**Man#6715-2020**

**Title:** Short-term perceptually regulated interval-walk training in hypoxia and normoxia in overweight-to-obese adults

**Heading title:** Perceptually regulated interval walks in hypoxia

**Abstract**

***Objectives:*** We compared the effects of short-term, perceptually regulated interval-walk training in hypoxia *vs.* normoxia on health outcomes in overweight-to-obese individuals.

***Design:*** Between-participants repeated measures.

***Methods*:** Sixteen adults(body mass index = 33 ± 3 kg.m-2) completed eight interval-walk training sessions (15 × 2 min walking at a rating of perceived exertion of 14 on the 6-20 Borg scale; rest = 2 min) either in hypoxia (FiO2 = 13.0%) or normoxia during two weeks.

***Results*:** Treadmill velocity did not differ between conditions or over time (*p* > 0.05). Heart rate was higher in hypoxia (+10 ± 3%; *p* = 0.04) during the first session, with no changes within condition across the training sessions (*p* > 0.05). Similarly, arterial oxygen saturation was lower in hypoxia than normoxia (83 ± 1% *vs*. 96 ± 1%, *p* < 0.05), but did not vary over time (p > 0.05). After training, perceived mood state (+11.8 ± 2.7%, *p* = 0.06) and exercise self-efficacy (+10.6 ± 4.1%, *p* = 0.03) improved in both groups. Body mass (*p* = 0.55), systolic and diastolic blood pressure (*p* = 0.19 and 0.07, respectively) and distance covered during a 6-min walk test (*p* = 0.11) did not change from pre- to post-tests.

***Conclusions*:** Eight perceptually regulated interval-walk training sessions with or without hypoxia had no effect on exercise-related sensations, health markers and functional performance. Hypoxic conditioning is not recommended to modify some cardiometabolic risk factors and improve exercise tolerance in overweight-to-obeseindividuals, at least over a short training period.

**Key words:** Obesity; Hypoxic conditioning; Perceptually regulated exercise; Cardio-metabolic health; Interval training.

**Introduction**

In recent years, there has been a surge in the use of hypoxic conditioning (HC) as a strategy for promoting health and weight loss in individuals with obesity (Hobbins et al. 2017; Ramos-Campo et al. 2019). When comparing to normoxic, constant-load exercise training programmes (60–90 min walking/running, cycling or cross-training at 60–75% maximal oxygen uptake [V̇O2max] or heart rate [HRmax]), HC (inspired fraction of oxygen or FiO2 = 13–16.5%) for 4–8 weeks elicits further reductions in body mass and fat mass (Netzer et al. 2008; Wiesner et al. 2009), along with improvements in blood glucose concentrations (Haufe et al 2008; De Groote et al. 2018), blood pressure (Kong et al. 2014) and exercise capacity (Chacaroun et al. 2020). Comparatively, perceptual responses and exercise-related sensations associated with this type of training have so far been overlooked.

Early weight loss success (within 2–4 weeks of initiating an intervention) is a prominent predictor of exercise adherence (Burgess et al. 2017). The great majority of HC studies involving individuals with obesity have implemented a training duration of 4–8 weeks (Hobbins et al. 2017; Ramos-Campo et al. 2019). Reportedly, similar improvements in V̇O2max and mean blood pressure occurred in adults with obesity following completion of twelve, 60-min cycling sessions at constant intensity (65% of relative V̇O2max) in hypoxia (FiO2 = 15.0%) over 2 weeks compared to the same number of sessions spread over 4 weeks in normoxia (Morishima et al. 2015). Therefore, achieving similar (or better) results in a shorter time frame may improve the confidence and likelihood of obese individuals in adhering to regular exercise.

Perceptually regulated exercise intensity is defined as the trade-off between maintaining target intensity such as rating of perceived exertion (RPE) by self-adjusting the absolute intensity (i.e., velocity or power output) (Tucker, 2009). Previously, it has been suggested that this mode of exercise may be considered more enjoyable than absolute fixed-intensity exercise (Hobbins et al. 2017; Ramos-Campo et al. 2019). Interestingly, available HC studies have typically implemented a continuous, fixed-intensity of exercise (60–75% V̇O2max),1,2 which may prove detrimental in terms of exercise adherence and other indicators of exercise satisfaction and pleasure (Burgess et al. 2017).

