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Effects of Ultratrail Running on Skeletal Muscle Oxygenation Dynamics

Running Title: Muscle Oxygenation and Ultratrail

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ABSTRACT

**Purpose:** To quantify changes in skeletal muscle oxygenation and pulmonary O$_2$ uptake after an extreme ultratrail running bout.

**Methods:** Before (PRE) and after (POST) the race (330-km, 24000 D±), profiles of vastus lateralis muscle oxygenation (i.e., oxyhaemoglobin, [O$_2$Hb], deoxyhaemoglobin, [HHb], and tissue oxygenation index [TOI]) and O$_2$ uptake ($\dot{V}$O$_2$) were determined in 14 athletes (EXP) and 12 control adults (CON) during two 4-min constant-load cycling bouts at power outputs of 1 (p1) and 1.5 (p1.5) W·kg$^{-1}$ performed in randomized order.

**Results:** At POST, normalized [HHb] values increased (p1, +38.0%; p1.5, +27.9%; $P < 0.05$), while normalized [O$_2$Hb] (p1, -20.4%; p1.5, -14.4%; $P < 0.05$) and TOI (p1, -17.0%; p1.5, -17.7%; $P < 0.05$) values decreased in EXP. $\dot{V}$O$_2$ values were similar ($P > 0.05$). An ‘overshoot’ in normalized [HHb]/$\dot{V}$O$_2$, though the increase was significant only during p1.5 (+58.7%, $P = 0.003$). No difference in the aforementioned variables was noted in CON ($P > 0.05$).

**Conclusions:** The concentric and, particularly, the eccentric loads characterizing this extreme ultratrail running bout may have led to variations in muscular structure and function, increasing the local muscle deoxygenation profile and the imbalance between O$_2$ delivery to working muscles and muscle O$_2$ consumption. This highlights the importance of
incorporating graded training, particularly downhill bouts, to reduce the negative influence of concentric and severe eccentric loads to the microcirculatory function and to enhance the ability of runners to sustain such loading.

Keywords: mountain ultramarathon; muscle deoxygenation; near-infrared spectroscopy; pulmonary oxygen uptake; trail running.
INTRODUCTION

Ultratrails (UT) typically involve running a long distance over a course with extreme changes in elevation.\(^1\)\(^3\) In the last 40 years, there has been an exponential growth of participation in UT events\(^4\) and research conducted to date has shown that the energy demand is at the extremes of human tolerance.\(^5\) Particularly, the extreme fatigue state such events induce originates at both central and the peripheral levels.\(^6\)\(^8\)

Many different mechanisms underlie exercise-related fatigue and these may include changes in skeletal muscle microvascular oxygenation dynamics. It is thought that the adequacy of muscle blood flow and \(O_2\) delivery affects muscular performance and the rate of development of both central and peripheral fatigue.\(^9\) Indeed, during dynamic exercise, muscle oxygenation increases to meet the demands of the working muscles.\(^10\) Thus, given the importance of blood flow to muscle metabolism, any alteration in muscle oxygenation would increase the development of fatigue.\(^9\)

Muscle oxygenation, as reflected by muscle end-capillary and muscle tissue \(O_2\) content, is dependent on the dynamic balance of \(O_2\) delivery to working muscles (i.e., muscle blood flow \(\times\) arterial \(O_2\) content) and muscle \(O_2\) consumption.\(^9\) Changes in local muscle (de)oxygenation status can be monitored non-invasively using the near-infrared spectroscopy (NIRS).\(^11\) Further, NIRS may provide complementary information
concerning muscle fatigue, since a strong correlation has been observed between muscle oxygenation measured by NIRS and muscle fatigue by electromyography. To date, few studies have evaluated the effects of fatigue on muscle oxygenation response after prolonged running exercise. For instance, Sear et al. showed a ~56% increase in muscle oxygenation in the vastus lateralis after a 45-min high-intensity intermittent running protocol in subjects wearing whole-body compression garments. Vercruyssen et al. observed a ~60% increase in oxygen uptake of the vastus lateralis after a 15.6-km trail run (825 m of D-)

