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Changes in skeletal muscle oxidative capacity after a trail running race

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Abstract

Purpose: to evaluate the effects of a trail-running race on muscle oxidative function by measuring pulmonary gas exchange variables and muscle fractional O_2 extraction. **Methods**: Eighteen athletes were evaluated before (PRE) and after (POST) a trail running competition of 32-km or 50-km with 2000 m or 3500 m of elevation gain, respectively. During the week before the race, runners performed an incremental uphill running test and an incremental exercise by utilizing a one-leg knee-extension (KE) ergometer. The KE exercise was repeated after the end of the race. During the KE test we measured oxygen uptake ($V'O_2$) and micromolar changes in deoxygenated hemoglobin (Hb)+myoglobin (Mb) concentrations (Δ[deoxy(Hb+Mb)]) on vastus lateralis with a portable near-infrared spectroscopy. **Results**: $V'O_2$peak was lower at POST vs. PRE (-23.9±9.0%, $p<0.001$). $V'O_2$peak at POST was lower than $V'O_2$ at the same workload at PRE (-8.4±15.6%, $p<0.050$). Peak power output and time to exhaustion decreased at POST by -23.7±14.3% and -18.3±11.3%, respectively ($p<0.005$). At POST the increase of Δ[deoxy(Hb + Mb)] as a function of work rate, from unloaded to peak, was less pronounced (from 20.2±10.1 to 64.5±21.1% of limb ischemia at PRE to 16.9±12.7 to 44.0±18.9% at POST). Peak Δ[deoxy(Hb+Mb)] values were lower at POST (by -31.2±20.5%; $p<0.001$). **Conclusions**: trail running leads to impairment in skeletal muscle oxidative metabolism, possibly related to muscle damage from repeated eccentric contractions. In association with other mechanisms, the impairment of skeletal muscle oxidative metabolism is likely responsible of the reduced exercise capacity and tolerance during and following these races.

Keywords: NIRS; downhill running; eccentric; ultra-endurance; mountain running

Introduction

Trail-running is defined by the International Trail Running Association (ITRA) *"[…]a pedestrian race open to all, in a natural environment (mountain, desert, forest, plain…) with minimal possible paved or asphalt road […]"* (www.i-tra.org). The racecourses can reach and exceed 200 km of length with more than 20000 m of elevation gain. In particular, races longer than the classical marathon distance are defined ultra-trails (UTs). Trail- and ultra-trail running become more popular among amateur and professional runners and they present significant differences in elevation gain, with multiple up- and downhill sections.[1] Whereas uphill sections stress to a greater extent aerobic metabolism, in downhill sections, as a consequence of the repeated and forceful eccentric contractions muscle damage and inflammation responses ensue.[2]

In the last few years several physiological aspects of trail running have been studied, such as the cost of running, neuromuscular alterations and biomechanical changes following trail-running races.[3-6] More recently, the availability of new portable or wearable devices allowed the determination of other physiological parameters, also during sport activities. For example, near-infrared spectroscopy (NIRS) allows to evaluate the tissue oxygenation dynamics under different conditions.[7] In particular, during the last few years, different authors used NIRS to evaluate exercise intensity and muscle oxygenation dynamics in different sport situations.[8-11] Snyder and Parmenter[10] proposed to use NIRS to determine the maximal steady state running intensity since the results were comparable to those obtained from blood lactate concentration test. Born et al.[9] monitored the intensity during a trail-running event comparing the heart rate and the NIRS-obtained parameters. They suggested that NIRS is more accurate than heart rate to monitor running intensity, in particular when the exercise is performed on hilly terrain.

Vernillo et al.[8] showed by NIRS that an UT (330 km, 24000 m of elevation gain) leads to an alteration in the balance between O_2 delivery ($Q'O_2$) and O_2 utilization ($V'O_2$) within the working muscles at submaximal (1 and 1.5 W/kg) intensity. They proposed that the continuous concentric and eccentric contractions, due to the up- and downhill sections, might impair microcirculatory function. The effects of downhill running on muscle oxidative functions in rats were investigated by Kano et al. (2005).[12] These authors observed that one and three days after a downhill run to exhaustion, capillary hemodynamic and $Q'O_2/V'O_2$ matching were still compromised. Moreover, Kano et al. (2005)[12] suggested that the fiber damage after eccentric exercise is the main determinant for these impairments. Also, Fernstrom et al.[13] studied the functional aspects of mitochondria isolated from muscle biopsies before and after a 24-h ultra-endurance exercise performed at ~60% of $V'O_2$max, demonstrating that after ultra-endurance exercise also mitochondrial efficiency is reduced.

