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Inspiratory muscle training at sea level improves the strength of inspiratory muscles during load carriage in cold-hypoxia.

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ABSTRACT

Inspiratory muscle training (IMT) and functional IMT (IMT_F: exercise-specific IMT activities) has been unsuccessful in reducing respiratory muscle fatigue following load carriage. IMT_F did not include load carriage specific exercises. Fifteen participants split into two groups (training and control) walked 6 km loaded (18.2 kg) at speeds representing $\sim 50\% \text{VO}_{2\text{max}}$ in cold-hypoxia. The walk was completed at baseline; post 4 weeks IMT and 4 weeks IMT_F (five exercises engaging core muscles, three involved load). The training group completed IMT and IMT_F at a higher maximal inspiratory pressure (P_{imax}) than controls. Improvements in P_{imax} were greater in the training group post-IMT (20.4%, $p = 0.025$) and post-IMT_F (29.1%, $p = 0.050$) compared to controls. Respiratory muscle fatigue was unchanged ($p = 0.643$). No other physiological or subjective measures were improved by IMT or IMT_F. Both IMT and IMT_F increased the strength of respiratory muscles pre-and-post a 6 km loaded walk in cold-hypoxia.

Keywords

Environmental physiology, respiratory muscle fatigue, functional training, inspiratory muscle training

Practitioner summary:

To explore the interaction between inspiratory muscle training (IMT), load carriage and environment, this study investigated 4 weeks IMT and 4 weeks functional IMT on respiratory muscle strength and fatigue. Functional IMT improved inspiratory muscle strength pre-and-post a loaded walk in cold-hypoxia but had no more effect than IMT alone.

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INTRODUCTION

Respiratory muscle fatigue (RMF) can be defined as an exercise-induced reduction in the ability of the respiratory muscles to generate pressure or airflow, which can be improved by rest (NHLBI, 1990). The level of force reduction that qualifies as RMF is unclear. Many acknowledge that a statistically significant reduction in either maximal inspiratory pressure ($P_{i\max}$) and/or maximal expiratory pressure ($P_{e\max}$) from baseline values is indicative of RMF (McConnell, Caine and Sharpe, 1997; Faghy and Brown, 2014; Phillips, Stickland and Petersen, 2016). While others, using direct measures of fatigue (twitch transdiaphragmatic pressure), have employed absolute thresholds of $\geq -10\%$ (Luo *et al.*, 2001) or -15% (Mador, Khan and Kufel, 2002).

Load carriage has been shown to challenge ventilation, through reductions in forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and end-expiratory lung volume (EELV) (Armstrong and Gay, 2015; Walker *et al.*, 2015; Phillips, Stickland and Petersen, 2016; Hingley *et al.*, 2017). Recent studies have progressed knowledge by demonstrating that RMF can occur at lower intensities than had previously been shown during load carriage (Faghy and Brown, 2014; Faghy and Brown, 2016) and in hypoxic environments (Downey *et al.*, 2007). Downey *et al.* (2007) observed a $\sim 17\%$ reduction in $P_{i\max}$ following exercise at $85\%VO_{2\max}$ to exhaustion in both normoxia and hypoxia ($\sim 14\%FiO_2$). RMF influences dyspnea, effort perception and limb discomfort and could ultimately lead to exercise termination and/or a reduction in performance (Romer and Polkey, 2008). Activities such as trekking, mountaineering and occupational tasks which predominately use load carriage, also frequently occur in cold-hypoxic environments. Combined cold with hypoxia [4300 m ($\sim 11.8\%FiO_2$) at $-10\text{ }^\circ\text{C}$] led to reductions in $P_{i\max}$ which was exacerbated when carrying $\sim 18\text{ kg}$ (-5.5% when unloaded, -13.6% when loaded (Hinde *et al.*, 2018). RMF was attributed to the additive effect of both environments. Cold exposure is known to reduce muscle strength (Oksa

et al., 2004) and has been reported to reduce FVC (Gavhed *et al.*, 2000). This reduction in FVC is also seen during hypoxic exposure (Deboeck, Moraine and Naeije, 2005) alongside increases in EELV (Johnson, Saupe and Dempsey, 1992). Reduced FVC attenuates the volume of air the lungs can support and together with increased EELV, the work of breathing is increased. In order to sustain hyperpnea, breathing frequency (BF) must also be increased (Babcock *et al.*, 1995). Dyspnea has been observed during load carriage, with greater levels reported when carrying heavier loads (Faghy and Brown, 2016; Phillips *et al.*, 2019). Thus, changes in breathing mechanics due to load carriage could impair performance by increasing perceived levels of effort (Faghy and Brown, 2016), leading to a reduction in work and prolonging exposure, which in cold environments affects energy expenditure (Hinde *et al.*, 2017). Identification of RMF in such environments could lead to the development of strategies to mitigate fatigue.

Inspiratory muscle training (IMT) has been shown to increase inspiratory muscle strength during exercise in normoxia and hypoxia (Romer, McConnell and Jones, 2002b; Downey *et al.*, 2007). IMT may serve to assist in cold-hypoxia, by increasing the muscle fibre cross-sectional area and reducing the relative intensity of inspiratory work (Turner *et al.*, 2016). Furthermore, when hypoxia causes a greater ventilatory demand, muscular changes in fibre type as a result of IMT may enhance the endurance of the respiratory musculature, maintaining adequate ventilation (Turner *et al.*, 2016). Stronger inspiratory muscles may delay the recruitment of accessory muscles which can distort the chest wall and reduce efficiency (Sheel and Romer, 2012). When exercising in hypoxia, IMT (performed at sea level) has been shown to reduce VE during exercise. These reductions have been observed alongside an increase arterial oxygen saturation (SaO₂) of ~6 % (Downey *et al.*, 2007). Although IMT alone is unlikely to have reduced the work of breathing, there is no agreed explanation for this

mechanism, it was suggested to be a result of slowed red blood cell transit time as a reduction in blood flow may have prolonged gas exchange at the lung, however more research is needed.

Inspiratory muscle training has also been associated with reductions in cardiovascular strain (-7 % in HR) and perceptual responses (-4 % in breathing discomfort and -10 % in leg discomfort) (Faghy and Brown, 2016). Despite increases in P_{imax} of 26-36 % and improved time-trial performance, these findings were not associated with a reduction in RMF (ΔP_{imax}) following a 6.5 km walk with load carriage (Faghy and Brown, 2016). McConnell and Lomax (2006) also reported that the reduction in P_{imax} was similar (~ 30 cmH₂O) pre- and post-IMT following an inspiratory muscle fatiguing task. It was suggested that following IMT, the task to fatigue the respiratory muscles became insufficient in exceeding the threshold required to elicit the respiratory metaboreflex, but that the muscle work done was still enough to reduce P_{imax} (McConnell and Lomax, 2006). In addition, it was noted that static IMT does not activate the non-ventilatory roles of the diaphragm and respiratory muscles thus, performing IMT alongside sport-or-activity specific exercise may improve diaphragm activity, providing a greater training stimulus (Hellyer *et al.*, 2015; Ramsook *et al.*, 2016). Recently, functional inspiratory muscle training (IMT_F) employed by Faghy and Brown (2019) encompassing core muscle training exercises (raised alternating crunches, swiss ball crunches, prone bridge and dynamic bird dog) did not reduce RMF after load carriage despite post-intervention P_{imax} values being greater and time-trial performance improved. The IMT_F used did not involve load carriage, which may be a reason for the lack of attenuated RMF following load carriage (Faghy and Brown, 2019). Load carriage elicits trunk forward lean and performing IMT when posture is compromised has been shown to be advantageous over static IMT in cycling and rowing (Tong *et al.*, 2016). Faghy and Brown (2019) suggested that future research should seek to investigate IMT_F with load carriage to attempt to reduce RMF post- load carriage activities.