Running has always been defined as a stretch-shortening exercise, where the preactivated muscle is first stretched (eccentric action) and then shortened (concentric action). UT presents significant challenges and runners need to combine a variety of activation patterns and types of muscle contraction (concentric and severe eccentric loads) as they continue along the trail. Recently, Giandolini et al. showed that a 6.5-km downhill trail running bout with 1264 m of altitude drop induced muscle alterations similar to those induced by UTs. Further, during UTs the related muscle damage and inflammation responses are relevant. Indeed, Saugy et al. found ~3200% and ~1600% increases in creatine kinase and myoglobin concentrations, respectively, after the same UT investigated in the present study. Thus, due to its eccentric
component, one may consider the downhill sections of an UT as the main factor inducing lower-limb tissue stress.\textsuperscript{6,7} In this regard, Davies et al.\textsuperscript{17} suggested that muscle-damaging eccentric exercise alters the matching of O$_2$ delivery and utilization during severe-intensity exercise.

However, the effects of an UT on leg muscle oxygenation dynamics are unknown. Specifically, whether an imbalance between muscle perfusion and oxygenation consumption occurs after an UT, quantifying local muscle deoxygenation status, remains to be determined. Thus, the purpose of this study was to examine the skeletal muscle oxygenation responses to an extreme UT (eUT). We hypothesized that the eUT would lead to structural and functional alterations in the microvasculature sufficient to increase local muscle deoxygenation.
METHODS

Race Characteristics

The international race supporting the study was the Tor des Géants®. Considered the world’s most challenging UT, it entails running or walking a course of 330 km with 24000 m D± (for further details, see http://www.tordesgeants.it).

Subjects

All runners taking part in the eUT were invited to participate in this study via a letter prepared and sent by e-mail by the race organizer informing them about the study purposes and design. Twenty-five healthy, experienced male trail runners underwent the pre-eUT testing protocol (PRE). Six subjects did not complete the eUT and three others did not undergo the post-eUT testing protocol (POST) due to time constraints. Two other subjects were physically capable of completing only a small portion of POST testing and were eliminated from the final analysis. In all, 14 subjects performed both PRE and POST protocols and constituted the experimental group (EXP). In parallel, a control group (CON) of 12 healthy, physically active male adults participated in this study and performed the PRE and POST protocols. The EXP and CON groups were similar except for age (Table 1). Prior to PRE, a questionnaire was administered to collect data on EXP training experience. On average, subjects had 11.3 ± 2.9 years of training in running.
and 5.1 ± 0.9 years of ultra-endurance running experience. Pre-
race weekly training consisted of 3-5 sessions comprising 9.1 ±
3.5 h and 60.2 ± 21.0 km with a cumulative elevation change
between 1000 and 5000 m. All participants were fully informed
about the procedures and risks involved in the study and that
they could withdraw at any time. Written informed consent was
obtained from all participants. The study was approved by the
local Institutional Ethics Committee and was performed
according to the ethical standards laid out in the 2013 revision
of the Helsinki Declaration.

****Table 1 about here****

**Design**

Subjects underwent two test sessions: the first was performed
1-2 days before the eUT (PRE) and the second immediately
after the eUT (POST). Shortly after the subjects had crossed the
finishing line, they were brought by car to the laboratory (~1
km away). The time between the finishing the race and POST
evaluations was approximately 25 min. PRE and POST tests
were organized in a similar fashion, and the timing of the
measurements was nearly identical for both groups. On arrival
at the laboratory, the subjects’ body mass was measured to the
nearest 0.1 kg using a digital scale (Model HF8000, Philips,
Eindhoven, The Netherlands) to determine the cycling
workload. Skinfold thickness of the vastus lateralis muscle area
(see ‘Near-infrared spectroscopy’ section) was measured using a skinfold caliper (Holtain Ltd., Crymmych, UK) and divided by two to determine the adipose tissue thickness (fat + skin layer) covering the muscle. Table 1 presents the tissue thickness values, all of which were below the 1.5 cm necessary to allow the NIRS photons to penetrate through to the muscle. The subjects were then seated comfortably while the NIRS optodes were positioned and secured on the leg (see ‘Near-infrared spectroscopy’ section). The laser diodes were activated approximately 15 min before beginning the cycling exercise protocol to ensure that the diodes had reached optimal working temperatures. After this period, the subject moved to a cycle ergometer (Lode Excalibur Sport, Lode, Groningen, The Netherlands) and was instrumented for pulmonary gas exchange assessment. The NIRS system was initialized while the subject rested quietly on the ergometer with the legs relaxed and still. When a steady baseline signal was achieved, the system was again zeroed and all subsequent changes in NIRS signal intensity were made relative to this resting value. The NIRS signal was monitored continuously for the remainder of the test, with no further adjustments being made to the NIRS system zero value. The exercise protocol consisted of two 4-min constant-load exercise bouts at power outputs of 1 (p1) and 1.5 (p1.5) W·kg⁻¹ at ~90 rpm performed in randomized order. Each test condition was preceded by a 2 min of baseline (BL)
cycling at a power output of 0.5 W·kg\(^{-1}\). The workloads selected for this study were the highest rates, previously observed\(^3\) and determined from pilot research, which would allow for accurate measurement of muscle deoxygenation levels while maximizing comfort for the subjects, especially during the POST test protocol. Fatigue-induced changes in running technique were previously observed after this eUT.\(^3,20\)