Therefore, our intention was to compare the effects of a short-term (eight, 60-min sessions over two weeks) perceptually regulated (RPE = 14) interval training intervention (15 × 2 min walking, 2 min of rest) in hypoxic and normoxic conditions on exercise-related sensations, cardio-metabolic markers, and functional performance in overweight-to-obeseindividuals. It was hypothesised that health outcomes will be improved after training in hypoxia compared to normoxia, despite a lower training workload (i.e., slower treadmill walking velocity) as a result of controlling exercise intensity perceptually.

\*\*\* Table 1 near here \*\*\*

**Methods**

Male and female adults were recruited to participate in this study from a University staff population of approximately 1700 individuals. Inclusion criteria required that participants were sedentary (<1 h of moderate-intensity exercise/week), did not smoke, had no current or recent (within 3 months) musculoskeletal injury or recent URT infection. Recruited participants were also classified in the BMI range of 27–35 kg.m-2 (overweight: 25-29.9; obesity: 30+) and had not been exposed to hypoxic conditions within the previous 6 months prior to the start of the study (see Table 1 for anthropometric data of the sample). Written informed consent was obtained from all participants. This study was carried out in accordance with the *Declaration of Helsinki*. Ethical approval was received from the School of Applied Sciences Ethics Committee (SAS1822).

This study compared two separate groups of adults who were classified as overweight or obese, whereby, half of the participants completed their sessions in hypoxic conditions (FiO2 = 13.0%, equivalent to ~3500 m elevation above sea level, HYP; n=8), and the other half completed their sessions in normoxic conditions (sea level, NOR; n=8). Participants were randomly allocated to training conditions through simple randomization. Participants completed eleven separate visits across three consecutive weeks and were instructed to maintain their normal daily habits in terms of activity, diet, social and sleep patterns. The first session (visit 1) consisted of eligibility determination, familiarisation with the measures and treadmill walking (Pulsar, h/p/cosmos, Germany), and identification of the walking velocity associated with a RPE of 14, as described previously (Hobbins et al. 2019). Within 72 h, they returned to the lab for pre-tests (visit 2), which consisted of assessment of anthropometrics (body mass and stature), physiological responses (blood pressure), exercise-related sensations (perceived mood change and exercise self-efficacy) and functional fitness (6-min walk test). After 24–72 h, participants undertook the first of eight supervised 60-min self-paced interval-walk sessions (visits 3–10), which were completed within a 2-wks period. Participants were asked to continue their current diet and daily physical activity during the intervention. Within 72 h of the final perceptually regulated interval walking session, participants returned to the lab (visit 11) for post-tests (identical test battery than pre-tests). Testing and training environments were maintained at 23°C and 45% relative humidity.

Participants completed eight supervised 60-min (15 × 2 min walking at RPE = 14 with 2 min of rest) perceptually regulated interval-walk sessions across two consecutive weeks in a commercial gym (Academy of Sport, London South Bank University). Each session began with a 5-min warm up at 3.0 km.h-1 on the treadmill (Fusion Run Series3, Pulse Fitness, UK). A facemask connected to a portable hypoxic generator (*see below*) was then attached and remained for the entire session. During all sessions, the first 30 s of each 2-min interval began at participants’ perceptually regulated walking velocity (RPE = 14). Following this, participants were able, every 30 s, to decide if and how treadmill velocity needed to be altered (i.e., increased or decreased by 0.5, 1.0 or 1.5 km.h-1, or maintained) to ensure maintenance of an RPE of 14 whilst walking.

Participants wore a facemask (Altitude Training Mask, Hypoxico Altitude Training Systems, USA) connected *via* corrugated plastic tubing to a hypoxic generator (Everest Training Summit II, Hypoxico Altitude Training Systems, USA) to create hypoxic conditions. The FiO2 provided in this study was 13.0% (simulated altitude of ~3500 m), while the total hypoxic exposure corresponded to exactly 480 min for those in the HYP group.

