scientific reports

Check for updates

OPEN

Time-course of muscle fatigue development during intense exercise in hypoxia and normoxia

Jacob Bejder[1], Jonathan Graae[1], Jakob Boye Andersen[1], Ricardo Augusto Barbieri[2], Eduardo Zapaterra Campos[3], Jens Bangsbo[1], Lars Nybo[1] & Nikolai Baastrup Nordsborg[1]✉

This study is the first to determine how hypoxia affects human muscle fatigue kinetics and metabolic perturbations during intense dynamic exercise. Using randomized, single-blinded crossover designs, three trials of two-legged knee extensions were performed under hypoxic (HYP, FiO_2 0.135) and normoxic (NOR) conditions. Trial 1 ($n = 8$): quadriceps femoris twitch force (F_{tw}) was measured before, during, and after 4 min intense exercise followed by exhaustive exercise. Maximal voluntary contraction (MVC) was measured pre- and post-exercise. Trial 2 ($n = 8$): muscle lactate and pH were determined before and after 4 min intense exercise. Trial 3 ($n = 6$): blood was sampled frequently from the femoral artery and vein during intense exhaustive exercise. Dynamic F_{tw} decreased more ($P < 0.05$) in HYP from 60s of exercise and onwards. After 4 min, isometric F_{tw} decreased more ($P < 0.05$) in HYP, whereas MVC was similar between conditions. At exhaustion, isometric F_{tw} and MVC were similar between conditions despite HYP exercise time being 55 ± 17% of NOR ($P < 0.01$). Muscle lactate and pH in- and decreased more ($P < 0.001$), respectively, after 4 min in HYP. Exercise-induced blood metabolites disturbances were largely unaffected by hypoxia. Conclusively, moderate hypoxia accelerated muscular fatigue from 60s and onwards. Hypoxia caused higher muscle but not blood lactate and H^+ accumulation rates.

Keywords Hypoxemia, Exercise performance, Peripheral muscle fatigue

Exercise in hypoxic environments is associated with reduced exercise capacity[1–5]. When exercising in moderate hypoxic exposure (inspired O_2 fraction (FiO_2) = 0.15) accelerated muscular fatigue development appears to be the primary cause of the compromised exercise capacity as opposed to the possible effects on the central nervous system[3]. Importantly, studies of human fatigue development in hypoxia have mostly applied an evaluation of muscle function before and after exercise but not during the task. Therefore, the present study determined the kinetics of human muscular fatigue development during intense exercise in hypoxia by applying a recently developed model to determine gradual peripheral muscular fatigue development during intense exercise[6]. This approach allows inference of possible underlying metabolic causes.

The impact of moderate hypoxia (F_iO_2 ~13%) on human muscular metabolism during constant-load exercise has not frequently been determined. It is clear from ^{31}P-MRS that two-legged intense exercise in a supine position accelerates PCr breakdown and proton accumulation in human muscle[7]. Thus, it appears likely that muscular lactate also accumulates faster during moderate hypoxic exposure. However, this has not been determined although well described in severe hypoxic conditions[8,9]. Therefore, exercise-induced shifts in muscle lactate and pH were measured in the present study.

Moreover, blood metabolite accumulation contributes to fatigue development. In particular, the combined effect of lactate, pH, and ATP shifts has been demonstrated to cause fatigue[10]. Hypoxia causes elevated blood lactate levels during graded cycling exercise[11] but it has not been determined whether this is also the case during intense constant-load dynamic exercise. Therefore, in the present study we determined changes in blood metabolite accumulation during intense exhaustive exercise in moderate hypoxia.

The aim of the present study was to evaluate the kinetics of changes in muscle contractile properties as well as the intra- and extracellular metabolic milieu in response to moderate hypoxia during dynamic intense exercise in humans. The hypothesis was that the gradual decline in muscle contractile function during constant-load,

[1]Department of Nutrition, Exercise and Sports (NEXS), Faculty of Science, University of Copenhagen, Nørre Allé 51, Copenhagen 2200, Denmark. [2]Department of Life Quality Studies, University of Bologna, Rimini, Italy. [3]Graduate Program in Physical Education, Federal University of Pernambuco, Recife, Brazil. ✉email: nbn@nexs.ku.dk

high-intensity dynamic exercise is faster under moderate hypoxia, and that the difference in fatigue development between normoxia and hypoxia is related to altered metabolism detectable in muscle and blood.

Methods

Design

The study consisted of three trials (Fig. 1). Trials 1 and 2 were completed by the same group of participants ($n=8$), while a separate group ($n=6$) completed trial 3. Each trial followed a randomized, counterbalanced, single-blind crossover design. Within each trial, participants were randomly assigned to either normoxia (NOR) or hypoxia (HYP) as their first condition. After completing the first condition, a washout period of at least four days elapsed before the participant crossed over to the opposite condition.

Following the completion of trial 1, at least 30 days elapsed before the same participants proceeded to trial 2. Meanwhile, trial 3 was conducted separately with a different group of participants. Throughout the study, all participants were instructed to maintain their usual diet and physical activity levels while avoiding caffeine, alcohol, and exercise for 24 h before each experiment. All tests were performed in a seated position, with each foot secured in a specially designed boot attached to a two-legged knee-extensor ergometer[12].

Participants

Eight regularly physically active and non-smoking healthy males with a mean ± SD age, weight and height of 24 ± 2 years, 83 ± 3 kg, and 185 ± 5 cm, respectively, participated in trials 1 and 2. Another six regularly physically active and non-smoking healthy males with a mean ± SD age, weight and height of 26 ± 1 years, 80 ± 4 kg and 180 ± 2 cm participated in trial 3. The local ethics committee of Copenhagen, Denmark, approved the applied protocols (H-1-2011-052, H-15000130 and H-A-2009-016), which were performed in accordance with the Declaration of Helsinki. All participants were informed both orally and in writing of potential risks and discomforts associated with participation before written informed consent was obtained.