To our knowledge the study of Vernillo et al.[8] is the first one in which authors used NIRS for the evaluation of muscle oxidative metabolism before and after an UT. However, these authors used a protocol that does not provide information on the maximal capacity to oxidize substrates by the skeletal muscle, since the exercise they analyzed was not isolated to a single muscle group (cycling at 1 and 1.5 W/kg). Thus, the purpose of the present study was to evaluate the effects of a trail-running race on muscle oxidative function by measuring pulmonary gas exchange variables and muscle fractional O_2 extraction. Measurements were carried out during an incremental one-leg knee extension (KE) exercise performed before and after a trail-running competition. We hypothesized a significant impairment of skeletal muscle oxidative function following the race, with specific reference to the maximal capacity of O_2 extraction and to the relationship between O_2 uptake and O_2 delivery.

Materials and Methods

Subjects

We enrolled thirty-two subjects in this study as participants in the "Trail dei 3 Castelli" (www.trail3castelli.com), but only eighteen athletes (17 men, 1 woman; mean ± SD; age: 36.8 ± 9.2 y; maximal oxygen uptake [$V'O_{2max}$]: 64.3 ± 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$) (Table 1) completed the race and were evaluated before (PRE) and after (POST) the competition. The participants were experienced ultra-endurance runners (5.9 ± 3.2 years of ultra-endurance experience, 78.9 ± 39.2 $km \cdot week^{-1}$). The experimental protocol was conducted according to the Declaration of Helsinki and the Ethics Committee of the University of Udine approved it. Before the study began, the purpose and objectives were carefully explained to each participant and written informed consent was obtained from all of them.

Design

Athletes enrolled in the present study participated to a 32-km race with 2000 m of elevation gain (n=9), or to a 50 km race with 3500 m of elevation gain (n=9). Racecourses included several up- and downhill sections (Figure 1). Participants were invited twice to the laboratory during the week before the race (PRE) in order to perform an incremental uphill running test (day 1) and an incremental exercise by utilizing a one-leg knee extensor (KE) ergometer (day 2), previously described and used by our group [14, 15]. The KE exercise was repeated as soon as possible after the end of the race (8±4 min, POST).

Physiological measurements

Anthropometry. We measured the body mass (BM) to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) before and after the race. Also, we measured stature to the nearest 0.001 m on a standardized wall-mounted height board. Then, we calculated the body mass index (BMI). Moreover, we calculated the lean (fat-free) volume of

the right lower limb using the Jones and Pearson method [16], which is based on the summation of truncated cones.

Incremental uphill running test. During the week before the race, we measured maximal oxygen uptake ($V'O_{2max}$), maximal heart rate (HR_{max}) and maximal vertical velocity ($V_{vert}max$) during a graded exercise test on a treadmill (Saturn, HP Cosmos, Germany) under medical supervision. After a ten-minutes warm-up at self-selected speed, athletes started the test at the speed of 6 $km \cdot h^{-1}$ and a slope of 10%. Maintaining the same speed, we increased the slope of the treadmill by 2% every two minutes until 24%, which is the maximum slope that the treadmill can reach. After this step, we increased the speed by 0.5 $km \cdot h^{-1}$ every two minutes until the volitional exhaustion of the subject. We choose this protocol because it allowed increasing the vertical velocity linearly by ~3.2 $m \cdot s^{-1}$ every two minutes. During the test, we measured oxygen uptake ($V'O_2$), carbon dioxide production ($V'CO_2$), ventilation (V'E), tidal volume (V'T), and respiratory frequency (fR) breath-by-breath using a portable metabolic unit (K4 b^2, Cosmed, Italy). Before every test, we calibrated the volume and gas analyzers using a 3-L calibration syringe and calibration gas (16.00 $\%O_2$ and 4.00% CO_2), respectively. During the tests, we recorded heart rate (HR) by using a belt positioned on the chest (T31 sender, Polar, Kempele, Finland).