Thus, the aim of this study was to evaluate the effects of a four-week pressure threshold loading IMT programme followed by four weeks of IMT_F on respiratory muscle strength, RMF and physiological responses to load carriage in a cold-hypoxic environment. It was hypothesised that, i) four weeks of IMT would significantly increase P_{imax} ii) P_{imax} would be further increased by an additional four weeks of IMT_F , iii) increased P_{imax} would reduce the degree of RMF seen following a loaded walk and, iv) IMT and IMT_F combined would reduce heart rate (HR) and subjective responses during loaded walking.

MATERIALS AND METHODS

Experimental approach to the problem

The current investigation used a randomised, single blind, placebo-controlled intervention to determine whether four weeks of IMT followed by four weeks of IMT_F could increase inspiratory muscle strength (P_{imax}), reduce RMF (ΔP_{imax}) and reduce the physiological [minute ventilation (VE), oxygen consumption (VO_2), HR] and perceptual strain associated with loaded walking in cold-hypoxia. The intervention consisted of two groups, experimental inspiratory muscle training (EXP, $n = 8$) and control sham (CON, $n = 6$). EXP performed four weeks of IMT at 50 % P_{imax} followed by four weeks of IMT_F performed at the same intensity whereas CON performed four weeks of IMT and IMT_F at 15 % P_{imax} . Loaded walking trials in cold-hypoxic conditions were performed pre, mid and post the eight-week training programme.

Participants

Following ethics approval from Leeds Beckett University, 14 (6 males, 8 females) healthy, habitually active and “low risk” individuals [as classed by the ACSM Guidelines (ACSM, 2013) for cardiovascular, pulmonary and metabolic diseases] with normal lung function (FVC and FEV_1 greater than 80 % of predicted) volunteered to participate. Participant characteristics

are reported in Table 1. Written informed consent was gained prior to data collection. Participants were assessed via a venous blood sample for sickle cell trait as under normal physiological conditions, sickle cell trait is harmless, however, a change in environment, i.e. hypoxia, enhances sickling which increases the risk of microvasculature blocking, increased blood viscosity, and risk of a splenic infarction (Weisman, Zeballos and Johnson, 1988). No participants presented a positive test.

Procedures

Preliminary assessment

To familiarise participants with the load, all participants performed sub-maximal loaded (18.2 kg) walking at $-10.0 \pm 1.0^{\circ}\text{C}$ in both normoxia and hypoxia ($\text{FiO}_2 = 11.8 \pm 0.3\%$, ~ 4300 m, wind speed was $\sim 2.9 \text{ m}\cdot\text{s}^{-1}$) for 18-minutes (3-minute bouts at $\sim 2, 3$ and $4 \text{ km}\cdot\text{hr}^{-1}$ at 0 and 10 % gradient). The weight of the load was justified from previous research (Simpson, Munro and Steele, 2011; Hinde *et al.*, 2017) and represented items that would be carried on a trekking/mountaineering trip. Participants were familiarised with measures of respiratory muscle pressure, spirometry and the POWERbreathe device (POWERbreathe classic series, Warwickshire, UK). Measures were performed in accordance with the American Thoracic Society and European Respiratory Society guidelines (American Thoracic Society/European Respiratory, 2002; Miller *et al.*, 2005). Using a respiratory pressure meter (MicroRPM, Carefusion, Basingstoke, UK) participants performed the ‘Mueller’ and ‘Valsalva’ manoeuvre. Maximal inspiratory and expiratory efforts from either residual volume or total lung capacity for a minimum of two seconds were measured. Maximal efforts were repeated at least 3 times with a minimum of 30 seconds between measures ($<10\%$ variance in 3 consecutive manoeuvres) (McConnell, Caine and Sharpe, 1997). The highest value was used in all measurements. Spirometry measures: FVC and forced expiratory volume in 1 second (FEV_1)

were obtained through hand-held spirometry (Micro I, Carefusion, Basingstoke, UK). Maximal inhalation followed immediately by maximal exhalation was performed standing. Manoeuvres were considered acceptable if they were free from artefacts (i.e. leaks or obstructed mouthpieces), had no hesitation and showed satisfactory exhalation (duration of ≥ 6 seconds). Measurements were repeated three times, with 30 seconds rest between each effort. Additional measures were taken if the two largest FEV₁ or FVC values exceeded 0.15 L of each other (Miller *et al.*, 2005). Baseline respiratory pressure and spirometry measures were performed without load and in a normoxic, thermo-neutral environment. All subsequent measures were taken loaded, in a cold-hypoxic environment immediately upon cessation of exercise.

Data collection was completed in a normobaric environmental chamber (TISS, Peak Performance Chamber Series 2009, Hampshire, UK) with conditions controlled at 50 ± 5 % humidity, -10 ± 1 °C and ~ 83 mmHg partial pressure of oxygen $FiO_2 = 11.8 \pm 0.3$ %, ~ 4300 m,) with a wind speed of $2.9 \text{ m}\cdot\text{s}^{-1}$. As a widely researched altitude, 4300 m represents the height of Pikes Peak in Colorado, a medical research laboratory used for high altitude research. An ambient temperature of -10 °C also reflected many of the environments experienced in popular trekking/mountaineering areas (OnTopLtd, 2018).

Participants performed baseline respiratory pressure and spirometry measures. Participants were advised to avoid caffeine, alcohol, and heavy strenuous exercise 12 hrs and 24 hrs prior to exercise respectively. Unloaded $VO_{2\text{max}}$ tests were completed at -10 °C in normoxia and hypoxia as no differences between $VO_{2\text{max}}$ values at 20 °C and -10 °C have been reported (mean $\Delta = 1.14 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p = 1.00$, $d = 0.11$, unpublished data). The $VO_{2\text{max}}$ protocol (Jones, 2007) began at a treadmill gradient of 1 % and increased 1 % every minute. Participants selected a suitable speed to run at which remained constant throughout the test. Running speeds ranged from 7–12 $\text{km}\cdot\text{hr}^{-1}$ at sea level and 6–10 $\text{km}\cdot\text{hr}^{-1}$ at 4300 m. Expired gas was sampled using the metalyzer (Cortex Metalyzer 3B, Leipzig, Germany) and measured

VO_2 and ventilation. Using regression analysis from baseline $\text{VO}_{2\text{max}}$ data, speeds representing 50 % $\text{VO}_{2\text{max}}$ were generated for each gradient and employed in the loaded walking trials.

Loaded walking trials

Participants undertook a loaded walk in cold-hypoxic conditions at least 48 hrs following maximal exercise. Before entering the chamber, unloaded baseline measures of resting heart rate (HR) using a Polar T31 codedTM transmitter with a FT1 watch (Polar Kempele, Finland), spirometry measures (FEV_1 and FVC) and respiratory muscle strength (P_{imax} and P_{emax}) were recorded. A respiratory muscle warm-up (RWU) was performed prior to the determination of spirometry and respiratory muscle strength measures, as this has been shown to improve the reliability of baseline P_{imax} measurements, reduce heteroscedasticity and negate a possible learning effect which is reported to influence the tests variability (Volianitis, McConnell and Jones, 2001; Lomax and McConnell, 2009). The RWU included 2 x 30 breaths using a POWERbreathe device set at 40 % P_{imax} . Between the 2 sets of 30 breaths, participants took a short rest, whereby an intermediate P_{imax} measurement was made and the POWERbreathe adjusted accordingly (Volianitis, McConnell and Jones, 2001).