Procedures

Familiarization for trials 1 and 2

Day 1: Initially, participants warmed up for 10 min at 20 W. Next, peak power output was determined during a graded two-legged knee-extensor exercise protocol starting at 60 W (60 rpm) and increasing by 12 W per minute

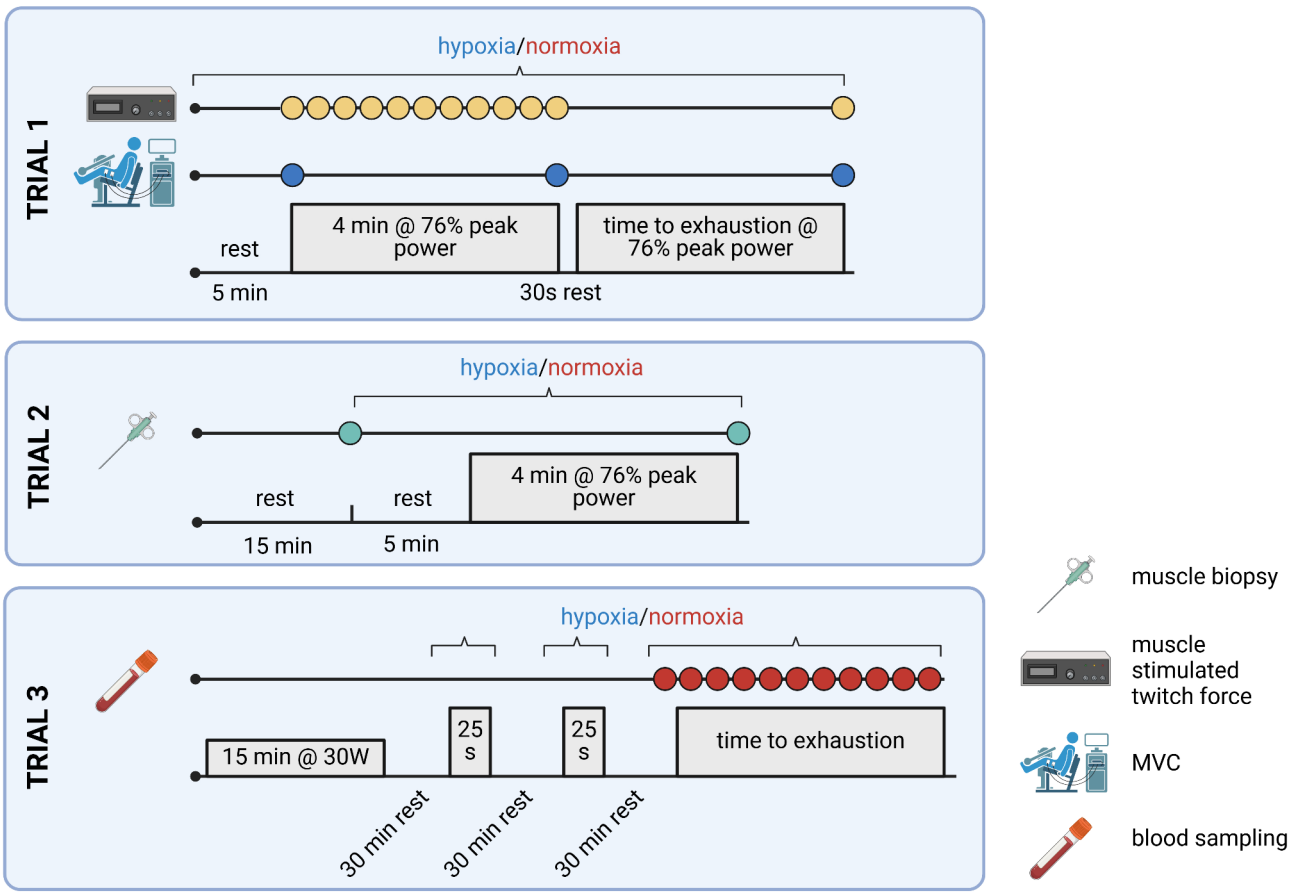

Fig. 1. Study overview. Illustration of the study design. MCV: maximal voluntary contraction.

until exhaustion. The test was terminated when the cadence was lower than 55 rpm for five seconds. The last completed workload was registered as peak power output.

Day 2: Participants were familiarized with subcutaneous electrical stimulation of m. quadriceps femoris as described in the *preparation* section of '*Trial 1*'. Subsequently, the participants performed a 4 min all-out exercise bout with the instruction to maintain the highest possible cadence at all times during the test. The initial workload corresponded to 80% of the peak power output recorded at familiarization day 1.

Day 3: Seated in the two-legged knee-extensor model, the participants inspired hypoxic air (F_iO_2 of 0.135, Hypoxico Altitude Training Systems, Everest Summit II, Hypoxico Inc., NY, USA). Following five minutes of hypoxic exposure the participants exercised at the mean power recorded during the 4 min all-out exercise on familiarization day 2. Exercise was continued until exhaustion, defined as a cadence below 55 rpm for 5 s. If time to exhaustion was not within 4–6 min, a fourth familiarization test day was conducted with an adjusted workload. This continued until a workload was determined where the time to exhaustion was within 4–6 min.

Familiarization for trial 3

The familiarization procedure for trial 3 is described elsewhere[13]. Briefly, three to five exercise tests similar to the main trial were performed on sepa rate days with the purpose of familiarizing the participants with the equipment and establishing experimental day workloads causing exhaustion within 3–6 min of exercising.

Trial 1

Preparation Self-adhesive 5 × 9 cm^2 electrodes with multistick gel (Model 895240, PALS platinum, Axelgaard, Lystrup, Denmark) were placed one-third proximally and another one-third distally over m. vastus lateralis of the right leg for transcutaneous muscle stimulation. The electrodes were connected to a constant-current stimulator (maximum voltage 400 V, DS7A, Digitimer, Hertfordshire, UK), which was connected to a train/delay generator (DG2A, Digitimer, Hertfordshire, UK). This enabled the triggering of a double stimulus square-wave pulse with a width of 1 ms and an interpulse interval of 10 ms. The signal was recorded using LabChart7 Pro (ADInstruments, Dunedin, New Zealand).

Following preparation, participants were seated in the two-legged knee-extensor model and strapped across thorax and pelvis to minimize movement during testing. The muscle was then stimulated with increasing intensity until the twitch force plateaued, which was recorded at 1 kHz by a calibrated strain gauge (Model 615, Tedea-Huntleigh Electronics, UK) attached to the heel of the boot. The maximal intensity plus 20% was used in the experiments.

Exercise protocol The participants inspired normoxic (F_iO_2 = 0.209) or hypoxic (F_iO_2 = 0.135) air at rest for five minutes followed by three 5 s maximal voluntary contraction (MVC) force measurements (F_{MVC}). During F_{MVC} determination, the knee was fixed in a 90° angle and force was recorded using a strain gauge. Five seconds after the maximal contraction the m. quadriceps femoris was stimulated to determine resting isometric potentiated twitch force ($F_{tw,iso}$). F_{MVC} and $F_{tw,iso}$ were determined as the average of the measurements obtained.