One-leg knee extensor incremental test. During the week before the race and as soon as possible (8±4 min) after the finish of the race, athletes underwent an incremental KE exercise on an ergometer that was previously used by our group.[14, 15] We measured the time to exhaustion, peak power (Ppeak), muscle oxygenation dynamics by NIRS and cardiorespiratory parameters ($V'O_2$, $V'CO_2$, V'E, VT, fR and HR) by a metabolimeter (K4 b^2, see above). Subjects were seated on an adjustable seat, and the angle of the hip was maintained at ~90° by a safety belt. We attached the right leg to a lever arm and participants were instructed to actively extend the leg from 90° to 175°. This range of motion was limited by two blocks specifically

arranged. The return of the leg to the starting position was performed passively (i.e. no contraction was required). Before each test, athletes familiarized with testing procedures. After an initial 3 min of unloaded KE exercise an operator adjusted the workload every minute by increasing the workload by ~8 $W \cdot min^{-1}$ (8.4±3.2 $W \cdot min^{-1}$) (see Ref. Salvadego et al.[14] for details). Since the regulation of the workload was done manually, we collected the force applied to the lever arm and we allowed an error of (±5%) to the force used to extend the leg. Throughout the test, the active KE and passive knee flexion cycle was carried out 40 times per minute as imposed by a metronome. During each cycle (total duration of 1.5 s), KE lasted ~1 s. Thus, muscle contraction corresponded ~65% of the duty cycle. Athletes performed the exercise until the exhaustion, which was defined as the inability to maintain the imposed workload at the required frequency despite vigorous encouragements by the operators. During the incremental test we evaluated oxygenation changes in *vastus lateralis* muscle by continuous-wave near-infrared spectroscopy (NIRS, PortaLite, Artinis, The Netherlands) (see Grassi and Quaresima[7] for a recent review on the method). NIRS was used for measuring micromolar changes in oxygenated (Hb)+myoglobin (Mb) concentrations Δ[oxy(Hb+Mb)], and deoxygenated [Hb+Mb] Δ[deoxy(Hb+Mb)], with respect to an initial value arbitrarily set equal to zero and obtained during the resting condition preceding the test. Δ[deoxy(Hb + Mb)] is relatively insensitive to changes in blood volume and has been considered an estimate of skeletal muscle fractional O_2 extraction (ratio between O_2 consumption and O_2 delivery).[17] Values of Δ[deoxy(Hb+Mb)] were expressed as a percentage of the values of maximal muscle deoxygenation obtained during a transient ischemia of the limb by a pressure cuff inflation (at 300–350 mmHg), maintained until Δ[deoxy(Hb+Mb)] reached a plateau. Δ[deoxy(Hb+Mb)] kinetics during the incremental tests were fitted by a sigmoid function, as previously presented by other authors and by our group.[14, 18] Then, we calculated mean values of cardiorespiratory and muscle oxygenation variables during the last 20 s of each workload. $V'O_2$ and

Δ[deoxy(Hb+Mb)] values were grouped for discrete workload intervals, which were determined in order to have, in each interval, each subject represented by one data point. When the subject had more than one "original" data point in the interval, mean individual values were calculated, both for the *x* and *y* variables, and were taken in consideration to obtain the figure [14].

Statistical analysis

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $P<0.05$. All results are expressed as means ± SD. Normal distribution of the data was tested using the Shapiro-Wilk test. Sphericity (homogeneity of covariance) was verified by the Mauchly's test. Differences in anthropometric characteristics and metabolic parameters of two groups, before (PRE) and at the end (POST) of the race, were studied with General Linear Model repeated measures of the main effects of group (G: Short vs Long), time (T: PRE *vs* POST) and group x time interaction. Since no significant effects of distance race was observed on reported parameters, the two groups were pooled together to further analyze the changes of metabolic and mechanical parameters by General Linear Model repeated measures.

Results

Age, BM pre- and post- race, stature, BMI, quadriceps muscle mass (QM), $V'O_2max$, HR max, $V_{vert}max$ and race time of the eighteen participants in the study are reported in Table 1. BM decreased by -2.1 ± 1.6%, $p<0.001$ in POST *vs.* PRE. The range position among the athletes participating in the study was 1^{st}-132^{nd} (04:59:07 ± 01:16:30, hh:mm:ss) in the 32-km race (total of 143 finishers) and 1^{st} – 84^{th} (08:17:53 ± 1:23:40, hh:mm:ss) in the 50-km race (total of 84 finishers).