Treadmill velocity, HR (M400, Polar, Finland) and arterial oxygen saturation (SpO2)(iHealth Air, iHealthLabs, USA) were recorded every 30 s during interval walking. Before each session, perceived recovery was assessed in response to a numeric scale, ranging from 0 being ‘*very poorly recovered*’ to 10 being ‘*very well recovered*’ (Laurent et al. 2011). Perceived motivation was assessed *via* a 20 cm visual analog scale, with 0 being ‘*not very motivated*’ (white colored) and 20 being ‘*very motivated*’ (black colored) (Crewther et al. 2016). Immediately after each training session, perceived breathlessness and limb comfort were determined in response to a numeric scale ranging from 0 being ‘*nothing at all*’ to 10 being ‘*very, very severe*’ (Ward and Whipp, 1989), whilst perceived pleasure was assessed *via* a 20-cm visual analog scale ranging from 0 being ‘*not very pleasant*’ (white colored) and 20 being ‘*very pleasant* ’(black colored).

Participants arrived at the lab following an 8-h fasting period (water exempt). Stature and body mass, and subsequently BMI, were assessed using an electric stadiometer (220, Seca GmbH, USA). After 10 min of rest, participants were asked ‘*how are you feeling right now?’* and instructed to verbally specify a number on an 11-point scale anchored ‘*very bad*’ (-5) up to ‘*very good*’ (+5) for perceived mood state (Hardy and Rejeski, 1989). Exercise self-efficacy was determined by participants completing six items, 11-point Likert scales (Smith et al. 2012). Blood pressure (systolic and diastolic) was assessed *via* an automated pressure cuff (Omron M4, Omron, Japan) attached, secured and inflated around the upper arm.

Participants completed a standardised warm up (5 min at 3 km.h-1) before 6 min of perceptually regulated continuous walking in normoxic conditions. The treadmill velocity was set at 50% of their self-selected walking velocity, and participants stepped on whilst the velocity was increased to their velocity associated with an RPE of 14 within 10 s. Participants were instructed to ‘*walk as far as possible in six minutes without running or jogging*’. They were able to increase/decrease (by 0.5, 1.0 or 1.5 km/h-1) or maintain the current velocity every 30 s. The total distance covered during the 6 min was determined (Gibson et al. 2015).

Preliminary analysis (paired-sample, equal variance *t*-test) was carried out to determine whether pre-tests measurements were statistically significantly different between HYP and NOR. If statistical differences were found, data collected during and post-training were normalized to the pre-training measurement. Velocity, HR and SpO2 were averaged across each 60-min session. Velocity, HR and exercise-related sensations recorded during sessions 2–8 were calculated as a percentage change from session 1 (100%) due to differences in the initial velocity deemed equal to RPE 14 between HYP and NOR.

Data are presented as mean ± standard deviation. A *t*-test was used to determine any statistically significant differences in the absolute velocity, HR, SpO2, and perceived recovery, motivation, breathlessness, limb discomfort and pleasure values (averaged across the session) during session 1. A two-way repeated-measures ANOVA was used to investigate the main effect of condition (hypoxia *vs.* normoxia), time (pre-training *vs.* post-training or session 1 *vs.* 2, 3, 4, 5, 6, 7 and 8) and the condition × time interaction. A *Bonferroni* post hoc multiple comparison was performed if a significant main effect was observed. Effect-sizes were described in terms of partial eta-squared (ηp², with ηp²≥0.06 representing a moderate effect and ηp²≥0.14 a large effect). All statistical calculations were performed using SPSS statistical software (IBM Corp., Armonk, NY, USA). The significance level was set at *p* < 0.05.

\*\*\* Figure 1 and Table 2 near here \*\*\*

**Results**

Treadmill velocity did not differ between conditions or over time (i.e., session 1: 6.5 ± 0.3 *vs*. 6.3 ± 0.6 km.h-1; *p* > 0.05, Figure 1A; Table 2). During the first session, HR was higher in HYP *vs.* NOR (144 ± 16 *vs*. 129 ± 20 bpm; +10 ± 3%; *p* = 0.04; Table 2). While this difference persisted between conditions there were no changes within condition across the training sessions (*p* > 0.05, Figure 1B). Similarly, SpO2 was lower during HYP *vs.* NOR (83 ± 1% *vs.* 96 ± 1%, *p* < 0.05), but did not vary over time (*p* > 0.05, Figure 1C; Table 2).

Perceived recovery was lower prior to session 4 (76 ± 16%, *p* < 0.01) and 7 (75 ± 19%, *p* < 0.01) compared to session 1, irrespective of condition (Figure 2A). Perceived motivation was lower prior to session 2 (75 ± 12%), 3 (69 ± 20%), 4 (69 ± 14%), 5 (77 ± 16%), 7 (73 ± 17%) and 8 (71 ± 20%) compared to session 1, irrespective of condition (*p* < 0.04, Figure 2B). Perceived breathlessness, limb discomfort and pleasure did not change (*p* > 0.05, Figure 2C–E; Table 2).