For the loaded walks, participants wore trousers, a long-sleeved top, hat, gloves, a winter jacket weighing 1.49 kg and their own gym shoes. Upon entering the environmental chamber, participants rested for a standardised period of 10 minutes during which the researcher made assessments of acute resting adjustments. Following rest, participants completed a 6 km loaded (18.2 kg in a backpack) walk at speeds representing 50 % hypoxic $\text{VO}_{2\text{max}}$. The 6 km walk was divided into 4 equal stages. Each stage consisted of 3 x 0.5 km sections performed at 0%, 5 % and 10 % gradient (Figure 2). The distance of the walk represented half a day trekking on popular trekking routes and to ensure ecological validity, a range of gradients were employed. Average walking speeds for 0 %, 5 % and 10 % gradients were $4.9 \pm 0.8 \text{ km} \cdot \text{hr}^{-1}$, $3.5 \pm 0.6 \text{ km} \cdot \text{hr}^{-1}$ and $2.1 \pm 0.5 \text{ km} \cdot \text{hr}^{-1}$ respectively. Rating of perceived

exertion for the whole body (RPE_{whole}) using the 6-20 point Borg Scale, rating of perceived exertion of breathing ($RPE_{\text{breathing}}$), legs (RPE_{legs}) using a Borg CR10 Scale, HR and finger pulse oximetry (SpO_2 , measured by PM10N, NellcorTM, Covidien, Mansfield, MA) were measured every 0.5 km. Due to issues with freezing sample lines, expired gas using the metalyzer (Cortex Metalyzer 3B, Leipzig, Germany) was measured during the first 0.2 km of each 0 % section and the last 0.2 km of each 10 % gradient section. It was also used to monitor patterns of ventilation, oxygen consumption (VO_2) and carbon dioxide production. At 6 km, spirometry measures and respiratory muscle pressures were measured.

Intervention

Prior to IMT, baseline P_{imax} were collected from each participant, they were then matched for P_{imax} and randomly assigned to either a control sham (CON) or an experimental inspiratory muscle training group (EXP) using a single blind, sham-controlled design. There were no differences between groups for any of the participant characteristics ($p \geq 0.210$, $d \leq 0.68$, Table 1). Participants in EXP were told that they were participating in a study investigating the influence of strength IMT. Whereas those in CON were told they were participating in endurance IMT. Consequently, participants were blinded to the true purpose of the study and expected outcomes.

[Table 1 about here]

Both CON and EXP performed IMT using POWERbreathe, a commercially available pressure threshold device. For EXP, the training load of the device was individually calibrated to $\sim 50\%P_{\text{imax}}$ for four weeks. Training comprised of 30 consecutive dynamic inspiratory efforts, twice daily for four weeks with each inspiratory effort initiated from residual volume. CON training involved 60 consecutive dynamic inspiratory efforts, once daily for four weeks. Training load was set at $15\%P_{\text{imax}}$ which is known to elicit negligible changes in P_{imax} (Caine and McConnell, 1998). Assessment of P_{imax} was made at the end of every week to allow for

adjustments to the training load to ensure 15 % or 50 % P_{imax} . IMT was performed four weeks prior to four weeks of IMT_F as it was used as foundation training, regarded as the first part of a phased approach to functional training (McConnell, 2011).

To monitor training adherence, participants completed an IMT log and photographed themselves at each session using a POWERbreathe via a mobile phone (or similar) and sent to the lead researcher using a smartphone application (WhatsApp version 2.17.202, WhatsApp Ltd, Dublin, Ireland). Once received and training logged, photographs were immediately deleted from the researcher's mobile phone. In addition, participants were asked to keep physical activity logs for the duration of the intervention to evaluate typical activity outside of the training intervention.

Mid-way through the intervention (week 4) participants performed a second 6 km loaded walk. Following this, EXP and CON reduced their training to three times a week (typically Monday, Wednesday and Friday) and visited the laboratory to complete a functional training programme so adherence could be monitored. Five exercises designed to strengthen and develop core strength relative to the demands of hiking and mountaineering were chosen for IMT_F (Figure 1) (McConnell, 2011); i) loaded backpack lift- wearing the backpack inhale forcefully, lifting shoulders and chest against the weight of the backpack and hold for 3-5 seconds before relaxing and exhaling slowly for 3 seconds; ii) loaded statue- standing on one leg with the lifted leg at 90 ° at the hip and knee, and the plantar surface of the foot parallel to the ground. Brace abdominal muscles, swing arms as though walking quickly and complete 5-6 breaths in 30 seconds.; iii) loaded shuttle walk- with load, walk at a comfortable pace, inhaling forcefully and exhaling slowly and fully; iv) plank- facing the floor rest on elbows and toes maintaining a straight body line, inhale forcefully and exhale whilst in this position and, v) twisted abdominal crunches- sit on floor with hips flexed, knees bent and feet off the floor, move left elbow towards right knee, as elbow and knee are brought together inhale forcefully,

exhale whilst returning to start position and twist in opposite direction. Loaded exercises were performed wearing the backpack (18.2 kg) and POWERbreathe devices were set to $\sim 50\%P_{i_{max}}$ for EXP and $\sim 15\%P_{i_{max}}$ for CON. Progression through IMT_F was achieved through a combination of increasing the number of sets and reducing the recovery time between sets. At week 8, participants performed a third and final 6 km walk. Trials took place at least 48 hrs after the final training session. An overview of the whole intervention is shown in Figure 2.

Statistical analysis

Data were analysed using IBM SPSS 22, with significance tested at 95% confidence intervals, $p < 0.05$. Descriptive statistics (mean \pm SD) were calculated for all outcome measures. All data were normally distributed (Shapiro-Wilk, $p > 0.05$). To measure for the effects of the IMT and IMT_F programme, between and within differences and interaction effects for dependent variables were assessed using a 2 (group: EXP and CON) \times 3 (trial: 1, 2 and 3) \times 3 (distance: rest and 6 km) mixed model analysis of variance (ANOVA) with Bonferroni post-hoc analysis. To identify any between and within differences in physiological variables (VO_2 , VE, BF, HR and SpO_2) main effects and interactions effects were assessed using a 2 (group: EXP and CON) \times 4 (stage: 1, 2, 3 and 4) \times 3 (trial: 1, 2 and 3) \times 2 (gradient: 0% and 10%) mixed model ANOVA with Bonferroni post-hoc analysis. For rating of perceived exertion, main effects and interactions effects were assessed using a 2 (group: EXP and CON) \times 4 (stage: 1, 2, 3 and 4) \times 3 (trial: 1, 2 and 3) \times 3 (gradient: 0%, 5% and 10%) mixed model ANOVA with Bonferroni post-hoc analysis. In cases when the assumption of sphericity was violated, if $\epsilon < 0.75$, the Greenhouse Geisser correction factor was applied, if $\epsilon > 0.75$, the Huynh-Feldt correction factor was applied. Effect sizes for repeated measures ANOVA were calculated using partial eta squared (η_p^2) which can be classified in accordance with Cohen's (1969) criteria using F values of trivial (≤ 0.1) small (0.11 - 0.24), medium (0.25 - 0.39) and large (≥ 0.4) (Richardson,

2011). Post-hoc paired t-tests for significant main effects were conducted using a Bonferroni adjustment while independent t-tests were used to identify within differences and presented alongside effect sizes (Cohen's d). Interpretation of Cohen's d is ≤ 0.2 is considered trivial, 0.21 - 0.5 is small, 0.51 - 0.8 is moderate and ≥ 0.8 is deemed large (Cohen, 1988).