To avoid mechanical changes potentially biasing interpretation of the results, cycling was used to focus only on metabolic adaptations.

**Methodology**

*Near-infrared spectroscopy*

Oxyhaemoglobin (\(\Delta[O_2Hb]\)), deoxyhaemoglobin (\(\Delta[HHb]\)) and total haemoglobin (\(\Delta[tHb] = (\Delta[O_2Hb] + \Delta[HHb])\)) concentrations and tissue oxygenation index [TOI (%)] = (\(\Delta O_2Hb/\Delta tHb\) \times 100) of the vastus lateralis of the dominant leg were measured by NIRS\(^{21}\) (NIMO, Nirox srl, Brescia, Italy). The extent of the contribution which haemoglobin and myoglobin make to the near-infrared signal is presently unclear,\(^{11}\) thus the above abbreviations refer to the combined signal due to haemoglobin and myoglobin. The transmitting and receiving optodes were positioned with 4-cm interoptode spacing on the vastus lateralis muscle of the dominant leg, midway between the lateral epicondyle and greater trochanter.
of the femur, parallel with the long axis of the muscle. To enable reproducible probe placement at the POST session, the location was marked with indelible ink and outlined on tracing paper. The optodes were housed in an optically dense plastic holder to ensure that the position of the optodes relative to each other was fixed and invariant. The optode assembly was secured on the skin surface with tape and then covered with an optically dense, black nylon sleeve to minimize the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize optode movement. Light absorption at different wavelengths (from 670 to 980 nm) was sampled at a frequency of 40 Hz and analyzed according to the modified Beer-Lambert law. A differential pathlength factor (DPF) of 3.8 was used for the vastus lateralis muscle.22

To provide equivalence for both $\Delta [O_2Hb]$ and $\Delta [HHb]$ signal sensitivity (i.e., allowing for minor differences arising from differences in optode placement and/or day-to-day variability in signal intensity), $\Delta [O_2Hb]$ and $\Delta [HHb]$ profiles were normalized from the minimum value (min) measured during the two cycling workloads and the maximum value (max) measured during each test condition according to the following equation:23

$$ Eq. 1: Y_{norm} (%) = \frac{(Y_t - Y_{\text{min}}) \times (Y_{\text{max}} - Y_{\text{min}})^1}{100} $$
where \( Y_{\text{norm}} \) is the [Hb] normalized signal value, \( Y_t \) is the [Hb] signal value at time \( t \), \( Y_{\text{min}} \) is the minimum [Hb] signal value, and \( Y_{\text{max}} \) is the [Hb] signal value during each test condition. As a consequence, at the end of the two conditions, the [Hb] normalized signal value represented the percentage of maximal muscle deoxygenation (i.e. 100\% \( \Delta[Hb] \)). This procedure does not affect the TOI value because TOI is measured as an absolute value instead of a change with respect to the arbitrary initial zero value. Then normalized \( \Delta[O_2\text{Hb}] \), \( \Delta[HHb] \), and TOI values were averaged during the last 60 s of each workload to obtain steady-state values aligned in time with pulmonary gas exchange data.