Body mass, body mass index, systolic and diastolic blood pressures and functional fitness did not change (*p* > 0.05, Table 1). Exercise self-efficacy (+7 ± 5%, *p* = 0.03) improved from pre- to post-tests, irrespective of condition (*p* > 0.05). Despite failing to reach statistical significance, perceived mood state (+12 ± 2%, *p* = 0.06) followed a similar trend.

\*\*\* Figure 2 near here \*\*\*

**Discussion**

The aim of the current study was to investigate whether the addition of hypoxia during short-term, perceptually regulated (RPE = 14) interval walking training in adults with obesity leads to similar (or superior) improvements in psycho-physiological responses. During the training, treadmill velocity and perceptual responses did not differ between conditions, despite hypoxia-induced elevations in physiological strain (i.e., higher heart rate and lower SpO2). From pre- to post-training perceived mood state improved in both groups. However, body mass, BMI, blood pressure and functional fitness did not change.

Over the course of our intervention, treadmill velocity that was self-selected by our participants to maintain a RPE of 14 did not differ either between conditions or across sessions. Despite similar external workload in both groups, higher HR and lower SpO2 were measured during session 1 in HYP *vs.* NOR, and this internal load due to hypoxia exposure persisted during all remaining sessions. When training three times per week for 3–4 weeks, lower workload (-7–28%) during cycling (Haufe et al. 2008; Pramsohler et al. 2017) or running(Wiesner et al. 2009) exercise modes in moderate hypoxia (FiO2 = ~15.0%) produced similar HR values compared to normoxia. In the aforementioned studies, training included 30–60 min of continuous exercise at a fixed, moderate intensity. In our study, the lack of a difference for treadmill velocity between conditions may be due to our original approach of maintaining a constant RPE target during 2-min interval walking workouts.

We further observed that neither external (treadmill velocity) nor internal load (HR, SpO2) metrics changed during the course of the eight training sessions. Contrastingly, Fernández Menéndez et al. (2018) showed that preferred walking speed (corresponding to RPE ~10) in obese adults became progressively faster over the course of a 3-wks walking intervention in both hypoxic and normoxic training groups, despite selection of a ~7% slower velocity in hypoxia than normoxia. In the present study when exercise intensity is perceptually regulated (RPE = 14) during interval walking workouts, internal and external loads metrics remained unchanged from the first to the eighth training session over the course of the 2-wks intervention. Comparing this to the findings of Fernández Menéndez et al. (2018), this may be explained by the interval nature of the exercise carried out in the current study and the rest periods allowing for appropriate recovery and initiation of exercise in the next interval at a constant perceptually regulated intensity.

Because walking velocities did not differ between conditions, one would expect that exercise-related sensations to be negatively impacted in HYP compared to NOR in the presence of lower SpO2 and higher HR readings. One interesting finding, however, was that none of the perceptual measures were negatively affected by the addition of moderate hypoxia. Conversely, higher difficulty breathing and limb discomfort readings were reported by Soo et al. (2020) when completing repeated cycle sprints (8 × 5-s sprints, 25 s of rest) and by Hobbins et al. (2019) during perceptually regulated (RPE = 16), high-intensity intermittent runs (4 × 4-min, 3 min of rest) in deprived-O2 conditions (FiO2 = 13–15%). Jefferies et al. (2019) reported progressive arterial hypoxemia (lower SpO2) and increases in ventilation as primary cues as an explanation for a shorter time to exhaustion during a cycling task (clamped at RPE 16) in severe hypoxia (FiO2 = 11.4%) than normoxia. This suggests that more severe hypoxia levels than those used in this study (FiO2 = 13.0%) may be required to observe a negative influence on exercise-related sensations. The nature of our perceptually regulated exercise, involving short (2 min) exercise intervals, followed by similar recovery duration, may also explain why we failed to observe apparent differences between conditions in perceptual variables. Importantly, decreases in perceived recovery and motivation (i.e., already visible after the initial session) occurred across sessions, irrespective of environmental condition. A possible explanation would be the large number of training sessions in a short-time frame. This is an important consideration for implementation since, as described by Ekkekakis and Lind (2006), exercise-related sensations are important for adherence to regular exercise training.