The participants then performed two-legged knee-extension for 4 min at the workload determined during the final familiarization day. The m. quadriceps femoris was stimulated 5, 30, 60, 90, 120, 150, 180, 210, and 235 s into the exercise period to investigate the time course of fatigue development evaluated as changes in potentiated twitch force during dynamic exercise ($F_{tw,dyn}$). The stimulation was delivered in the final part of the muscle relaxation, i.e., when the leg moves backwards. Identical timing of stimulation during exercise and between trials was secured by an automated switch. A 5 s MVC was determined immediately following exercise. Five seconds after the maximal contraction the m. quadriceps femoris was stimulated to determine resting $F_{tw,iso}$.

Following a 30 s break, the participants exercised at the same workload at 60 rpm until exhaustion defined as a cadence below 55 rpm for five seconds. Finally, F_{MVC} and $F_{tw,iso}$ were determined immediately after exhaustion. The placement of electrodes was marked before removal to ensure similar placement in the crossover experiment.

Trial 2

The participants rested 15 min in a supine position followed by collection of a muscle biopsy from m. vastus lateralis under local anesthesia using the Bergström needle[14]. The participants were then seated in the knee-extensor model and mounted with a facemask providing a F_iO_2 of 0.209 (NOR) or 0.135 (HYP). Following 5 min of rest, a 4 min two-legged knee-extensor task was completed at the workload determined during the final familiarization day. A second biopsy was collected immediately after the 4 min exercise bout. Furthermore, blood oxygen saturation was measured immediately prior and post the exercise test using finger pulse-oximetry (CMS-50E, Contec Medical Systems CO., Ltd).

Trial 3

At rest, catheters were placed in the femoral artery and vein under local anesthesia – see elsewhere for details[13]. Next, the participants performed 4 bouts of two-legged exercise, i.e., 15 min at 30 W, two 25 s bouts and a time to exhaustion test, which were separated by 30 min of rest. The workload for the latter three bouts were determined at familiarization and caused exhaustion within 3–6 min. Here we report the results from the time to exhaustion trial. The results for the first three exercise bouts are reported elsewhere[13]. Arterial blood samples were collected at rest, 10 s before and at the onset of exercise as well as 10, 17, 20, 30, 50, 110, 230, 350 and 470 s into the exercise bout and at exhaustion. Venous blood samples were collected at rest, 5 s before exercise as well as 3, 6, 9, 12, 15, 20, 40, 60, 120, 240 and 360 s into the exercise bout and at exhaustion.

Blood gas and metabolite measurements
Arterial and venous samples were collected in heparinized syringes and stored on ice (< 60 min) until analysis of pH, lactate concentration, hemoglobin concentration, oxygen saturation and oxygen partial pressure (ABL800, Radiometer, Copenhagen, Denmark) as well as K^+ concentration (Hitachi 912, Boehringer Mannheim).

Muscle biopsies
Muscle biopsies were frozen immediately upon collection in liquid nitrogen and stored at -80˚C until analyzed. After freeze-drying, the muscle samples were dissected free of blood, fat, and connective tissue and ~ 1 mg dry weight tissue was extracted in a solution of 0.6 M perchloric acid and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M KHCO3 and analyzed for lactate by a flurometric assay[15]. Muscle pH was measured by a small glass electrode (Radiometer GK2801, Copenhagen, Denmark) after homogenizing ~ 2 mg dry weight freeze-dried muscle sample in a non-buffering solution containing 145 mM KCl, 10 mM NaCl and 5 mM iodoacetic acid. After having adjusted pH of the sample to 7.1 with 0.01 M NaOH, the sample was titrated to pH 6.5 by serial additions of 0.01 M HCl. The pH was measured after each addition. The non-HCO3 physiochemical buffer capacity was determined from the number of moles of hydrogen required to change pH from 7.1 to 6.5 and was expressed as millimoles hydrogen per kilogram dry weight per pH[16].

Statistics
SPSS (IBM SPSS Statistics Corp, New York, USA, v27) was used for statistical analyses and differences were considered significant if $P \leq 0.05$. To assess the effect of hypoxia, a mixed linear model for repeated measurements was used for all variables, except for time to exhaustion in trial 2 and blood variables at exhaustion in trial 3, which was compared by a paired t-test. The mixed model included time, treatment, and time × treatment as fixed factors, and repeated measures over time were considered by including a random effect of participant. If a fixed main effect was significant, a post hoc analysis was performed using a Sidak adjusted pairwise comparison. The results are expressed as mean ± SD.

Results
In trial 1 and 2, the participants' age, height and weight were 24 ± 2 years, 83 ± 3 kg, and 185 ± 5 cm, respectively. The average two-legged exercise power output was 118 ± 15 W, corresponding to 76% of the recorded peak power output. Furthermore, the average F_iO_2 was 0.135 ± 0.002 and 0.209 ± 0.003 during the HYP and NOR trials, respectively. Accordingly, the oxygen saturation was lower ($P < 0.001$) in HYP compared with NOR prior (88 ± 1 vs. 97 ± 2%) and immediately after (86 ± 2 vs. 98 ± 1%) the four min exercise. In trial 3, the participants' age, height and weight were 26 ± 1 years, 80 ± 4 kg and 180 ± 2 cm. The average two-legged workload was 100 ± 6 W, and the oxygen saturation was lower ($P < 0.01$) in HYP (92 ± 3%) than NOR (98 ± 0%).

Trial 1: Muscle performance, maximal voluntary and stimulated contraction force
Time to exhaustion, evaluated 30 s after the initial 4 min exercise period, was shorter ($P < 0.01$) in HYP compared with NOR (185 ± 101 s vs. 353 ± 173 s, respectively), corresponding to 55 ± 17% of time in NOR.

When evaluating muscle contractile properties during dynamic exercise, a main effect was apparent for treatment ($P < 0.001$) and time ($P < 0.001$) for $F_{tw,\,dyn}$. Specifically, $F_{tw,\,dyn}$ was similar 5 s and 30 s into the exercise between treatments, but a larger decrease in $F_{tw,\,dyn}$ was present in HYP from 60 s ($P < 0.05$) and $F_{tw,\,dyn}$ remained lower in HYP than NOR for the remainder of the trial (Fig. 2).