**Pulmonary gas exchange**

The volume was calibrated with a 3-liter syringe before the start of each protocol. Ventilation (\( \dot{V}E \)), breathing patterns (\( V_T \), \( f_R \)), gas exchange (\( O_2 \) uptake, \( \dot{V}O_2 \)) were measured breath-by-breath throughout each cycling exercise by means of an indirect calorimetry system (Quark b2, Cosmed, Rome, Italy). Data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. Heart rate (HR) was recorded continuously during each condition using a HR monitor (Polar S610i, Polar Electro, Kempele, Finland). Average values were calculated during the last 60 s of each test condition. \( \dot{V}O_2 \) data
were further normalized according to Eq. 1 and a normalized
$\Delta[\text{HHb}] / \Delta\dot{V}_O^2$ ratio derived from each subject was calculated for the steady-state during p1 and p1.5 as the average value of the last 60 s to identify an ‘overshoot’ or a ‘no-overshoot’ when the $\Delta[\text{HHb}] / \Delta\dot{V}_O^2$ ratio was above or below a ‘perfect’ matching of 1.0, respectively.

**Statistical Analysis**

Data are expressed as mean ± standard deviation (SD). Results were tested for normal distribution using the Shapiro-Wilk W test. Repeated-measures ANCOVA was performed to determine possible differences by examining the group (EXP vs. CON) × time (PRE vs. POST eUT) interaction, with the PRE values used as a covariate. Bonferroni’s *post hoc* test was applied when a significant $F$-value was found. To assess the $\Delta[\text{HHb}] / \Delta\dot{V}_O^2$ overshoot, a Student’s *t*-test was performed between the testing conditions and the hypothetical perfect matching of blood flow and utilization (i.e. 1.0 score of $\Delta[\text{HHb}] / \Delta\dot{V}_O^2$). All statistical analyses were performed using IBM™ SPSS™ Statistics (version 20.0.0, IBM Corporation, Somers, NY) and the level of significance was set at $\alpha < 0.05$. 
RESULTS

The current best record for the race is 70h 04min 15s and the average time of the study participants was 129h 54min 54s (range: 110h 47min 23s to 147h 58min 42s; ranking: 37th to 325th). There were no differences in height, body mass, body-mass index, adipose tissue thickness or cycling workload between EXP and CON, nor between PRE and POST in EXP (Table 1).

Near-infrared spectroscopy

The vastus lateralis muscle deoxygenation profiles for a representative EXP subject are presented in Figure 1a. The HHb signal increased rapidly in all subjects without discernible delay at the onset of both cycling bouts and approached a plateau both during p1 and p1.5. The average changes in both absolute and normalized Δ[HHb] data recorded during the test are illustrated in Figure 2. There were no differences in Δ[HHb] between EXP and CON at PRE. In both groups, the absolute and normalized Δ[HHb] values during p1.5 were significantly higher than p1 at PRE and POST. EXP showed higher absolute Δ[HHb] values compared to CON at POST; however, this difference disappeared when Δ[HHb] data were normalized.

PRE and POST data showed a significant increase in both absolute (p1, +16.0%, \( P = 0.03 \); p1.5, +24.6%, \( P < 0.001 \)) and normalized (p1, +38.0%, \( P = 0.04 \); p1.5, +27.9%, \( P < 0.001 \))
Δ[Hb] values in EXP, whereas no significant increase was observed in CON. The increase in Δ[Hb] from PRE to POST was greater for EXP than for CON; the end-exercise values represented 62.5 ± 16.5% and 77.4 ± 12.1% of maximal muscle deoxygenation (i.e. 100% Δ[Hb]) during p1 and p1.5, respectively (Figure 2)).

There were also no differences in Δ[O$_2$Hb] between EXP and CON at PRE. Both groups showed higher absolute and normalized values during p1.5 than p1 at PRE and POST. EXP showed lower absolute Δ[O$_2$Hb] values compared to CON at POST; however, this difference disappeared when the data were normalized. From PRE to POST, there were significant decreases in both absolute (p1, -24.4%, $P$ = 0.002; p1.5, -21.5%, $P$ = 0.021) and normalized (p1, -20.4%, $P$ = 0.009; p1.5, -14.4%, $P$ = 0.03) Δ[O$_2$Hb] values in EXP, whereas no significant increase was observed in CON (Figure 3).

The observed increase in Δ[Hb] values reflect the decrease in TOI. TOI decreased between PRE and POST in EXP (p1, -17.0%; p1.5, -17.7%) but remained unchanged in CON. EXP showed higher decreases in TOI compared to CON at POST (Figure 4).