Pulmonary gas exchange during incremental KE exercise. Peak values of the main cardiorespiratory variables determined at exhaustion are presented in Table 2, along with the

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peak power and time to exhaustion. V'Epeak and HRpeak suggest that the exercise was not maximal from a cardiorespiratory perspective. In particular, HRpeak was markedly lower than the maximal values obtained during the incremental uphill running test, both in PRE (-29.3±11.7%; $p<0.001$) and in POST (-35.1±6.2%; $p<0.001$). V'Epeak, V'Tpeak and $V'O_2$peak (L/min) were significantly lower at POST vs. PRE (-17.4 ± 10.9%, -18.2 ± 14.6% -23.9 ±9.0%, respectively, $p<0.001$). Moreover, $V'O_2$peak at POST was lower than $V'O_2$ at the same workload at PRE (-8.4 ± 15.6%, $p<0.05$, Figure 2). When $V'O_2$peak was normalized for the quadriceps muscle mass, values were still significantly lower (by -24.7±8.2%, $p<0.001$) in POST vs. PRE. Peak power output and time to exhaustion decreased significantly (by -23.7±14.3% and -18.3±11.3%, respectively, $p<0.005$) after the race (Tab. 2). No differences were observed for the other peak variables. $V'O_2$ values *vs.* work rate are shown in Figure 2. No significant differences between values in PRE and POST were observed at the 3 lowest work rates. $V'O_2$ was significantly lower in POST *vs.* PRE at the work rate corresponding to about 60 watts. Further, no significant differences ($p>0.05$) were detected in the above-mentioned parameters between subjects participating in 30 or 50 km race.

Near-infrared spectroscopy. The dynamics of Δ[deoxy(Hb+Mb)] as a function of work rate during the incremental KE exercise PRE- and POST race are described in Figure 3. Both in PRE and in POST, Δ[deoxy(Hb+Mb)] values increased following a sigmoid-like pattern, approaching a plateau within the last one or two work rates. Values did not differ significantly in PRE vs. POST at the submaximal work rates. However in POST the increase as a function of work rate, from unloaded to peak, was much less pronounced (from 20.2±10.1 to 64.5±21.1% of limb ischemia at PRE to 16.9±12.7 to 44.0±18.9% at POST). Peak Δ[deoxy(Hb+Mb)] values were significantly lower at POST (-31.2±20.5%; $p<0.001$). No significant differences ($p>0.05$) were detected in peak Δ[deoxy(Hb+Mb)] between subjects participating in 30 or 50 km race.

Discussion

The main result of the present study shows that a few minutes after a trail running competition lasting between ~3 and ~11 hours the participants experienced a marked impairment of oxidative function intrinsic to skeletal muscle. This impairment was manifested by a reduced peak $V'O_2$ and peak fractional O_2 extraction (Δ[deoxy(Hb+Mb)]) of *vastus lateralis* muscle determined during an incremental one-leg KE exercise.

This exercise utilizes a relatively small muscle volume (the quadriceps muscle of one leg, ~2.5 kg), thereby reducing substantially any limitation to oxidative metabolism deriving from central-cardiovascular O_2 delivery, and allowing any limitation intrinsic to skeletal muscle to become fully manifest. [14,15] In fact, in the present study participants reached exhaustion at relatively low HR values (corresponding to ~70% at PRE and 65% at POST of the peak values determined during the incremental running test), confirming the absence of any cardiovascular limitation during KE. $V'O_2$peak was significantly lower after the race (-23.9±9.0%) compared with PRE, and this was associated with lower peak power output (-23.7±14.3%) and early exercise interruption (see Tab 2). Considering the nature of the KE exercise (see above), the functional impairment of oxidative metabolism can be located "downstream" of cardiovascular function, in other words at the skeletal muscles level.

At submaximal loads and at the beginning of the KE exercise there were no differences in $V'O_2$ in POST vs. PRE, suggesting that the exercise economy was not negatively affected by the race. This result is in agreement with other studies [4, 8, 19] that did not report an alteration in O_2 cost of cycling or walking after an UT of 330 km. However, other authors reported an increased cost of running following UT [6, 20]; the increase was related to exercise duration or distance covered. The conflicting results of these studies may be attributed, at least in part, to the different type of exercise (walking/running 330 km vs. a multi-stage competition or treadmill running) and/or to the time elapsed between the end of the race and the arrival of the

subjects at the laboratory for the test which was ~1h in the study of Vernillo et al.[4] vs. few minutes in our study. Also, we adopted a different protocol that may have affected the results (see below).

To our knowledge there are no studies in which the V'O_2peak was measured immediately after an ultra-endurance event, but we can speculate that this parameter is negatively affected by the fatigue state occurred after this type of exercise.[21] During a pilot study in which we involved three subjects, we compare power output and V'O_2 during an incremental test on cycle ergometer before and after an ultra-endurance cycling race of 606 km with 16000 m of elevation gain (Ultracycling Dolomitica). The peak power and the systemic V'O_2peak after the race were ~40% and ~25% lower than before the race, respectively. As well, Kasikcioglu et al.[22] and Sierra et al.[23] reported a decrease of ~9% in V'O_2peak during an incremental test one and three days after a marathon. According to some authors the decrease in V'O_2peak after a long-lasting effort may be attributed to some degree of "cardiac fatigue".[23] In the present study we have demonstrated that a significantly impaired oxidative function is present also downstream of cardiovascular function.