RESULTS

Adherence to IMT and IMT_F was excellent for both EXP and CON (>94 %, Table 1). Physical activity logs were completed fully (100 %) by 8 participants (4 in CON, 4 in EXP). There were no differences in the number of hours performing low, moderate or high intensity exercise over the intervention period between groups ($p = 0.959$, $\eta_p^2 = 0.039$, power = 0.105).

Respiratory muscle pressures and pulmonary function

Reductions in $P_{i\max}$ were reported following a loaded 6 km walk in cold-hypoxic conditions in all three walking trials ($p < 0.001$, $\eta_p^2 = 0.739$, power = 1.000). During the first walking trial, $P_{i\max}$ was reduced from 123 ± 28 cmH₂O and 127 ± 16 cmH₂O in CON and EXP respectively, to 108 ± 30 cmH₂O and 106 ± 15 cmH₂O at 6 km respectively. There were no reductions in $P_{e\max}$ over time ($p = 0.306$, $\eta_p^2 = 0.087$, power = 0.166). Neither IMT nor IMT_F increased $P_{e\max}$ ($p = 0.641$, $\eta_p^2 = 0.026$, power = 0.087).

There was an interaction observed for $P_{i\max}$ between trial \times group ($p = 0.013$, $\eta_p^2 = 0.302$, power = 0.778). Regardless of time point, $P_{i\max}$ was significantly greater post-IMT and post-IMT_F in EXP compared to CON (Table 2). Table 2 shows that when averaged across both time points (rest and 6 km), EXP showed greater $P_{i\max}$ post-IMT (WT2, $p = 0.005$, $d = 1.28$) and post-IMT_F (WT3, $p = 0.009$, $d = 1.68$) when compared to baseline values (pre-intervention), but no differences were found between WT2 and WT3 ($p = 0.205$, $d = 0.42$). Relative improvements from pre-intervention were also greater in EXP compared to CON both

post-IMT (20.4 % vs 4.0 %, $p = 0.025$, $d = 1.43$) and post-IMT_F (29.1 % vs 9.0 %, $p = 0.050$, $d = 1.23$). Although $P_{i_{max}}$ was greater at each time point post-IMT and IMT_F in EXP, reductions following the loaded 6 km walk ($\Delta P_{i_{max}}$ both absolute and relative) were similar to pre-intervention ($p \geq 0.643$, $\eta_p^2 \leq 0.036$, power ≤ 0.115).

[Table 2 about here]

Following a 6 km loaded walk in cold-hypoxic conditions, FVC and FEV₁ were reduced (main effect of time, $p < 0.001$, $\eta_p^2 \geq 0.712$, power ≥ 0.99). FVC was reduced by 11.0 % whilst FEV₁ decreased 10.9 %. There was no effect of IMT or IMT_F on FVC ($p = 0.899$, $\eta_p^2 = 0.003$, power = 0.055) or FEV₁ ($p = 0.146$, $\eta_p^2 = 0.148$, power = 0.387) as there were no significant interaction effects (trial x group).

Physiological responses

Increases in VE, BF and HR with subsequent stages during the loaded walks were reported (Tables 3 and 4, $p \leq 0.011$, $\eta_p^2 \geq 0.298$, power ≥ 0.802). For all walking trials SpO₂ was reduced with increasing stages ($p < 0.001$, $\eta_p^2 = 0.516$, power = 0.984). Post-hoc comparisons during the first walking trial showed significant reductions in stages 2 (67.3 ± 6.4 %), 3 (66.0 ± 4.2 %) and 4 (64.5 ± 5.3 %) compared to stage 1 (71.2 ± 6.4 %, $p < 0.001$, $d \geq 0.61$, power = 0.995).

Following IMT and IMT_F, there were no differences in any of the variables between groups ($p \geq 0.410$, $\eta_p^2 \leq 0.072$, power ≤ 0.596), demonstrating no effect of IMT or IMT_F on VE, BF, VO₂, HR or SpO₂.

[Tables 3 and 4 about here]

Perceptual responses

Perceptual responses (RPE_{whole} , RPE_{legs} and $RPE_{breathing}$) increased with subsequent stages, ($p < 0.001$, $\eta_p^2 \geq 0.589$, power ≥ 0.987) and increasing gradient ($p < 0.001$, $\eta_p^2 \geq 0.618$, power ≥ 0.994) with no differences between groups ($p \geq 0.069$, $\eta_p^2 \leq 0.234$, power ≤ 0.472), or between

trials ($p \geq 0.064$, $\eta_p^2 \leq 0.212$, power ≤ 0.541). There were no 2-way, 3-way or 4-way interaction effects for RPE_{whole} , RPE_{legs} and $RPE_{\text{breathing}}$.

DISCUSSION

This study is the first to examine the effect of load carriage specific IMT_F on the physiological burden of load carriage exercise in cold-hypoxia. The key findings were fourfold: i) IMT increased P_{imax} when compared to pre-intervention values. IMT_F did not significantly increase these values further, ii) RMF occurred following a loaded 6 km walk in cold-hypoxia, iii) Despite a greater P_{imax} following IMT , the change in P_{imax} (degree of RMF) was not reduced, and iv) Nor IMT or IMT_F had an effect on pulmonary measures, VE , VO_2 , HR , BF , SpO_2 or RPE .

Respiratory muscle pressures

Inspiratory muscle training significantly increased P_{imax} in the training group when compared to pre-intervention values and the control group. Increases in inspiratory muscle strength have been attributed to increased muscle hypertrophy, increased motor unit recruitment and/or neural adaptations (Kellerman, Martin and Davenport, 2000; Ramírez-Sarmiento *et al.*, 2002) which reduce the relative intensity of inspiratory work reducing the work of breathing (Ray, Pendergast and Lundgren, 2010). Following very similar IMT programmes and performing exercise in hypoxia, other studies have shown increases in P_{imax} comparable to the present study [24.5 % by (Downey *et al.*, 2007), 28.4 % by (Salazar-Martinez *et al.*, 2017) and ~13.5 % by (Lomax, Massey and House, 2017)]. In addition, post-hoc power calculations (G*Power) using P_{imax} results ($\eta_p^2 = 0.302$) showed a power of 0.99 implying the study was sufficiently powered. Although IMT_F resulted in greater P_{imax} when compared to pre-intervention, P_{imax} was not significantly different to post- IMT values, thus did not have any additional effect. Similar findings were reported by Tong *et al.* (2016) who found that while performance in the sport-

specific endurance plank test improved in the intervention group, P_{imax} did not increase any further when compared to post-IMT values. Similarly, Faghy and Brown (2019) reported P_{imax} to be unchanged post-IMT_F when compared to post-IMT values. The main differences between that of the present study and Faghy and Brown (2019) are the exercises used in IMT_F as they were focused on core muscle training specific to running rather than load carriage and furthermore, despite no further increase in P_{imax} following IMT_F, they reported an increase in performance on a loaded 2.4 km time-trial. A time-trial was not used to assess performance in the present study because this would have impaired ecological validity as it is not relevant to a recreational, mountaineering population. Therefore, the IMT_F used in this study is unlikely to bring any further physiological benefits to prolonged sub-maximal load carriage that IMT does not already provide. Faghy and Brown (2019) suggested that no change in P_{imax} following IMT_F may be due to the reduction in training volume when changing from IMT to IMT_F, as training reduced from every day to three times a week. Despite maintaining training intensity during IMT_F at 50 % P_{imax} , which with an increasing baseline P_{imax} meant that intensity progressively increased, this constant inspiratory load may have contributed to no further significant improvement in P_{imax} post-IMT_F.