Evaluation of isometric contractile properties before and after the exercise tests revealed a main effect of treatment ($P < 0.05$) and time ($P < 0.001$) for $F_{tw,\,iso}$. At rest $F_{tw,\,iso}$ was similar between trials, but the decrease during the 4 min exercise bout was larger ($P < 0.05$) in HYP than in NOR (Table 1). However, $F_{tw,\,iso}$ was similar between treatments at exhaustion.

A main effect of time ($P < 0.001$) existed for F_{MVC}. F_{MVC} which was similar between HYP and NOR at rest, following the 4 min exercise and at exhaustion (Table 1).

Trial 2: Muscle lactate, pH, and buffer capacity
A main effect of treatment ($P < 0.05$) and time ($P < 0.001$) existed for muscle lactate concentration. The pairwise comparison revealed that the muscle lactate was similar between treatments at rest (HYP: 5.6 ± 3.2; NOR: 5.3 ± 2.3 mmol × kg dry wweight^{-1}) but following the 4 min exercise bout, muscle lactate was higher ($P < 0.001$) in hypoxia (HYP: 84.5 ± 39.1, NOR: 69.5 ± 35.6 mmol × kg dry weight^{-1}).

A main effect of time ($P < 0.001$) was evident for muscle pH. In the post hoc analysis, muscle pH was similar at rest (HYP: 7.39 ± 0.10, NOR: 7.35 ± 0.10), but lower ($P < 0.001$) in HYP than NOR following the 4 min exercise bout (HYP: 6.94 ± 0.22, NOR: 7.04 ± 0.28).

Finally, no main effects existed for muscle buffer capacity, revealing a similar buffer capacity at rest (HYP: 142 ± 21, NOR: 143 ± 7 H^+ × kg dry weight^{-1} × pH^{-1}) and following the 4 min exercise period (HYP: 133 ± 27, NOR: 140 ± 8 H^+ × kg dry weight^{-1} × pH^{-1}, respectively) between treatments.

Trial 3: Blood metabolites during intense exercise
Time to exhaustion was shorter ($P < 0.05$) in HYP (267 ± 113 s) than in NOR (378 ± 137 s) and HYP corresponded to 73 ± 19% of time to exhaustion in NOR.

Both arterial oxygen saturation and partial pressure was reduced ($P < 0.001$) in HYP compared to NOR (Fig. 3). An effect of time ($P < 0.001$) but not treatment existed for arterial and venous lactate concentration (Fig. 4). However, post-hoc paired t-test revealed arterial lactate concentration was higher in HYP at 30 s ($P < 0.05$), 50 s ($P < 0.01$) and tended to be higher at 230 s ($P = 0.06$). Likewise, venous lactate concentration (Fig. 4) was higher in HYP at 40 s ($P < 0.05$), 60 s ($P = 0.07$) and 240 s ($P < 0.05$). A likely explanation for the lack

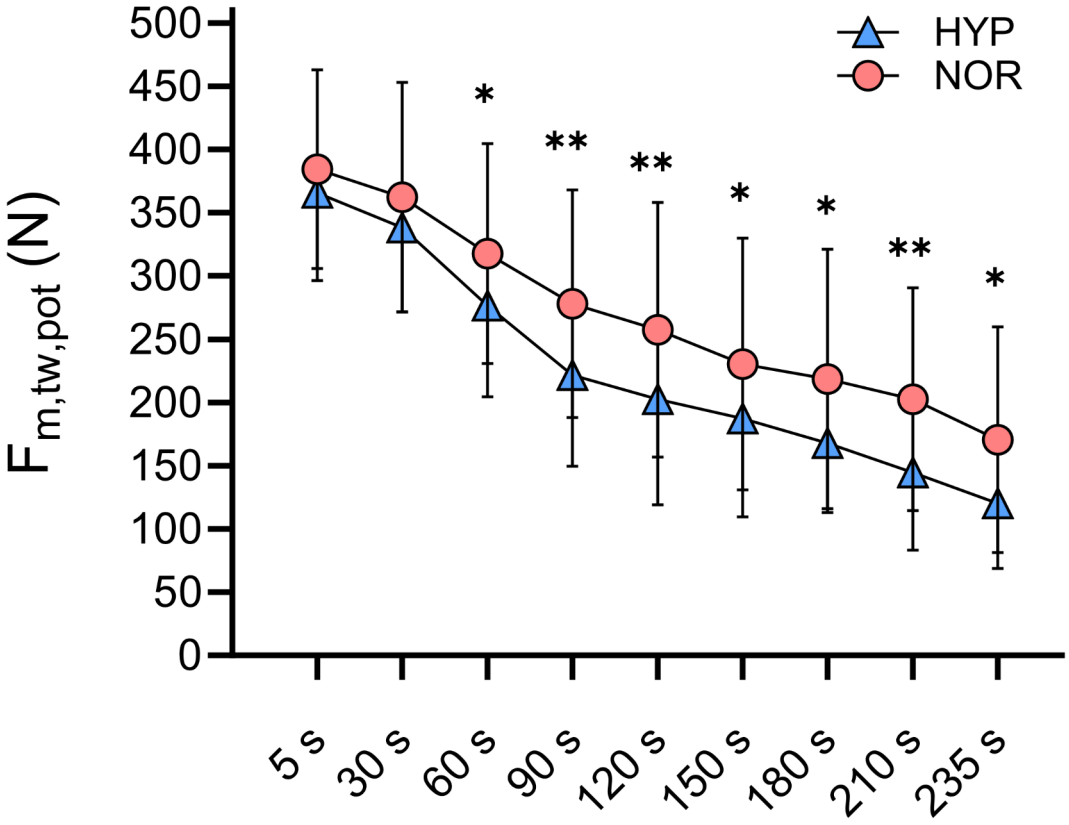

Fig. 2. Muscle contractile properties. Potentiated twitch force at m. vastus lateralis stimulation ($F_{m, tw, pot}$) during a four-min all-out kicking exercise in hypoxia (HYP) and normoxia (NOR). Significant differences are denoted by: $^{*}P<0.05$, $^{**}P<0.01$ between hypoxia and normoxia.