During the UT that we analyzed, muscle mass was not affected by the race, and thus the observed impairment in V'O_2peak was substantially qualitative, presumably related to functional impairments intrinsic to the muscle fibers. Millet et al.[21] reported that after an UT of 166 km and 9500 m of elevation gain the maximal voluntary contraction of the knee extensor muscles was markedly reduced, and was mainly associated with an early development of central fatigue. In particular, these authors reported a reduction in voluntary activation measured during KE exercise. It is also known that the repeated eccentric contractions during downhill running induce muscle damage and neuromuscular alterations, with a decreased central drive and strength loss.[24] In fact, UT induces muscle damage and inflammation which could affect the contractility properties and oxidative metabolism.[21] Thus, the repeated

downhill sections occurred during the competition may have affected KE performance by acting both at peripheral and central level. Moreover, prolonged exercise can induce an accumulation of metabolites within the muscle fibers [21, 25], which can disrupt Ca^{2+} release and uptake pathways in the sarcoplasmic reticulum, with a consequent failure of excitation-contraction coupling.[26] Overall, these alterations may lead to a lower force production during KE exercise, lower peak power and an early exhaustion of the subjects.

In the present study Δ[deoxy(Hb+Mb)] was utilized to estimate fractional O_2 extraction at the *vastus lateralis* muscle, in other words the ratio between intramuscular increases in $V'O_2$ and O_2 delivery.[18] Δ[deoxy(Hb+Mb)] did not change in POST (*vs.* PRE) at submaximal work rates. Considering that at these work rates $V'O_2$ was not different in the two conditions, it can be concluded that also O_2 delivery was unaffected. Peak values of O_2 extraction were significantly lower in POST (44.0±18.9% of limb ischemia) than in PRE (64.5±21.1%), in conjunction with a percentage-wise similar decrease in $V'O_2$peak. Again, this observation suggests a substantially maintained O_2 delivery, also at peak exercise, pointing to alterations more specifically related to muscle fibers as the potential cause of the observed oxidative impairment.

At a first glance, these findings may appear in contradiction with those obtained by Vernillo et al.[8] after an UT of 330 km and 24000 m of elevation gain; these authors found an increase in muscle deoxygenation during low-intensity cycling and attributed this response to an impaired intramuscular $Q'O_2/V'O_2$ matching consequent to the eccentric loads that characterize the downhill sections in such races. An association between eccentric exercise and impaired matching between $Q'O_2$ and $V'O_2$ was indeed demonstrated by Davies et al.[27] in physically active men during severe-intensity cycling, and by Kano et al.[12] in rats during moderate-intensity downhill running and was explained by an altered capillary hemodynamic. The apparent discrepancy between the responses observed in this study at submaximal work

rates with those observed by Vernillo et al.[8] are presumably due to the peculiarity of the exercise protocol proposed in the present study. Indeed, KE exercise recruits a small portion of the legs muscles and cardiovascular limitations in O_2 delivery are absent or significantly reduced compared to what observed in cycling exercise or treadmill running.[28] In other words, the adopted exercise protocol allowed us to identify the impairments in oxidative metabolism, more strictly related to muscle fibers, deriving from the race.

Of note, the results of the present study are similar to those observed in healthy young men after a 35-days bed rest period, suggesting that two opposite stimuli such as a trail running race and chronic physical inactivity can induce a similar substantial muscle dysfunction and a subsequent marked exercise limitation.[14, 29] Unlike from the bed rest studies, in the present study no loss of muscle mass was observed after the race, while a parallel decrease in $V'O_2$peak and Δ[deoxy(Hb+Mb)] peak was evident. These findings suggest the occurrence of qualitative impairments after a trail running race, which may involve the contractile function, such as a different recruitment of motor units towards the less oxidative and less resistant fast twitch fibers,[30] and/or the metabolic function, such as an impaired mitochondrial respiration. We did not perform biochemical or molecular analyses of muscle samples in this study. However, it can be hypothesized that the mitochondrial efficiency was lower after the race. Indeed, this result was observed by Fernstrom et al. after a 24-h ultra-endurance exercise at ~60% of $V'O_2$max [13] and it may be attributed to an increased oxidative stress.[31] Oxidative stress and inflammation are present in mountain marathon and ultra marathon [32-34] even if they may be counterbalanced by increased antioxidant defenses.[33]

Practical applications

The results of this study combined with the results of other authors [8, 13, 31] suggest that the impairments observed in the muscle oxidative capacity during a trail running race may be limited by adding specific exercises (e.g. downhill running at high intensity) in the training program of these athletes and enhancing the antioxidant defenses.