Respiratory muscle fatigue

The present study confirmed our earlier findings that RMF occurred in a cold-hypoxic environment when carrying loads of < 20 kg (Hinde *et al.*, 2018) as reductions in P_{imax} post-6 km (~14 %, $p < 0.001$) were reported. RMF reduces exercise tolerance by the activation of the respiratory metaboreflex (Harms *et al.*, 2000). In occupational settings, this could reduce operational time, increase recovery time and reduce overall physical performance (Armstrong *et al.*, 2019). In a recreational setting, this could also negatively impact performance and may increase time to complete an expedition and if prolonged, RMF could negatively impact dyspnea, effort perception and limb discomfort. Respiratory muscle fatigue and diaphragm

fatigue have been reported in hypoxia previously (Gudjonsdottir *et al.*, 2001; Deboeck, Moraine and Naeije, 2005; Sharma and Brown, 2007; Verges, Bachasson and Wuyam, 2010). The exact mechanisms for hypoxia-induced RMF are not clear, but previous literature has speculated that it may be a result of multiple factors; i) a greater ventilation requirement (increased VE, increased EELV and increased BF) which consequently increases work of breathing; ii) activation of the metaboreflex response which promotes blood flow competition between the respiratory muscles and the limbs and iii) due to a reduction in VO_{2max} , individuals working at a greater relative intensity resulting in a greater concentration of circulating metabolites (Verges, Bachasson and Wuyam, 2010). The evidence associated with spirometry, respiratory function and cold ambient temperatures however is equivocal. In healthy individuals, some literature has shown that cold exposure does not affect respiratory muscle strength (Hinde *et al.*, 2018) while others have reported reductions of FVC and FEV₁ (Gavhed *et al.*, 2000; Kennedy and Faulhaber, 2018). Gavhed *et al.* (2000) only observed reductions in spirometry measures when exposure involved significant facial cooling and was not influenced solely by the temperature of inhaled air while Kennedy and Faulhaber (2018) only measured responses in female participants. Nevertheless, reductions in FVC (seen both in hypoxia and at reduced ambient temperatures) causes reductions in the volume of air the lungs can support. This increases work of breathing, reduces lung compliance and may lead to RMF (Armstrong *et al.*, 2019). In the present study, the additive effect of both hypoxia and a cold ambient temperature likely led to reduction in FVC and thus P_{imax} .

Despite significantly stronger inspiratory muscles post-IMT and IMT_F , the magnitude of RMF (ΔP_{imax}) remained similar. The finding of no change in ΔP_{imax} is in agreement with existing studies (Griffiths and McConnell, 2007; Faghy and Brown, 2015; Lomax *et al.*, 2018; Faghy and Brown, 2019). Lomax *et al.* (2019) found that ΔP_{imax} was not correlated with changes in 100 or 200 m swim times showing that increases in P_{imax} did not determine the

magnitude of improvement in performance. Unchanged RMF could be due to potential ineffective training, however in the present study, $P_{i_{max}}$ was increased in EXP when compared to pre-intervention values which was not apparent in CON, indicating training was effective in increasing absolute strength. A more aggressive protocol with progressive increments of resistance [up to 80 % $P_{i_{max}}$ in the 3rd week of training (Hartz *et al.*, 2018)] may have been more successful in both reducing the RMF associated with a loaded 6 km walk and further increasing $P_{i_{max}}$ following IMT_F . No reduction in the magnitude of RMF is in contrast to others that reported attenuated RMF post time-trial in cyclists (Romer, McConnell and Jones, 2002a) and during exercise in hypoxia (Downey *et al.*, 2007). Following four weeks of IMT, Downey *et al.* (2007) reported that alongside increased $P_{i_{max}}$, participants in the intervention group showed less RMF following exercise at 85 % $VO_{2_{max}}$ breathing hypoxic gas ($FiO_2 = 0.14$) which was not observed in the control group.

Faghy and Brown (2016) hypothesised that no change in RMF may have been due to static IMT not influencing muscles involved in postural control. The function of respiratory muscles is not solely restricted to respiration, but also postural support and spinal stability. The addition of load carriage during IMT_F in the present study should have assisted in activating these functions to account for the shift in the length-tension curve when wearing a load (Brown and McConnell, 2012). In its non-ventilatory role, the diaphragm contracts and pushes down in the abdominal cavity, increasing intra-abdominal pressure. This is counteracted by contraction of the lumbar extensor muscles to stabilise the spine (Hodges and Gandevia, 2000). A stronger diaphragm and other respiratory muscles, as a result of static IMT and IMT_F , would delay the development of RMF and maintain the diaphragmatic contribution to trunk and spinal stability reducing potential injury risk (Brown and McConnell, 2012). A higher training resistance (> 50 % $P_{i_{max}}$) may therefore attenuate the fatigue associated with the non-respiratory functions of inspiratory muscles, but further research is warranted. Additionally, it could be

argued that the nature of the exercises performed during IMT_F (i.e., with or without load) may have been unsuccessful in activating the non-respiratory roles of the diaphragm. Load carriage increases the dependence on the respiratory muscles in stabilising the spine whilst increasing postural sway (Faghy and Brown, 2016) therefore it is likely that the exercises involving load carriage played a key role in the activation of the non-respiratory roles of the diaphragm and other respiratory muscles, but exercises without load carriage lacked sufficient stimulus. Thus, only load carriage specific exercises should be employed during IMT_F , this however needs confirming. Furthermore, Armstrong et al. (2019) reported greater respiratory mouth pressures in infantry soldiers when compared to civilian populations. The authors hypothesised that regular training with load carriage may increase respiratory muscle strength without the need to perform IMT or IMT_F . Currently the effect of regular load carriage on respiratory muscle strength is unknown and should be an area for future research.

Physiological measures

Other than increased inspiratory muscle strength, there were no additional physiological or subjective benefits of IMT or IMT_F . These findings agree with previous studies which demonstrated no physiological improvements post-IMT during exercise, except for a greater baseline P_{imax} (Williams *et al.*, 2002; Ramsook *et al.*, 2016) but in contrast with other IMT interventions that showed improvements in various physiological measures. Lomax et al. (2017) observed lower VE responses in the intervention group during exercise breathing a hypoxic gas mixture (14.6% O_2). However, this was not seen in exercise in normoxia so could not be attributed to a reduction in work of breathing, rather it was linked to a 3% increase in SpO_2 . Downey et al. (2007) found that following IMT, time to exhaustion did not change but during exercise, VO_2 and VE were lower during exercise in hypoxia in the intervention group alongside significant reductions in RPE and dyspnea in hypoxia. The specific mechanisms for these changes are yet to be determined but are also thought to be linked to increases in SpO_2

which may reduce the input to peripheral chemoreceptors and thus reduce VE (Downey *et al.*, 2007). Results regarding changes in perception are mixed, while some have reported improvements in effort perception and dyspnea (Edwards, 2013; Faghy and Brown, 2016), other studies have reported no changes in perceptual responses (Suzuki *et al.*, 1993; Sonetti *et al.*, 2001; Sperlich *et al.*, 2009). Suzuki *et al.* (1993) proposed three reasons why perception of effort may be unaffected by IMT. Firstly, during exercise in which RMF occurs, intercostal accessory muscles are recruited to maintain ventilation, it is unclear whether IMT affects these muscles, so recruitment of these during heavy, prolonged exercise would increase dyspnea (Suzuki *et al.*, 1993). Secondly, previous literature (Romer, McConnell and Jones, 2002b; Witt *et al.*, 2007) and the present study have shown that IMT can have minimal effect on expiratory muscle strength. During exercise, expiratory muscles, such as the abdominals, become active and therefore it may be the fatiguing of these expiratory muscles that contribute to respiratory effort (Suzuki *et al.*, 1992).