	Normoxia			Hypoxia		
	Pre exercise	Post 4 min	Post TTE	Pre exercise	Post 4 min	Post TTE
F_{MVC} (N)	665 ± 78	493 ± 116	410 ± 115	675 ± 131	485 ± 141	426 ± 130
$F_{tw, iso}$ (N)	248 ± 38	207 ± 42	170 ± 39	237 ± 22	181 ± 31*	161 ± 31

Table 1. Maximal voluntary contraction force (F_{MVC}) and potentiated twitch force during M.scle stimulation ($F_{tw, iso}$) of M. vastus lateralis before (pre) exercise, following a four-minute intense exercise bout (4 M.n) and after a time to exhaustion (TTE) trial completed in hypoxia and normoxia. Significant differences are denoted by: $^{*}P<0.05$ between hypoxia and normoxia.

of interaction but apparent pairwise effect is that participants exercised for different durations in HYP and NOR. Consequently, a given exercise time represents a different percentage of total exercise duration and thereby a difference in metabolic perturbation. At exhaustion, arterial, venous, and arterial-venous difference for lactate concentration and oxygen content of the blood was similar between conditions (Table 2).

An effect of time ($P<0.001$) was present for arterial and venous [K^+]. The pairwise comparison revealed a higher ($P<0.05$) arterial [K^+] after 230 s of exercise and a higher ($P<0.01$) venous [K^+] after 240 s of exercise in HYP compared to NOR (Fig. 3). Furthermore, an effect of time ($P<0.001$) and treatment ($P<0.01$) existed for arterial pH, whereas only an effect of time ($P<0.001$) existed for venous pH. A higher ($P<0.05$) arterial pH was evident 10 s before exercise as well as 10 s ($P<0.05$) and 230 s ($P<0.01$) into the exercise and at exhaustion ($P<0.05$) in HYP compared to NOR.

Discussion

The main findings were that moderate hypoxic exposure exacerbated peripheral fatigue development of m. quadriceps after 60 s of intense two-legged knee-extensor exercise and onwards. Accordingly, potentiated isometric contractile twitch force was lower after 4 min exercise in hypoxia compared to normoxia. Also, muscle contractile properties were similar at the point of exhaustion in hypoxia and normoxia despite the hypoxia trial lasting substantially shorter. Exacerbated peripheral fatigue after 4 min of exercise in moderate hypoxia

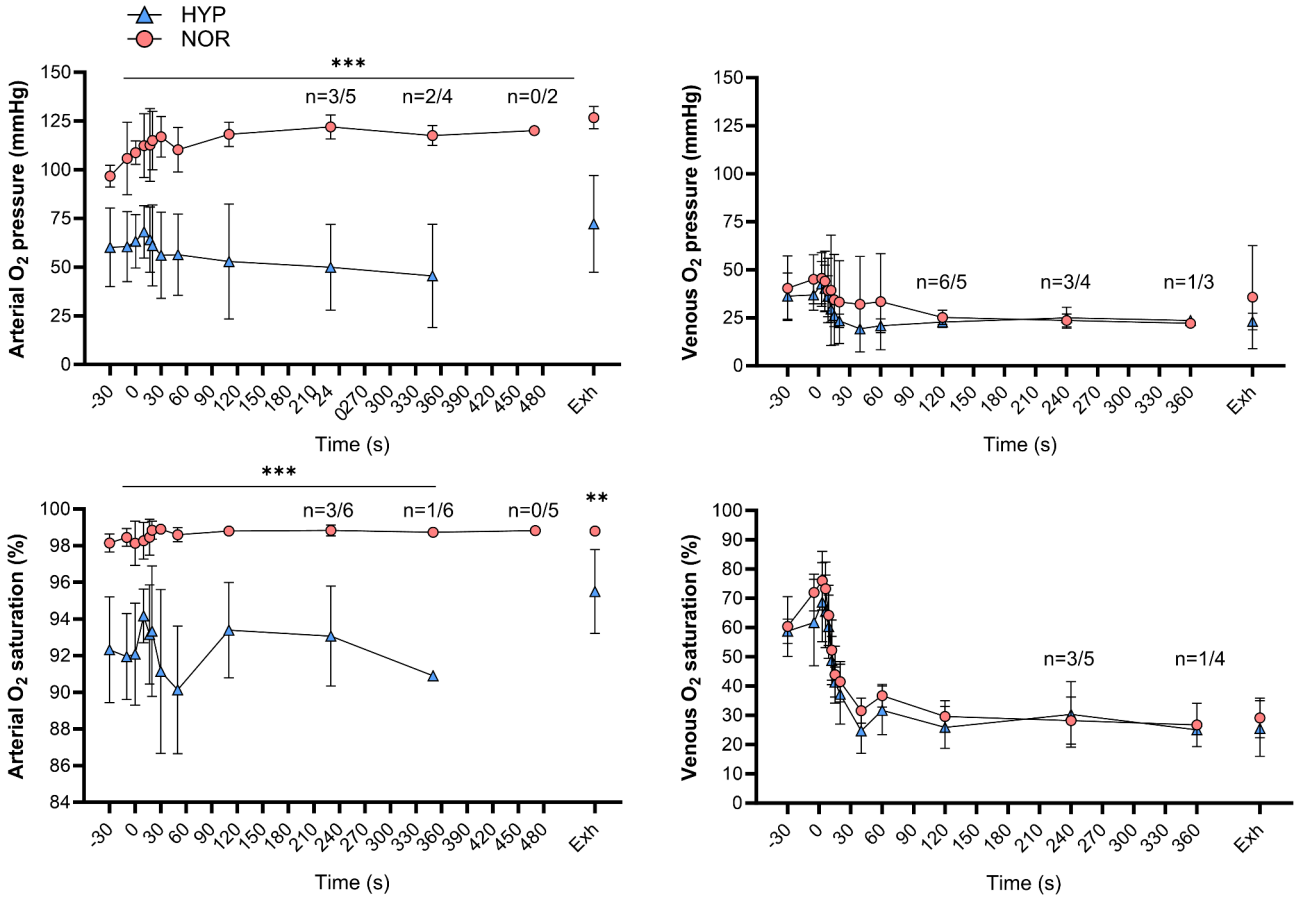

Fig. 3. Vascular oxygenation. Femoral artery and vein O_2 pressure and saturation during the time to exhaustion exercise in hypoxia (HYP) and normoxia (NOR). Number of subjects (n) is given for HYP/NOR when less than 6. Significant differences are: ***$P < 0.001$ between hypoxia and normoxia treatments. Exh: Exhaustion.

coincided with a higher muscle lactate accumulation and lower pH as compared with normoxia. Exercise-induced disturbances in blood metabolites were largely unaffected by the investigated conditions.