Total body mass, BMI and functional fitness did not change in response to either interventions. Previously, larger (Kong et al. 2014; Netzer et al. 2008) and similar improvements(Gatterer et al. 2015)of body composition have been found after training in hypoxic than normoxic conditions. Similarly, gains in functional fitness were larger after HC compared to the normoxic equivalent training (Haufe et al. 2008; Wiesner et al. 2009). In adults with obesity, a 2-wks training block may not be long enough to elicit positive changes in body composition and functional fitness, unlike the aforementioned studies implementing longer training periods (4–6 weeks) yet with a similar number (8–12) of training sessions (Haufe et al. 2008; Kong et al. 2014; Netzer et al. 2008). Other anthropometric measures not assessed in the current study (i.e., waist: hip ratio, fat mass and muscle mass) that are pertinent for improved body composition should be assessed in future investigations. In our study, blood pressure remained unchanged throughout the protocol. Perhaps a greater training dose (i.e., longer weekly duration) may be required in normotensive individuals (as recruited here) to positively impact on exercise capacity and cardio-metabolic health when in hypoxia compared to normoxia (Navarrete-Opazo and Mitchell, 2014). That said, despite greater improvement in exercise tolerance, HC (hypobaric hypoxia with a target SpO2 of 80%) thrice weekly for 8 weeks was not associated with larger improvement in either body composition or vascular and metabolic functions in overweight-to-obeseindividuals compared to normoxic equivalent (Chacaroun et al. 2020).

Irrespective of condition, perceived mood change and exercise self-efficacy improved from pre- to post-tests. To our knowledge, no investigation exists that has compared exercise-related sensations before and after HC in a similar population. In obese individuals, fixed-intensity interval training (60 × 8-s sprint at 90% V̇O2max/12 s recovery) including 20 sessions over 5 weeks in normoxia was perceived as being more enjoyable and easier compared to moderate-intensity, continuous training (40 min at 65% V̇O2max) (Kong et al. 2016). In our study, implementation of interval exercise at a perceptually regulated intensity, causing higher internal but similar external load levels between conditions, may have mitigated the potential onset of hypoxic-induced negative mood (Lane et al. 2004). Overall, similar improvements in perceived mood change and exercise self-efficacy result from perceptually regulated interval walking in HYP and NOR.

**Conclusion**

In overweight adults, or those with obesity, eight perceptually regulated interval-walk sessions over 2-wks training period led to similar treadmill velocity and perceptual responses between HYP and NOR, despite hypoxia-induced elevations in physiological strain (i.e., higher heart rate and lower SpO2). While both interventions improved exercise-related sensations, significant weight loss, and change in blood pressure and functional performance were not achieved from either training condition. Hypoxic conditioning does not appear to modify some cardiometabolic risk factors and improve exercise tolerance in overweight-to-obese adults, at least over a short training period.

**Limitations**

Limitations of this study include both the relatively small sample size and the approach to simple randomization increasing the likelihood of unequal distribution of participants (Kim & Shin 2014). This appears evident in both the mean age of the participants in the hypoxic and normoxic groups (32.1 and 41.1 years, respectively) and the assessment of baseline functional fitness (670 *vs*. 613 m, respectively). That being said, changes in functional fitness from pre- to post- the intervention did not differ between groups (hypoxic >1.5% and normoxic 1.0%). This implies that the ability to determine the impact of age on change in functional fitness is not evident. Rather the duration of the intervention was the key limiting factor in determining the impact of training under hypoxic compared to normoxic conditions. This suggests that adherence to exercise for longer than a 2-wks period is required if the beneficial effects of exercise in hypoxia *vs*. normoxia are to be realized.

**Key points**

• We compared the effects of a 2-wks (8 sessions) perceptually regulated interval-walk intervention in hypoxia versus normoxia in overweight-to-obese adults.

• Despite stronger hypoxia-induced physiological stimulus – yet essentially similar walking speeds – during training, psychological and physiological measures did not differ either between conditions or across sessions.

• Hypoxic conditioning does not appear to ameliorate exercise-related sensations, cardio-metabolic markers and functional performance, at least over a short training period.