****Figures 1, 2, 3, and 4 about here****
Pulmonary gas exchange

The breath-by-breath $\dot{V}O_2$ responses for a representative EXP subject are presented in Figure 1b. Steady-state $\dot{V}O_2$ was attained within 4 min at p1 and p1.5 without any apparent additional increase (i.e. slow component). $\dot{V}E$, $V_T$, $f_R$, and HR responses during the test protocols are presented in Table 2. $\dot{V}E$, $V_T$, $f_R$, and HR were similar in both groups at PRE. EXP showed a trend toward increased ventilation, albeit not significant, between PRE and POST during both p1 (+5.0%, $P = 0.3$) and p1.5 (+4.5%, $P = 0.5$). EXP showed higher $\dot{V}E$ responses compared to CON at POST at both power outputs ($P < 0.05$). Breathing pattern responses ($V_T$ and $f_R$) during p1 and p1.5 were significantly different between PRE and POST in EXP. At p1, $V_T$ decreased by 8.8% ($P = 0.01$) and $f_R$ increased by 18.0% ($P = 0.007$); whereas at p1.5, $V_T$ decreased by 8.5% ($P = 0.01$) and $f_R$ increased by 15.9% ($P = 0.02$). EXP showed lower $V_T$ ($P < 0.01$) and higher $f_R$ ($P < 0.001$) during both conditions compared to CON at POST. In EXP, HR increased from PRE to POST during both p1 (+18.5%, $P < 0.001$) and p1.5 (+19.3%, $P < 0.001$). EXP had higher HR responses compared to CON at POST during both exercise protocols ($P < 0.05$).

Absolute and relative $\dot{V}O_2$ values were similar across the conditions and between groups. The $\Delta[HHb]/\Delta\dot{V}O_2$ ratio
profiles for a representative EXP subject are presented in Figure 1c. In EXP, an apparent ‘overshoot’ (identified as a \( \Delta[Hb]/\Delta V\text{O}_2 \) ratio > 1.0) was observed during p1 \((P = 0.04)\) and p1.5 at POST \((P = 0.04)\). The normalized \( \Delta[Hb]/\Delta V\text{O}_2 \) ratio showed no significant increase between PRE and POST during p1 (+23.0%, \( P = 0.4 \)); whereas an increase was observed during p1.5 (+58.7%, \( P = 0.003 \)). No difference was noted in the normalized \( \Delta[Hb]/\Delta V\text{O}_2 \) ratio in CON \((P > 0.05)\).

****Table 2 about here****

DISCUSSION

Changes in the balance of \( \text{O}_2 \) delivery and muscle \( \text{O}_2 \) consumption reflect haemodynamic and metabolic responses to extreme physical challenges and influence the factors implicated in central and peripheral fatigue.\(^9\) Since both central and peripheral fatigue occur in runners after UTs,\(^6\)\(^-\)\(^8\) the aim of the present study was to investigate the balance between \( \text{O}_2 \) delivery to working muscles and muscle \( \text{O}_2 \) consumption using quantitative NIRS measurements in runners who had completed a 330-km eUT. EXP showed an increase in both absolute (p1, +16%; p1.5, +24.6%) and normalized (p1, +38.0%; p1.5, +27.9%) \( \Delta[Hb] \) values. Further, this was accompanied by an ‘overshoot’ in the normalized \( \Delta[Hb]/\Delta V\text{O}_2 \) ratio during exercise at p1 and p1.5 in EXP at POST and associated with a
significant 58.7% increase in normalized $\Delta[\text{HHb}] / \Delta\dot{\text{VO}}_2$ ratio between PRE and POST only during p1.5. The main results of the present study confirmed our hypothesis that an eUT would alter the balance between $\text{O}_2$ delivery to working muscles and muscle $\text{O}_2$ consumption, thus increasing the muscle deoxygenation profile of the runners.

A significant difference in the degree of muscle deoxygenation between the two conditions was noted (Figure 2). This observation is consistent with previous studies\textsuperscript{23,25} which showed that $\Delta[\text{HHb}]$ increases in an exercise intensity-dependent manner, implying that the magnitude of the release of $\text{O}_2$ by oxyhaemoglobin for aerobic energy production was higher during exercise at p1.5 than at p1 even in a fatigued state. Our data also demonstrate a significant decrease in the rate of the muscle oxygenation during both conditions after the eUT (Figure 2). Furthermore, TOI represents a dynamic balance of $\text{O}_2$ consumption and delivery.\textsuperscript{21} The observed decreased in the TOI data (Figure 4) are in line with [HHb] changes, revealing that muscle tissue oxygenation decreases significantly after the eUT at both workloads examined.