Respiratory muscle endurance

During prolonged load carriage, the endurance capacity of the respiratory muscles rather than absolute strength may become a significant factor. There have been various respiratory muscle training programmes that have reported increased respiratory muscle endurance (Inbar *et al.*, 2000; Williams *et al.*, 2002). It may be argued that due to an increase in inspiratory muscle strength, inspiratory muscles work at a lower relative intensity, resulting in an improved endurance capacity of inspiratory muscles. However, a number of studies reporting increases in P_{imax} following IMT, have also described no changes in respiratory muscle endurance (Sonetti *et al.*, 2001; Downey *et al.*, 2007). This evidence helps support the counterargument that improvements in absolute strength as a result of IMT, do not necessarily imply that endurance is also improved. Downey *et al.* (2007) reported no significant improvements in respiratory muscle endurance after an IMT programme (2 x 30 breaths a day at 50 % P_{imax} for

four weeks) following a run to exhaustion at 85 % $\text{VO}_{2\text{max}}$ but did report greater P_{imax} values. An improvement in ΔP_{imax} in the absence of any improvement in respiratory muscle endurance may therefore only occur during short duration, high intensity activities when endurance is less important. Respiratory muscle endurance was not measured in the present study therefore, it cannot be determined if respiratory muscle endurance was reduced post-6 km in cold-hypoxic conditions or improved post-IMT/IMT_F but it could be speculated that for low intensity prolonged exercise, the endurance of the respiratory muscles may take priority and may explain the lack of reduction in RMF in the present study and that reported by Faghy and Brown (2019). Studies using pressure threshold loading techniques demonstrating improvements in respiratory muscle endurance used incremental inspiratory intensities (Inbar *et al.*, 2000; Williams *et al.*, 2002), thus our earlier statement of utilising a protocol with progressive increments of resistance may be key. Data from the present study supports work by others that showed no change in the strength of the expiratory muscles (Griffiths and McConnell, 2007) or pulmonary function post-IMT (Inbar *et al.*, 2000; Williams *et al.*, 2002).

Limitations

P_{imax} and P_{emax} as volitional measures of respiratory muscle strength have been criticised as they do not directly reflect the strength of the diaphragm (Johnson *et al.*, 1993). However, measures are reliable, reproducible (Romer and McConnell, 2004) and widely used throughout the literature (Ross *et al.*, 2008; Hartz *et al.*, 2018; Faghy and Brown, 2019). These findings, combined with the considerations acknowledged in our previous study (Hinde *et al.*, 2018), justifies their use within the present study. Furthermore, in order to minimise any potential effects of reduced motivation, familiarisation sessions were used and maximal effort was supported by verbal encouragement. We are therefore confident that any potential effects of reduced effort were minimised. Nonetheless despite demonstrating RMF and increases in P_{imax} , it is important to note that due to methodological restrictions, we did not measure non-volitional

tests of respiratory muscle strength or calculate the work of breathing, end inspiratory or expiratory lung volume or total lung volumes, these should be measured in future research.

Variations in individual's anatomical features could have resulted in an imperfect backpack fit and strap tension was not standardized between trials. Nevertheless, back length adjustment was used to ensure a suitable fit and all subjects were satisfied with the fitting of the backpack.

As mentioned previously, a time-trial was not deemed appropriate for a recreational mountaineering setting. If investigating in an occupational setting however, new physical employment standards and operational exercises are timed and therefore increased performance as well as improved physiological measures would be beneficial. Thus, this should be considered an area for future research.

Furthermore, moderate hypoxia ($FiO_2 = 15.0\%$) has been shown to reduce central neural drive and muscle force/power output to ensure the rate of development of peripheral fatigue is attenuated and prevented from reaching a 'critical threshold' (Amann *et al.*, 2006). In severe hypoxia (arterial oxygen saturation $< 70\%$), termination of exercise is in part independent of peripheral feedback and suggestive of a non-peripheral influence (central nervous system) (Amann *et al.*, 2007). Without involuntary measures of respiratory muscle strength in the present study, the contribution of central and peripheral mechanisms to the reduction in respiratory muscle strength cannot be clarified. Thus, we are unable to attribute the finding of reduced $P_{i\max}$ solely to peripheral factors. This should be an area for future research.

Conclusion

This IMT_F intervention (performing IMT with load carriage) was, to the researchers' knowledge, the first of its kind, with the aim to lessen the degree of RMF during load carriage in a cold-hypoxic environment. The evidence leads to an acceptance of the first experimental

hypothesis as four weeks of IMT strengthened inspiratory muscles by ~20 % however, the second hypothesis can be rejected as IMT_F did not significantly further increase $P_{i_{max}}$. The third hypothesis can also be rejected as the $\Delta P_{i_{max}}$ following a 6 km loaded walk was unchanged suggesting that the endurance of the respiratory muscles play a role in influencing performance. Nonetheless, $P_{i_{max}}$ values post-6 km in EXP were greater than CON and higher than pre-intervention baseline values.

Activities that necessitate load carriage such as trekking, mountaineering and occupational tasks, frequently occur in extreme environments. If RMF is prevalent, as found following load carriage in cold-hypoxic environments, IMT programmes can be implemented to improve the absolute strength of the inspiratory muscles. Protocols employing greater and/or more progressive training loads have reported greater changes in respiratory muscle strength and reductions in RMF (Inbar *et al.*, 2000; Kellerman, Martin and Davenport, 2000) and could also reduce perceptions of exertion and breathlessness. Such interventions may be more beneficial and could present future lines of enquiry.

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Table 1 Mean \pm SD descriptive participant characteristics, pre-intervention for EXP and CON groups.

	Experimental inspiratory muscle training group (EXP, n=8, M=3, F=5)	Control sham group (CON, n=6, M=3, F=3)
Anthropometry		
Age (years)	27 \pm 6	27 \pm 6
Height (cm)	171.1 \pm 5.8	177.7 \pm 6.2
Body mass (kg)	68.3 \pm 11.2	75.3 \pm 10.3
Pulmonary function		
FVC (l)	4.5 \pm 1.0	4.7 \pm 0.7
FVC (% predicted)*	98 \pm 8	93 \pm 11
FEV ₁ (l)	3.7 \pm 0.9	3.8 \pm 0.7
FEV ₁ (% predicted)*	96 \pm 12	89 \pm 11
P _i max (cmH ₂ O)	127 \pm 16	123 \pm 28
P _e max (cmH ₂ O)	124 \pm 36	127 \pm 51
Aerobic power		
Sea level VO ₂ max (ml·kg ⁻¹ ·min ⁻¹)	47.9 \pm 7.1	49.5 \pm 7.1
Hypoxic VO ₂ max (ml·kg ⁻¹ ·min ⁻¹)	33.6 \pm 4.5	32.1 \pm 5.3
Training adherence (%)		
IMT	99 \pm 2	96 \pm 4
IMT _F	97 \pm 6	94 \pm 9

M= males, F = females, * using model published by (Stanojevic et al., 2008)

Table 2 Mean \pm SD P_{imax} (cmH₂O) for CON and EXP at baseline (pre-intervention), week 4 (post-IMT) and week 8 (post-IMT_F) at each time point (rest and 6 km) and averaged across time points. ^a denotes a significant difference from EXP pre-intervention values ($p < 0.009$), ^b denotes a significant difference from resting values ($p < 0.001$), ^c denotes a significant interaction effect between groups ($p = 0.013$).