• Adherence to exercise for longer than a 2-wks period is likely required if the beneficial effects of exercise in hypoxia *vs*. normoxia are to be realized.

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**Figure 1** -Changes in velocity (A), heart rate (B) and arterial oxygen saturation (C) during the interval walking workouts.

*Velocity and heart rate from sessions 2–8 are calculated as change from session 1. All data are presented as mean ± SD. ANOVA main effects of time, condition and interaction are stated along with partial-eta squared into brackets. \* denotes a statistically significant difference (p < 0.05) between hypoxiaand normoxia.*

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**Figure 2** - Changes in perceived recovery (A) and motivation (B), breathlessness (C), limb discomfort (D) and pleasure (E) assessed before and after the interval walking workouts.

*Data from sessions 2–8 are calculated as a percentage difference from session 1 (100%) and presented as mean ± SD. ANOVA main effects of time, condition and interaction are stated along with partial-eta squared into brackets. \* denotes a statistically significant difference (p < 0.05) for a given session in reference to session 1.*

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| **Table 1** Effect of a 2-wks exercise training program in hypoxia or normoxia on anthropometrics, blood pressure and functional fitness. |
|  |  | **Hypoxia** | **Normoxia** | **ANOVA *p* value (effect size)** |
| **Parameters** | **Pre-tests** | **Post-tests** | **Pre-tests** | **Post-tests** | **Condition** | **Time** | **Interaction** |
|  | Gender | 4 Males, 4 Females | 5 Males, 3 Females | - | - | - |
|  | Age (years) | 32.1 ± 10.2 | 41.1 ± 13.0 |
|  | Stature (m) | 1.7 ± 0.9 | 1.7 ± 0.1 |
| Body mass (kg) | 92.2 ± 12.0 | 91.7 ± 11.9 | 95.5 ± 9.5 | 95.5 ± 10.0 | 0.55 (0.05) | 0.45 (0.08) | 0.56 (0.05) |
| Body mass index (kg/m-2) | 31.9 ± 3.6 | 32.6 ± 3.6 | 33.0 ± 1.4 | 32.0 ± 2.0 | 0.75 (0.02) | 0.21 (0.21) | 0.72 (0.02) |
| Systolic blood pressure (mmHg) | 119 ± 8 | 117 ± 14 | 132 ± 14 | 125 ± 17 | 0.19 (0.23) | 0.22 (0.20) | 0.40 (0.10) |
| Diastolic blood pressure (mmHg) | 77 ± 9 | 74 ± 6 | 84 ± 8 | 81 ± 7 | 0.07 (0.39) | 0.10 (0.34) | 0.88 (0.01) |
| Functional fitness (m) | 670 ± 43  | 680 ± 72 | 613 ± 88 | 618 ± 102 | 0.11 (0.31) | 0.58 (0.05) | 0.74 (0.02) |
| Data presented as mean ± SD.  |

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| **Table 2** Velocity, HR, SpO2 and perceived recovery, motivation, breathlessness, limb discomfort and pleasure during session 1 (averaged across the session). |
|  | **Condition** |  |
| **Parameter** | **HYP** | **NOR** | ***t*-test *p* value** |
| Velocity (km/h-1) | 6.5 ± 0.3 | 6.3 ± 0.6 | 0.23 |
| HR (bpm) | 144 ± 16 | 129 ± 20 | 0.04 |
| SpO2 (%) | 83.2 ± 0.9## | 95.7 ± 0.7 | 0.01 |
| Perceived recovery (au) | 8.4 ± 1.6 | 8.1 ± 1.9 | 0.38 |
| Perceived motivation (au) | 13.8 ± 1.8## | 16.4 ± 2.3 | 0.01 |
| Perceived breathlessness (au) | 2.5 ± 1.3 | 2.3 ± 1.4 | 0.28 |
| Perceived limb discomfort (au) | 3.3 ± 2.1 | 2.4 ± 1.7 | 0.15 |
| Perceived pleasure (au) | 12.4 ± 1.5# | 14.3 ± 3.5 | 0.07 |
| Data presented as mean ± SD. HR = heart rate, HYP = hypoxic condition, NOR = normoxic condition, SpO2 = arterial oxygen saturation. ## denotes a statistically significant difference (*p* ≤ 0.05) *versus* NOR, # denotes a statistically significant trend (*p* ≤ 0.07) *versus* NOR. |

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