Conclusions

In conclusion, in the present study we provide evidence of a substantial impairment in skeletal muscle oxidative metabolism following a trail running exercise of 3-11 hours, possibly related to muscle damage from repeated eccentric contractions. In association with other mechanisms leading to impaired force generation, central fatigue, oxidative stress and damage, the impairment of skeletal muscle oxidative metabolism is likely responsible of the reduced exercise capacity and tolerance during and following these races.

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Figure 1A.

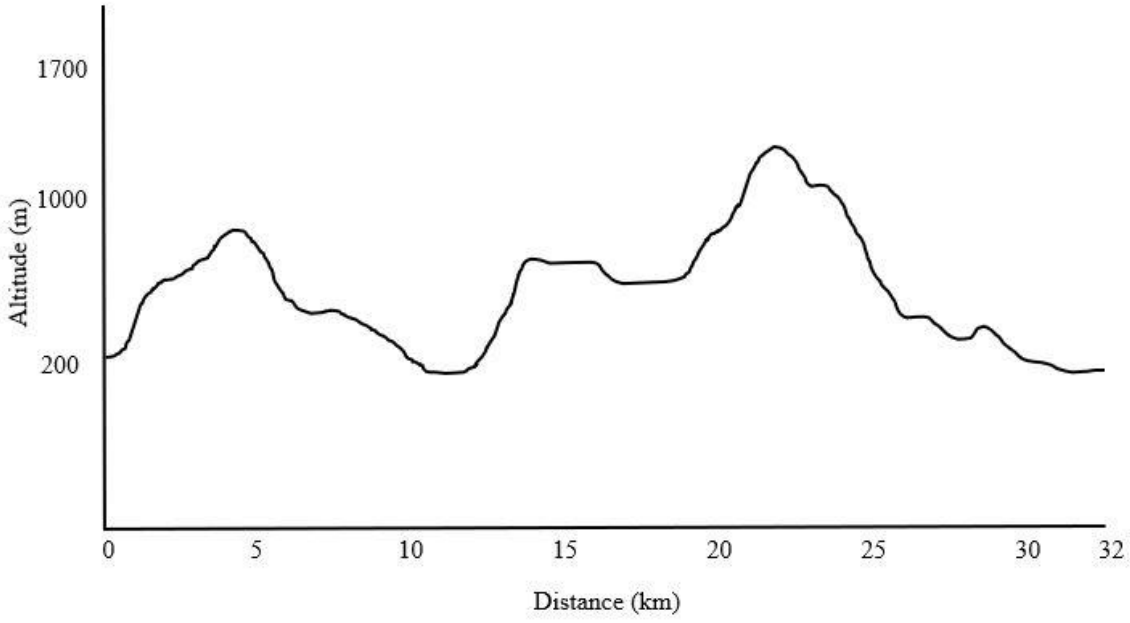

Figure 1B.

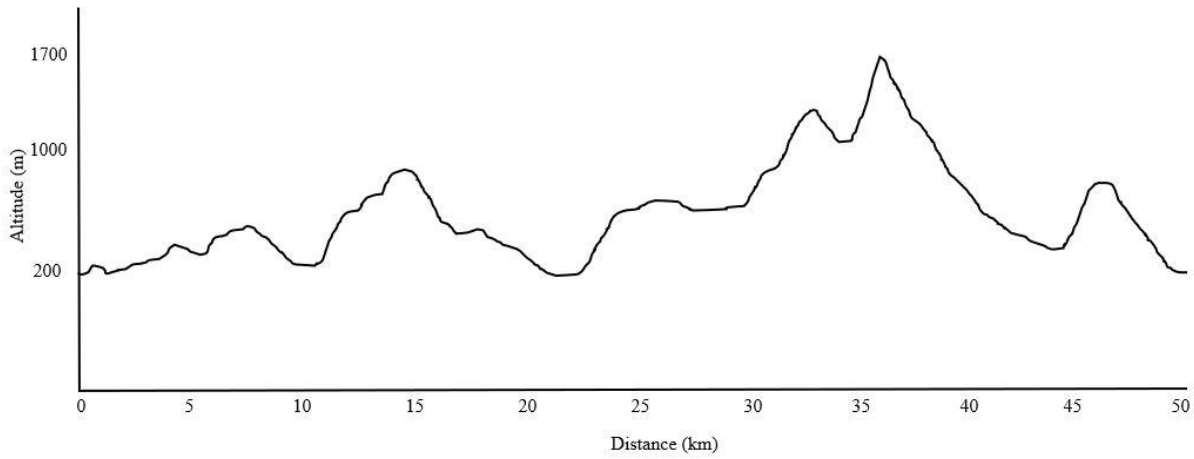

Figure 1. Race profiles for the 32 km (2000 m elevation gain, A) and 50 km (3200 m elevation gain, B).