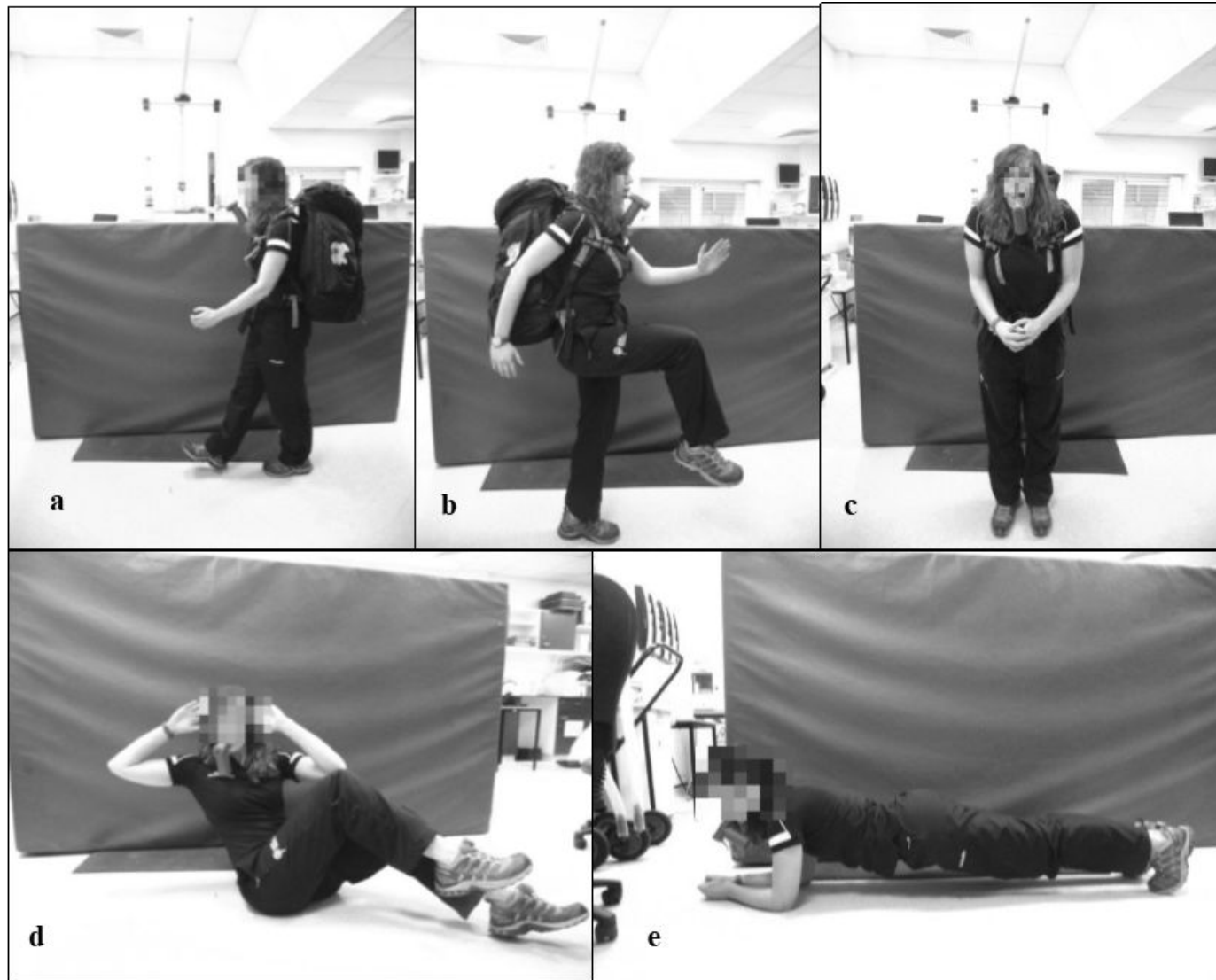
Time point	Time point	CON		EXP	
Pre-Intervention (cmH ₂ O)	Rest	123 \pm 28	116 \pm 28	127 \pm 16	117 \pm 15 ^c
	6 km	108 \pm 30 ^b		106 \pm 14 ^b	
Post-IMT (cmH ₂ O)	Rest	126 \pm 34	120 \pm 34	146 \pm 20	140 \pm 21 ^{ac}
	6 km	116 \pm 34 ^b		135 \pm 24 ^b	
Post- IMT_F (cmH ₂ O)	Rest	130 \pm 27	125 \pm 29	155 \pm 30	150 \pm 23 ^{ac}
	6 km	121 \pm 31 ^b		144 \pm 19 ^b	

Table 3 Mean ± SD physiological responses to a loaded 6 km walk pre and post IMT_F in the CON group. ^a denotes a significant difference to stage 1, ^b denotes a significant difference to stage 2, ^c denotes a significant difference to stage 3, ^d denotes a significant difference to 0% gradient

Stage	Pre-IMT								Post-IMT _F							
	1		2		3		4		1		2		3		4	
Gradient	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%
SpO ₂ (%)	72 ± 6	68 ± 9	67 ± 8 ^a	66 ± 6 ^a	66 ± 5 ^a	63 ± 3 ^a	62 ± 4 ^a	62 ± 4 ^a	76 ± 8	70 ± 8	68 ± 7 ^a	65 ± 5 ^a	67 ± 4 ^a	66 ± 5 ^a	67 ± 6 ^a	70 ± 5 ^a
VE (L·min ⁻¹)	43.7 ± 8.9	45.9 ± 8.8	45.5 ± 6.7	44.4 ± 6.8	45.1 ± 6.6	46.6 ± 5.6	46.3 ± 6.0 ^c	47.3 ± 6.0 ^c	42.6 ± 3.2	40.9 ± 3.3	45.2 ± 7.9	42.7 ± 5.8	44.6 ± 6.1	43.2 ± 5.6	44.8 ± 6.4 ^c	44.7 ± 5.9 ^c
VO ₂ (ml·min ⁻¹ ·kg ⁻¹)	14.8 ± 2.3	16.0 ± 3.8 ^d	15.6 ± 3.0	15.6 ± 2.8 ^d	15.5 ± 3.4	14.7 ± 2.1 ^d	14.6 ± 3.2	14.4 ± 2.4 ^d	13.7 ± 3.6	13.3 ± 3.6 ^d	14.5 ± 4.9	13.6 ± 4.8 ^d	14.2 ± 4.7	13.1 ± 4.2 ^d	13.8 ± 4.2 ^d	13.5 ± 4.8 ^d
HR (beats·min ⁻¹)	127 ± 11	133 ± 11 ^d	132 ± 12 ^a	137 ± 16 ^{ad}	133 ± 12 ^a	139 ± 15 ^{ad}	135 ± 11 ^a	139 ± 13 ^{ad}	123 ± 9	129 ± 7 ^d	127 ± 8 ^a	127 ± 9 ^{ad}	125 ± 8 ^a	131 ± 10 ^{ad}	127 ± 9 ^a	130 ± 11 ^{ad}
BF (breaths·min ⁻¹)	30.4 ± 4.9	34.1 ± 5.1	34.8 ± 5.3 ^a	33.0 ± 6.5 ^a	35.4 ± 6.1 ^a	35.9 ± 5.9 ^a	36.2 ± 7.1 ^{ab}	38.5 ± 7.8 ^{ab}	29.8 ± 1.2	32.6 ± 2.2	34.7 ± 3.4 ^a	33.3 ± 2.6 ^a	34.1 ± 3.8 ^a	34.5 ± 3.1 ^a	35.5 ± 4.5 ^{ab}	35.6 ± 3.0 ^{ab}