Although increased blood flow following both eccentric (100 squats with a load corresponding to 70% of body mass)\textsuperscript{17} and exhaustive stretch-shortening cycle (100 maximal, unilateral drop jumps)\textsuperscript{26} exercise have been reported, this is not the case in the present study. Rather, the lack of an increase in
pulmonary \( \bar{V}O_2 \) observed after the eUT (Table 2) suggests that 
\( \bar{V}O_2 \) is preserved in combination with an increase in [HHb] 
change. This would imply a decrease in local muscle blood 
flow, likely due to a microcirculatory dysfunction\(^{27}\) induced by 
the high concentric and (particularly) eccentric muscle loads 
during the eUT. Indeed, the activation patterns and types of 
muscle contraction (concentric and eccentric) characterizing 
UTs\(^6,7\) may have caused a greater muscle damage and a higher 
concentration of inflammatory markers. Further, mononuclear 
cell infiltration in damaged myocytes, the presence of multiple 
central nuclei in the muscle fibers as well as fiber swelling 
and/or fiber degeneration may be considered.\(^{27}\) All these 
aspects potentially altered muscular structure and function, 
likely influencing microcirculatory function\(^{27}\) and resulting in a 
significant increase in muscle deoxygenation after the eUT. 
The normalized \( \Delta[Hb]/\Delta \bar{V}O_2 \) ratio supports this statement. 
Although a significant increase in normalized \( \Delta[Hb]/\Delta \bar{V}O_2 \) 
ratio was observed only during exercise at p1.5 (+58.7\%, \( P = 
0.003 \)), there was a discernible ‘overshoot’ during exercise at 
both p1 and p1.5, suggesting a ‘non-optimal’ matching of \( O_2 \) 
delivery to \( O_2 \) utilization. To our knowledge, this is the first 
study to show changes in skeletal muscle oxygenation after an 
UT, limiting comparisons with literature reports.
Pulmonary $\dot{V}O_2$ did not change in either of the two exercise conditions between PRE and POST (Table 2). This result is in agreement with a recent finding from our laboratory that showed unchanged pulmonary $\dot{V}O_2$ under the same cycling condition after a 330-km eUT. The maintenance of pulmonary $\dot{V}O_2$, despite high muscle deoxygenation, suggests that ventilatory responses were not affected by the eUT-induced fatigue state. Of note, the higher HR values observed at POST did not influence either $\dot{V}O_2$ or local muscle blood flow dynamics. This is likely due to the fact cardiac output (that directly influences the leg blood flow) was maintained because the higher HR was counterbalanced by a decreased stroke volume.

Further, since the responses in pulmonary and muscle $\dot{V}O_2$ were different at POST in EXP, $\dot{V}O_2$ as an indicator of changes in muscle oxygenation during a fatiguing task appears to be inaccurate. This is likely due to a potential dissociation between pulmonary and muscle $\dot{V}O_2$, influenced by the systemic cardiovascular response to exercise and metabolic regulatory processes, respectively.

Finally, one of the main limitations of the present study is that subjects were not tested immediately after crossing the finish line. Despite efforts to conduct POST measures in a timely manner, there was a delay (24min 27s ± 5min 37s) in the
time between race completion and the start of POST testing procedures. Thus, the present results must be treated with caution since the magnitude of the changes was likely attenuated ~25 min POST-eUT. Another potential limitation is that the subjects could freely choose whether to run or walk during the eUT. However, this aspect is impractical, if not impossible, to control in a racing context. Thus, some differences in the results are expected depending on how long athletes run versus walk throughout the race. To control for the effects of potential biomechanical changes on the interpretation of our results, cycling was used so as to focus only on metabolic adaptations. However, future studies involving both treadmill running/walking and a larger number of participants of different performance levels should be undertaken to compare hemodynamic responses.

PRACTICAL APPLICATIONS

In the current study, the observed imbalance between $O_2$ delivery and muscle $O_2$ consumption in working muscles, i.e. muscle deoxygenation, was likely do to a microcirculatory dysfunction induced by high concentric and, particularly, eccentric muscle loads during the eUT. This highlights the importance of incorporating graded training, particularly downhill bouts, to reduce the negatively influence of concentric and severe eccentric loads to the microcirculatory function and
to enhance the ability of the UT runners to sustain such
loadings.