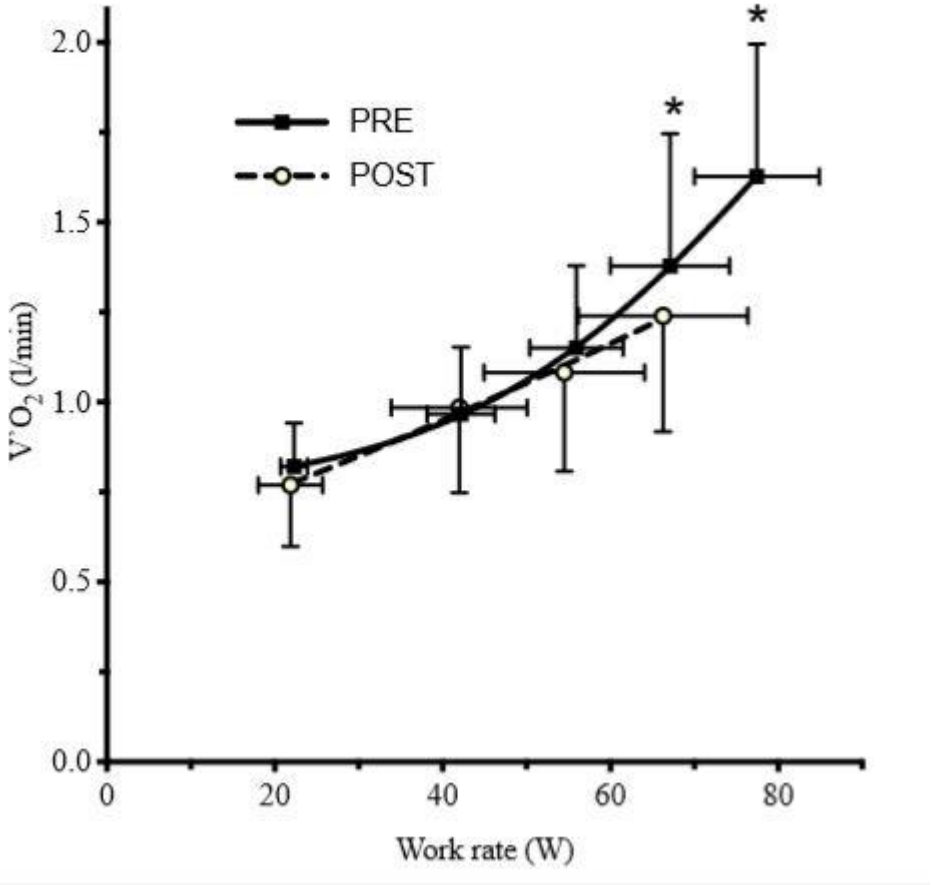

Figure 2. Oxygen uptake ($V'O_2$) as a function of work rate during one-leg knee extensor exercise before (PRE, black squares) and after (POST, white dot) the race. All values are means ± SD. *: $p<0.001$ compared to the last workload at POST.