The kinetics of muscle fatigue during exercise in moderate hypoxia

Here, we provide the first detailed description of the kinetics in peripheral muscle fatigue during dynamic high-intensity exercise in hypoxia. A gradual reduction in muscular twitch response to electrical stimulation during dynamic exercise was apparent from the onset of exercise. Specifically, the peripheral muscular contractility, measured by direct muscle stimulation, was unaffected by hypoxia during the initial 30 s of exercise, but accelerated muscle fatigue manifested from 60 s and onwards when compared with the normoxic trial. Notably, the exacerbated peripheral fatigue during hypoxia does not appear to gradually increase after ~60 s as the difference in $F_{tw,\,dyn}$ was ~57 N after 90 s of exercise and was maintained between 44 N and 57 N for the remainder of the 4 min exercise period. In accordance with our previous findings, the majority of the loss in muscle function during constant-load dynamic exercise occurred within 25% of the time to exhaustion[6]. Thus, the mechanisms causing gradual fatigue development during dynamic exercise appear initiated from the onset of exercise whereas the moderate hypoxia induced exacerbation of fatigue development occurs from ~60 s of exercise.

While hypoxia clearly induced accelerated loss of muscle function during the 4 min exercise bout, F_{MVC} determined after the initial 4 min of exercise was not detected to be different between conditions. A similar pattern has been demonstrated previously[5] albeit the F_{MVC} did tend to decrease more with hypoxia in that study. The reason for the apparent discrepancy between $F_{tw,\,dyn}$ and F_{MVC} is unclear but likely involves factors affecting the interaction between peripheral and central fatigue development.

At exhaustion, muscular contractility ($F_{tw,\,iso}$ and F_{MVC}) was similar between conditions despite the reduced exercise time in hypoxia. These observations indicate that a lower limit for loss of muscle contractile function as a result of intense exercise exists.

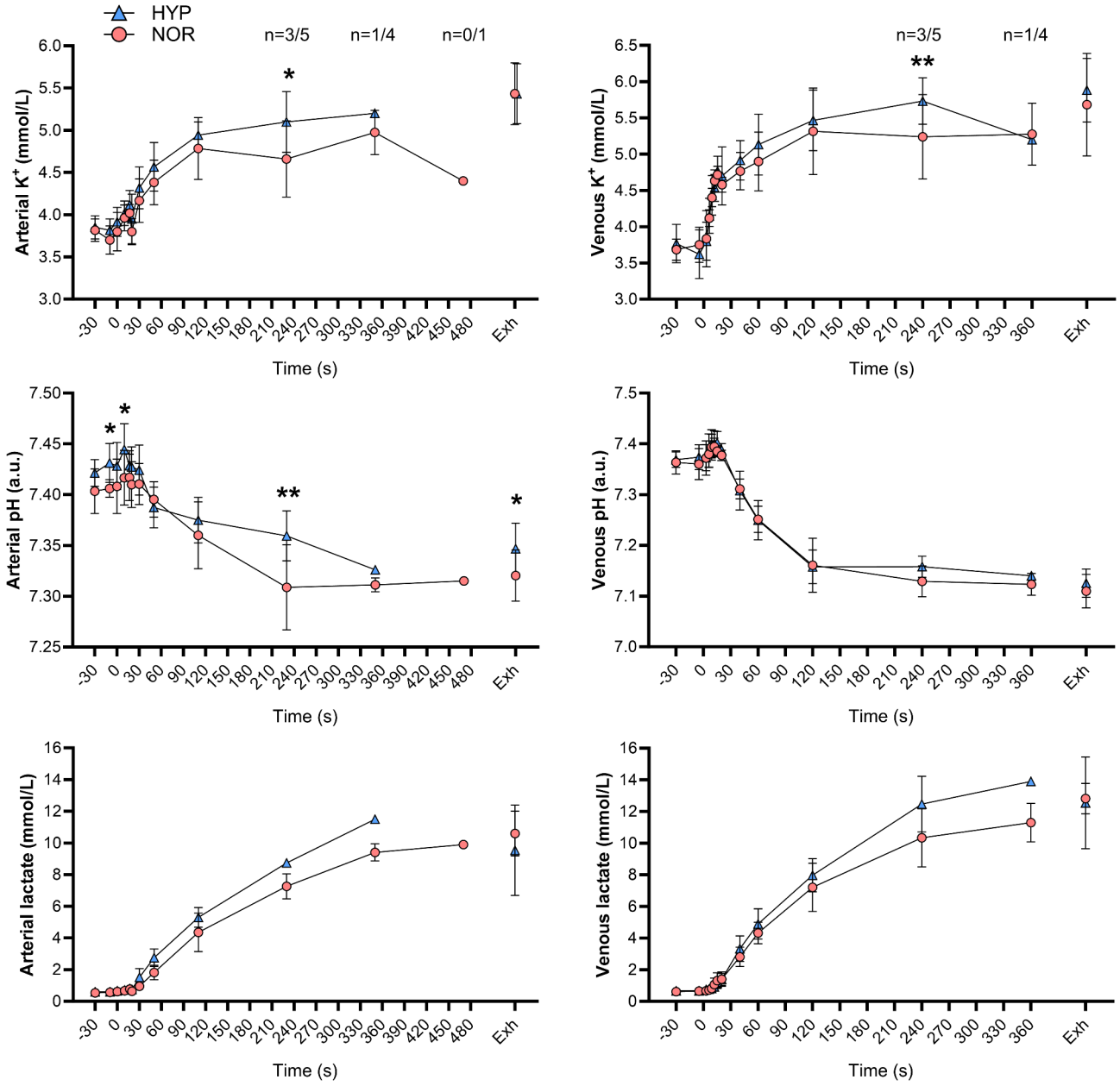

Fig. 4. Vascular metabolic milieu. Potassium (K^+), pH and lactate concentration in the femoral artery and vein during the time to exhaustion exercise in hypoxia (HYP) and normoxia (NOR). Number of subjects (n) is given for HYP/NOR when less than 6. Significant differences are: $^*P<0.05$, $^{**}P<0.01$ between hypoxia and normoxia treatments. Exh: Exhaustion.

	Normoxia	Hypoxia
Lactate, arterial (mM)	10.6 ± 1.4	9.5 ± 2.9
Lactate, venous (mM)	12.8 ± 1.0	12.5 ± 2.9
Lactate arterial-venous difference	2.5 ± 2.5	3.0 ± 3.0
Arterial oxygen content (ml/l)	197 ± 12	192 ± 12
Venous oxygen content (ml/l)	58 ± 16	51 ± 21
Oxygen content arterial-venous difference	139 ± 12	141 ± 15

Table 2. Femoral arterial and venous lactate and oxygen content at exhaustion of intense two-legged knee extensor exercise in normoxia or hypoxia.