Table 4 Mean \pm SD physiological responses to a loaded 6 km walk pre and post IMT_F in the IMT group. ^a denotes a significant difference to stage 1, ^b denotes a significant difference to stage 2, ^c denotes a significant difference to stage 3, ^d denotes a significant difference to 0% gradient

Stage	Pre-IMT								Post-IMT							
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Gradient	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%
SpO ₂ (%)	76 \pm 5	69 \pm 8 ^d	70 \pm 7 ^a	69 \pm 7 ^{ad}	70 \pm 5 ^a	66 \pm 6 ^{ad}	69 \pm 8 ^a	68 \pm 7 ^{ad}	78 \pm 4	70 \pm 6 ^d	71 \pm 7 ^a	66 \pm 5 ^{ad}	69 \pm 10 ^a	66 \pm 6 ^{ad}	68 \pm 4 ^a	70 \pm 8 ^{ad}
VE (L·min ⁻¹)	41.2 \pm 7.6	41.9 \pm 5.9	44.1 \pm 4.7	43.2 \pm 6.9	45.2 \pm 6.6	42.7 \pm 8.3	46.4 \pm 5.5 ^c	46.1 \pm 8.9 ^c	39.9 \pm 10.1	38.6 \pm 11.0	42.3 \pm 8.4	40.3 \pm 9.4	42.6 \pm 9.1	40.5 \pm 8.8	44.7 \pm 9.2 ^c	42.2 \pm 9.7 ^c
VO ₂ (ml·min ⁻¹ ·kg ⁻¹)	13.5 \pm 0.9	13.1 \pm 2.3 ^d	13.7 \pm 2.0	13.1 \pm 2.4 ^d	13.6 \pm 2.5	12.6 \pm 1.7 ^d	13.4 \pm 2.0	12.4 \pm 1.5 ^d	12.2 \pm 2.4	11.6 \pm 1.8 ^d	12.7 \pm 2.6	11.8 \pm 2.0 ^d	12.4 \pm 2.3	11.8 \pm 2.5 ^d	12.6 \pm 2.7	11.6 \pm 2.5 ^d
HR (beats·min ⁻¹)	115 \pm 9	125 \pm 10	126 \pm 13 ^a	131 \pm 14 ^a	127 \pm 14 ^a	131 \pm 11 ^a	128 \pm 14 ^a	132 \pm 13 ^a	119 \pm 9	126 \pm 11	123 \pm 13 ^a	127 \pm 14 ^a	126 \pm 12 ^a	128 \pm 12 ^a	125 \pm 14 ^a	129 \pm 13 ^a
BF (breaths·min ⁻¹)	28.7 \pm 8.2	34.5 \pm 9.2	34.9 \pm 9.6 ^a	33.6 \pm 6.8 ^a	36.7 \pm 6.7 ^a	34.9 \pm 9.1 ^a	38.5 \pm 8.3 ^{ab}	39.2 \pm 10.1 ^{ab}	30.1 \pm 5.6	31.7 \pm 6.4	33.9 \pm 7.2 ^a	34.0 \pm 7.7 ^a	36.7 \pm 7.3 ^a	34.2 \pm 8.4 ^a	37.1 \pm 7.0 ^{ab}	34.6 \pm 8.1 ^{ab}



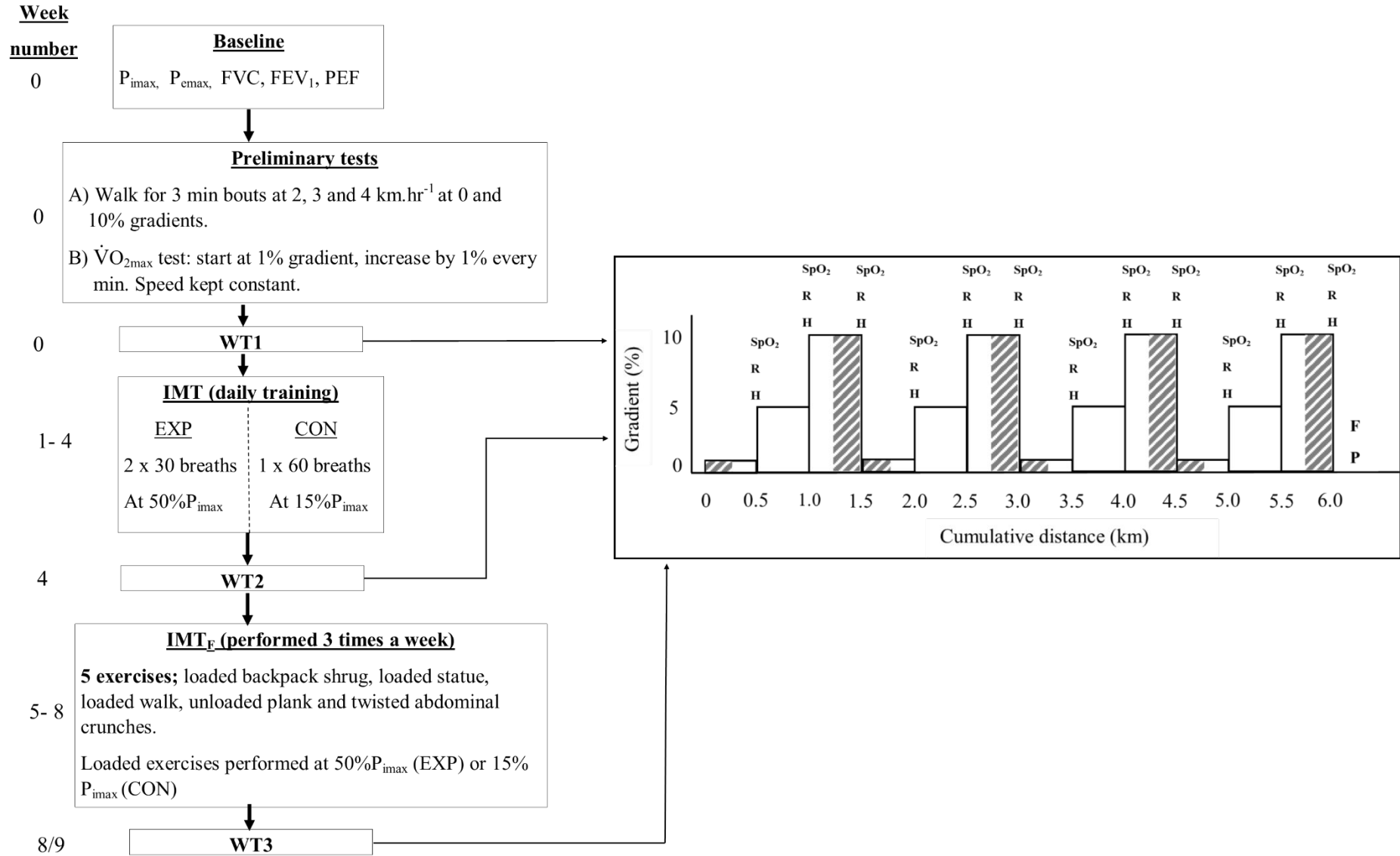


Figure Captions

Figure 1: The IMT_F programme; a) Loaded shuttle walk, b) Loaded statue, c) Backpack lift, d) Abdominal twists, e) Plank.

Figure 2: Schematic of IMT_F intervention including the 6 km loaded walk. **F**- Spirometry (FVC, FEV₁), **P**- P_{imax}/P_{emax} , **SpO₂**- Peripheral oxygen saturation, **R**- RPE (whole, leg and breathing) using Borg Scale and CR10 scale, **H**- Heart rate, **White block**- walk at 50 % VO_{2max} , **Hatched block**- expired gas collected, **EXP**- experimental group, **CON**- control sham group.