

634 Dependent Variable: Performance time (h)

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