Metabolic milieu associated with muscle fatigue during exercise in moderate hypoxia

During high-intensity exercise in hypoxia the muscular metabolism is shifted to a more anaerobic profile within the first few minutes, evidenced by accelerated muscle metabolic perturbations measured as the rate of phosphocreatine degradation and inorganic phosphate accumulation[7]. In accordance, muscle lactate concentration was higher and muscle pH was lower after four minutes of high-intensity exercise in moderate hypoxia compared to normoxia in the present study. The current study is the first to evaluate muscular lactate accumulation in human skeletal muscle 4 min into constant load exhaustive dynamic exercise where exhaustion occurs after ~4–6 min in hypoxia and normoxia, respectively. Our findings align with studies demonstrating higher muscle lactate concentration after moderate intensity exercise in severe hypoxia[8,17]. The present observation of higher muscular lactate accumulation in hypoxic conditions supports that hypoxia induces a higher anaerobic energy production during intense exercise.

The increased reliance on anaerobic energy production during exercise in hypoxia likely results from reduced oxygen availability to contracting muscle fibers due to decreased arterial oxygen content. Exercise modality appears to be a key determinant of how hypoxic exposure affects metabolic responses. If the reduction in arterial oxygen content is compensated by an increase in blood flow, oxygen delivery to the contracting fibers can be maintained. However, this occurs at the cost of a reduced mean transit time, which may impair oxygen diffusion. In our previous work, we reported that two-legged knee extensions, as performed in the present study, did not elicit a compensatory increase in leg blood flow during the initial two minutes of exercise[13]. In contrast, when a smaller muscle mass is engaged—such as in one-legged knee extensions—a clear compensatory increase in leg blood flow has been observed in hypoxia[18,19]. When a larger muscle mass is recruited, the severity of hypoxia appears to be a critical factor. In severe hypoxia ($FiO_2 = 0.11$), a compensatory increase in blood flow has been reported, whereas this response was absent in mild hypoxia ($FiO_2 = 0.16$)[20]. Thus, in the present study, the increased reliance on anaerobic metabolism appears to be a direct consequence of reduced arterial oxygen content, which was not offset by a compensatory increase in leg blood flow. Moreover, as a compensatory mechanism, oxygen extraction was increased[13] which resulted in a constant oxygen uptake. The compromised diffusion conditions in hypoxia present a challenge for oxygen delivery to individual muscle fibers. To compensate for this, fibers may rely on less efficient oxygen utilization as indicated by the increase in lactate production and possibly also compensate by recruiting additional fibers.

In a temporal perspective, lactate accumulates gradually during exercise and is associated with continued reduction in muscle pH[7]. Although both lactate and H^+ accumulates in muscle tissue during intense exercise, an important difference exists in the initial phase. Initially, muscle pH increases for up to 10 s and a reduction below resting levels only becomes apparent after >60 s of exercise[7]. The modest perturbations in muscular pH and lactate shifts in the initial phase of exercise (<60 s) thus appear unlikely determinants of the difference in fatigue development between hypoxia and normoxia at ~60 s of exercise in the present study. A likely explanation for the observed difference in muscle contractility from 60 s and onwards is accelerated PCr breakdown. This suggestion is based on the observations that ~50% of the change in PCr breakdown and P_i accumulation is evident after ~60 s and accelerated in hypoxic conditions[7].

Systemic changes in metabolite levels also play a role in fatigue development[10]. Although rapid initial changes in plasma K^+ occurred, leveling off was evident after a few minutes of exercise. Moreover, plasma K^+ accumulation in the initial phase of exercise was not affected by hypoxia. Thus, K^+ accumulation may have influenced the initial fatigue development but does not appear to explain the accelerated fatigue during hypoxic exposure. Regarding pH, mild systemic alkalosis was apparent at the onset of exercise in hypoxia, most likely caused by elevated ventilation. Also, in the initial phase of exercise, blood lactate accumulation was limited. Thus, blood metabolite shifts appear unlikely factors to explain the initial loss of muscle function during intense constant-load exercise. Likewise, shifts in blood metabolites also appear an unlikely causative factor in fatigue acceleration during exercise in moderate hypoxia because this manifested ~60 s into exercise where arterial blood pH was still within resting levels while venous values were ~7.2. Moreover, the lactate concentration only reached ~5 mM in venous blood after ~60 s of exercise.

The present study investigated muscle fatigue kinetics under mild hypoxia during exercise involving greater muscle mass than single-joint contractions but less than for example cycling. Muscle fatigue has been studied for centuries and arises from both central and peripheral mechanisms[21,22]. Hypoxia impairs muscle function through both pathways. In cycling, knee extension, and repeated-sprint protocols under reduced oxygen availability (FiO_2: 0.10–0.16), time to exhaustion decreases and force output declines[2]. Central mechanisms, such as a ~25% reduction in cerebral oxygen delivery and decreased motor cortex activation, play a key role[23]. However, peripheral fatigue is equally significant, with potentiated twitch force reductions of 39% in hypoxia versus 24% in normoxia, indicating impaired contractility[24]. In the present study, the kinetic evaluations revealed peripheral fatigue to emerge after 60 s of intense exercise, suggesting P_i accumulation as a key factor, since pH is likely unchanged at this stage. After 4 min, the increased lactate accumulation and pH decline suggests that multiple interacting factors contribute to the performance loss in hypoxia. Central fatigue development is possibly also of importance but that was not assessed here.

Limitations

This study has some limitations that should be considered when interpreting the findings. First, the sample size was relatively small, which may reduce statistical power, particularly for detecting subtle physiological effects. However, our use of a linear mixed model with restricted maximum likelihood estimation helped mitigate the impact of missing data and maximize the use of available measurements. Additionally, the observed differences in e.g. potentiated twitch force during exercise suggest that the study had sufficient power to detect meaningful effects. Second, only male participants were included in the study, which limits the generalizability of our findings to female athletes or the broader population.

Summary

Moderate hypoxic exposure exacerbated peripheral muscular fatigue after ~60 s of intense constant load two-legged exercise. Hypoxia also caused higher muscle lactate concentrations and lower muscle pH after 4 min of exercise. Blood metabolite shifts were largely unaffected by hypoxia except for a slightly increased lactate accumulation towards the end of exercise.

Based on temporal considerations, it is suggested that hypoxia-induced acceleration of fatigue development is related to accelerated PCr breakdown and P_i accumulation. However, this requires further investigation.

Data availability

Data is available upon reasonable request to the corresponding author.