CONCLUSIONS

This is the first study to apply the NIRS technique to UT
runners to investigate the effects of extreme-duration exercise
on local muscle deoxygenation and gain insight into the
balance of O$_2$ delivery to working muscles and muscle O$_2$
consumption. The results showed that an UT is a sufficient
stimulus to promote an increase in local muscle deoxygenation,
thus reflecting an alteration in the balance between O$_2$ delivery
to working muscles and muscle O$_2$ consumption. This
mismatch is likely due to the concentric and eccentric loads
characterizing this eUT and negatively influencing
microcirculatory function.

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FIGURE AND TABLE CAPTIONS

**Table 1.** Physical characteristics for experimental (EXP, $n = 14$) and control (CON, $n = 12$) groups.

**Table 2.** Changes in the pulmonary gas exchange parameters for experimental (EXP, $n = 14$) and control (CON, $n = 12$) groups before (PRE) and after (POST) the race.

**Figure 1.** Profiles of changes in deoxyhemoglobin concentration ($\Delta[HHb]$, absolute ($\mu$M) and normalized (%) values, Panel a), oxygen uptake ($\dot{V}O_2$, absolute (L·min$^{-1}$) and normalized (%) values, Panel B) and the $\Delta[HHb]/\Delta\dot{V}O_2$ ratio (Panel C) during the three cycling conditions. Data are from a representative subject who took part in the race.

BL: baseline at 0.5 W·kg$^{-1}$

p1: cycling at 1 W·kg$^{-1}$

p1.5: cycling at 1.5 W·kg$^{-1}$

**Figure 2.** Average changes in deoxyhemoglobin concentration ($\Delta[O_2Hb]$) in absolute ($\mu$M) and normalized (%) values during cycling at p1 (1 W·kg$^{-1}$) and at p1.5 (1.5 W·kg$^{-1}$) in the athletes (EXP) and the control (CON) groups before (PRE) and after (POST) the race. Standard deviations were omitted for clarity.

* Significantly different from the corresponding PRE value ($P < 0.05$).
‡ Significantly different as compared with CON ($P < 0.05$).

# Significantly different from the corresponding p1 value ($P < 0.05$).

§ $P < 0.01$, significant group × time interaction.

**Figure 3.** Average changes in deoxyhemoglobin concentration ($\Delta[\text{HHb}]$) in absolute (µM) and normalized (%) values during cycling at p1 (1 W·kg$^{-1}$) and at p1.5 (1.5 W·kg$^{-1}$) in the athletes (EXP) and the control (CON) groups before (PRE) and after (POST) the race. Standard deviations were omitted for clarity.

* Significantly different from the corresponding PRE value ($P < 0.05$).

‡ Significantly different as compared with CON ($P < 0.05$).

# Significantly different from the corresponding p1 value ($P < 0.05$).

§ $P < 0.01$, significant group × time interaction.

**Figure 4.** Changes in tissue oxygenation index (TOI) during the first (1 W·kg$^{-1}$) and second (1.5 W·kg$^{-1}$) workload for athletes (EXP) and control (CON) group before (pre) and after (post) the race. Standard deviations were omitted for clarity.

* Significantly different from the corresponding pre value ($P < 0.05$).

‡ Significantly different compared to CON ($P < 0.05$).
# Significantly different from the corresponding 1 W·kg⁻¹ value

(P < 0.05).

§ P < 0.01, significant group × time interaction.
<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Stature (cm)</th>
<th>Body Mass (kg)</th>
<th>BMI (kg·m⁻²)</th>
<th>Adipose tissue thickness (mm)</th>
<th>p1</th>
<th>p1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>EXP</td>
<td>47.6±7.2</td>
<td>175±0.05</td>
<td>69.8±7.1</td>
<td>68.4±6.9</td>
<td>22.7±1.6</td>
<td>22.3±1.8</td>
</tr>
<tr>
<td>CON</td>
<td>32.7±7.1*</td>
<td>179±0.06</td>
<td>73.8±8.8</td>
<td>73.2±8.0</td>
<td>23.1±3.0</td>
<td>22.8±2.9</td>
</tr>
</tbody>
</table>

BMI, Body Mass Index; cycling workload at a power of 1 (p1) and 1.5 W·kg⁻¹ (p1.5). Data are presented as mean ± SD.