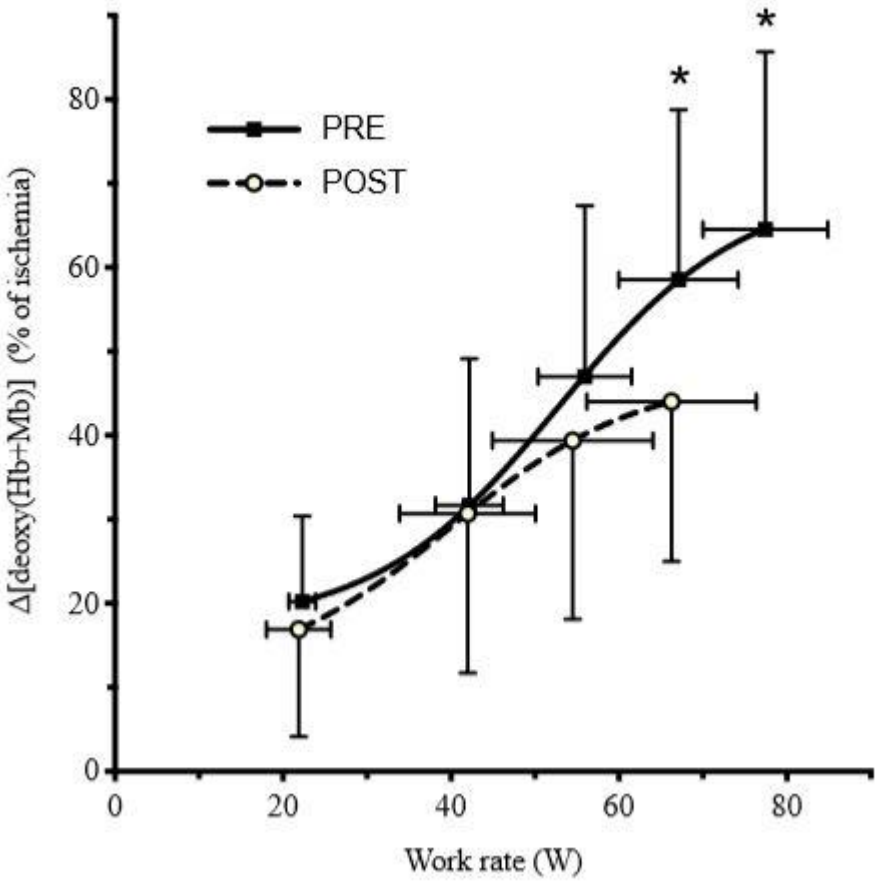

Figure 3. Deoxygenated hemoglobin and myoglobin Δ[deoxy(Hb+Mb)] measured by NIRS as a function of work rate during one-leg knee extensor exercise before (PRE, black squares) and after (POST, white dot) the race. The percentage is related to the maximum value obtained during a transient limb ischemia induced at the end of the test. All values are means ± SD. *: $p<0.001$ compared to the last workload at POST.

Table 1. Physical characteristics of the participants and race time.

	All runners (n:18)	Range
Age (years)	36.8 ± 9.2	[23 - 56]
BM (kg) PRE	68.1 ± 8.1	[46 - 82]
BM (kg) POST	66.7 ± 8.3	[44 - 82]
Stature (m)	1.75 ± 0.07	[1.54 - 1.83]
BMI ($kg \cdot m^{-2}$)	22.1 ± 1.6	[18.8 - 25.3]
VO2max	64.3 ± 6.3	[55.3 - 77.5]
HRmax (bpm)	179.0 ± 9.5	[163 - 198]
Vvert max (m/s)	0.42 ± 0.07	[0.33 - 0.55]
Race time (hh:mm:ss)	06:32:39 ± 02:08:15	[03:20:15 - 10:48:50]

All values are means ± SD.

BM: body mass; BMI: body mass index; $V'O_2max$: maximal oxygen uptake; HRmax: maximal heart rate; QM: quadriceps muscle mass; $V_{vert}max$: maximal vertical velocity.

Table 2. Peak physiological parameters during the one-leg knee extensor exercise measured before (PRE) and after (POST) the race.

	PRE	POST	P
$V'Epeak$ ($L \cdot min^{-1}$)	55.1 ± 14.5	45.8 ± 14.9	<0.001
V'Tpeak (L)	1.73 ± 0.40	1.39 ± 0.32	<0.001
$V'O_2peak$ ($L \cdot min^{-1}$)	1.63 ± 0.37	1.24 ± 0.32	<0.001
fRpeak ($breaths \cdot min^{-1}$)	32.9 ± 7.0	33.5 ± 7.6	0.566
Rpeak	0.96 ± 0.09	0.91 ± 0.08	0.164
HRpeak ($beats \cdot min^{-1}$)	126.9 ± 20.1	116.6 ± 13.5	0.085
$V'O_2peak$ /QM ($ml \cdot min^{-1} \cdot 100g^{-1}$)	83.50 ± 12.70	63.1 ± 13.1	<0.001
Ppeak (W)	79.5 ± 15.5	58.5 ± 17.5	<0.01
Time to exhaustion (min:sec)	08:43 ± 01:27	07:00 ± 01:34	<0.01

All values are means ± SD.

$V'Epeak$: peak ventilation; V'Tpeak: peak tidal volume; $V'O_2peak$: peak oxygen uptake; fRpeak: peak respiratory frequency; Rpeak: peak respiratory exchange ratio; HRmax: maximal heart rate; QM: quadriceps muscle mass; Ppeak: peak power.