Received: 15 November 2024; Accepted: 14 April 2025
Published online: 23 April 2025

References

1. Amann, M. et al. Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans. *J. Physiol.* **575**, 937–952. https://doi.org/10.1113/jphysiol.2006.113936 (2006).
2. Amann, M. et al. Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *J. Appl. Physiol.*. **101**, 119–127. https://doi.org/10.1152/japplphysiol.01596.2005 (2006).
3. Amann, M., Romer, L. M., Subudhi, A. W., Pegelow, D. F. & Dempsey, J. A. Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *J. Physiol.* **581**, 389–403. https://doi.org/10.1113/jphysiol.2007.129700 (2007).
4. Calbet, J. A. et al. Determinants of maximal oxygen uptake in severe acute hypoxia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **284**, R291–303. https://doi.org/10.1152/ajpregu.00155.2002 (2003).
5. Romer, L. M., Haverkamp, H. C., Lovering, A. T., Pegelow, D. F. & Dempsey, J. A. Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290**, R365–375. https://doi.org/10.1152/ajpregu.00332.2005 (2006).
6. Rannou, F., Nybo, L., Andersen, J. E. & Nordsborg, N. B. Monitoring muscle fatigue progression during dynamic exercise. *Med. Sci. Sports. Exerc.* **51**, 1498–1505. https://doi.org/10.1249/mss.0000000000001921 (2019).
7. Vanhatalo, A. et al. Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *J. Physiol.* **589**, 5517–5528. https://doi.org/10.1113/jphysiol.2011.216341 (2011).
8. Green, H. J. et al. Altitude acclimatization and energy metabolic adaptations in skeletal muscle during exercise. *J. Appl. Physiol.*. **73**, 2701–2708. https://doi.org/10.1152/jappl.1992.73.6.2701 (1992).
9. Katz, A. & Sahlin, K. Effect of decreased oxygen availability on NADH and lactate contents in human skeletal muscle during exercise. *Acta Physiol. Scand.* **131**, 119–127. https://doi.org/10.1111/j.1748-1716.1987.tb08213.x (1987).
10. Amann, M., Sidhu, S. K., Weavil, J. C., Mangum, T. S. & Venturelli, M. Autonomic responses to exercise: group III/IV muscle afferents and fatigue. *Auton. Neurosci.* **188**, 19–23. https://doi.org/10.1016/j.autneu.2014.10.018 (2015).
11. Nordsborg, N. B. et al. Human muscle net K(+) release during exercise is unaffected by elevated anaerobic metabolism, but reduced after prolonged acclimatization to 4,100 m. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299**, R306–313. https://doi.org/10.1152/ajpregu.00062.2010 (2010).
12. Andersen, P., Adams, R. P., Sjøgaard, G., Thorboe, A. & Saltin, B. Dynamic knee extension as model for study of isolated exercising muscle in humans. *J. Appl. Physiol.*. **59**, 1647–1653. https://doi.org/10.1152/jappl.1985.59.5.1647 (1985).
13. Christensen, P. M. et al. Thigh oxygen uptake at the onset of intense exercise is not affected by a reduction in oxygen delivery caused by hypoxia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **303**, R843–849. https://doi.org/10.1152/ajpregu.00201.2012 (2012).
14. Bergström, J. Muscle electrolytes in man. *Scand. J. Clin. Lab. Invest.* **68**, 1–110 (1962).
15. Lowry, O. H. & Passonneau, J. V. Some recent refinements of quantitative histochemical analysis. *Curr. Probl. Clin. Biochem.* **3**, 63–84 (1971).
16. Mannion, A. F., Jakeman, P. M. & Willan, P. L. Determination of human skeletal muscle buffer value by homogenate technique: methods of measurement. *J. Appl. Physiol.*. **75**, 1412–1418. https://doi.org/10.1152/jappl.1993.75.3.1412 (1993).
17. Parolin, M. L. et al. Regulation of glycogen phosphorylase and PDH during exercise in human skeletal muscle during hypoxia. *Am. J. Physiol. Endocrinol. Metab.* **278**, E522–534. https://doi.org/10.1152/ajpendo.2000.278.3.E522 (2000).
18. DeLorey, D. S., Shaw, C. N., Shoemaker, J. K., Kowalchuk, J. M. & Paterson, D. H. The effect of hypoxia on pulmonary O_2 uptake, leg blood flow and muscle deoxygenation during single-leg knee-extension exercise. *Exp. Physiol.* **89**, 293–302. https://doi.org/10.1113/expphysiol.2003.026864 (2004).
19. Gonzalez-Alonso, J., Richardson, R. S. & Saltin, B. Exercising skeletal muscle blood flow in humans responds to reduction in arterial Oxyhaemoglobin, but not to altered free oxygen. *J. Physiol.* **530**, 331–341. https://doi.org/10.1111/j.1469-7793.2001.0331l.x (2001).
20. Koskolou, M. D., Calbet, J. A., Rådegran, G. & Roach, R. C. Hypoxia and the cardiovascular response to dynamic knee-extensor exercise. *Am. J. Physiol.* **272**, H2655–2663. https://doi.org/10.1152/ajpheart.1997.272.6.H2655 (1997).
21. Allen, D. G., Lamb, G. D. & Westerblad, H. Skeletal muscle fatigue: Cellular mechanisms. *Physiol. Rev.* **88**, 287–332. https://doi.org/10.1152/physrev.00015.2007 (2008).
22. Gandevia, S. C. Spinal and supraspinal factors in human muscle fatigue. *Physiol. Rev.* **81**, 1725–1789. https://doi.org/10.1152/physrev.2001.81.4.1725 (2001).
23. Mira, J. et al. Neuromuscular fatigue of cycling exercise in hypoxia. *Med. Sci. Sports. Exerc.* **52**, 1888–1899. https://doi.org/10.1249/mss.0000000000002331 (2020).
24. Romer, L. M. et al. Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R598–606. https://doi.org/10.1152/ajpregu.00269.2006 (2007).

Acknowledgements

We sincerely thank all subjects for participating in the study, Jens Jung Nielsen (Department of Nutrition, Exercise and Sports) for technical assistance as well as all the students affiliated with various parts of the protocol.

Author contributions

NBN, LN, JBa conceived and designed the study. NBN, LN, JBA, RAB, EZC performed the experiments. NBN, JB, JG analyzed and interpreted the results. JB, JG prepared figures and drafted the manuscript. All authors edited and revised the manuscript and approved the final version.

Funding
No funding was received for the present study. The authors declare no conflicts of interest.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to N.B.N.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.