* Significantly different from EXP group (P < 0.05).
<table>
<thead>
<tr>
<th></th>
<th>EXP</th>
<th>CON</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
</tr>
<tr>
<td>p1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\dot{V}O_2$ (L·min$^{-1}$)</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>HR (beats·min$^{-1}$)</td>
<td>97.8 ± 7.8$</td>
<td>$</td>
<td>108.8 ± 8.3*$</td>
</tr>
<tr>
<td>$\Delta$ $\dot{V}O_2$ (%)</td>
<td>54.7 ± 25.2</td>
<td>58.6 ± 22.2</td>
<td>59.7 ± 24.9</td>
</tr>
<tr>
<td>$\Delta$[HHb]/$\Delta$ $\dot{V}O_2$ ratio</td>
<td>1.06 ± 0.02</td>
<td>1.15 ± 0.05$</td>
<td>$</td>
</tr>
<tr>
<td>p1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\dot{V}O_2$ (L·min$^{-1}$)</td>
<td>1.7 ± 0.2$</td>
<td>$</td>
<td>1.6 ± 0.3$</td>
</tr>
<tr>
<td>HR (beats·min$^{-1}$)</td>
<td>109.2 ± 7.4$</td>
<td>$</td>
<td>122.4 ± 8.9$</td>
</tr>
<tr>
<td>$\Delta$ $\dot{V}O_2$ (%)</td>
<td>76.5 ± 10.1$</td>
<td>$</td>
<td>65.8 ± 14.8$</td>
</tr>
<tr>
<td>$\Delta$[HHb]/$\Delta$ $\dot{V}O_2$ ratio</td>
<td>0.81 ± 0.03$</td>
<td>$</td>
<td>1.25 ± 0.02*$</td>
</tr>
</tbody>
</table>

$\dot{V}$ $O_2$, $O_2$ uptake; HR, heart rate; $\Delta$ $\dot{V}O_2$, normalized $O_2$ uptake; $\Delta$[HHb], normalized deoxyhaemoglobin; cycling workload at a power of 1 (p1) and 1.5 W·kg$^{-1}$ (p1.5). Data are presented as mean ± SD.

* Significantly different from the corresponding PRE value ($P < 0.05$)

‡ Significantly different compared to CON ($P < 0.05$)

# Significantly different from the corresponding p1 value ($P < 0.05$)

$ Significantly different from 1.0 ($P < 0.05$)
Average changes in deoxyhemoglobin concentration (Δ[O₂Hb]) in absolute (µM) and normalized (%) values during cycling at p1 (1 W·kg⁻¹) and at p1.5 (1.5 W·kg⁻¹) in the athletes (EXP) and the control (CON) groups before (PRE) and after (POST) the race. Standard deviations were omitted for clarity.

* Significantly different from the corresponding PRE value (P < 0.05).
‡ Significantly different as compared with CON (P < 0.05).
# Significantly different from the corresponding p1 value (P < 0.05).
§ P < 0.01, significant group × time interaction.
Average changes in deoxyhemoglobin concentration ($\Delta[\text{HHb}]$) in absolute ($\mu$M) and normalized (%) values during cycling at p1 (1 W·kg$^{-1}$) and at p1.5 (1.5 W·kg$^{-1}$) in the athletes (EXP) and the control (CON) groups before (PRE) and after (POST) the race. Standard deviations were omitted for clarity.

* Significantly different from the corresponding PRE value ($P < 0.05$).
‡ Significantly different as compared with CON ($P < 0.05$).
§ Significantly different from the corresponding p1 value ($P < 0.05$).
§§ $P < 0.01$, significant group $\times$ time interaction.

177x127mm (300 x 300 DPI)
Changes in tissue oxygenation index (TOI) during the first (1 W·kg⁻¹) and second (1.5 W·kg⁻¹) workload for athletes (EXP) and control (CON) group before (pre) and after (post) the race. Standard deviations were omitted for clarity.

* Significantly different from the corresponding pre value (P < 0.05).
‡ Significantly different compared to CON (P < 0.05).
# Significantly different from the corresponding 1 W·kg⁻¹ value (P < 0.05).
§ P < 0.01, significant group × time interaction.