Acute psycho-physiological responses to cyclical variation of intermittent hypoxic exposure in adults with obesity

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**Title:** Acute psycho-physiological responses to cyclical variation of intermittent hypoxic exposure in adults with obesity

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Authorship Confirmation Statement

All authors contributed to the design on the study. Liam Hobbins was responsible for data collection, processing and analysis. All authors contributed to production and revision of the manuscript. All authors have reviewed and approved the final manuscript prior to submission.

Disclosure statement

The authors declare no conflicts of interest.
Abstract

Background: We compared acute psycho-physiological responses to a single intermittent hypoxic/normoxic exposure (IHE) trial with varying cycle lengths in adults with obesity.

Materials and methods: Eight obese adults (BMI = 33.0 ± 2.2 kg/m²) completed three 60-min IHE trials (passive seating), separated by seven days. Trials comprised 30-min hypoxia/30-min normoxia (inspired oxygen fraction = 12.0%/20.9%) over Short (15 × 2/2 min), Medium (10 × 3/3 min) and Long (5 × 6/6 min) hypoxic/normoxic cycles, and a control trial (60-min normoxia).

Results: Arterial oxygen saturation was lower during hypoxic periods of Long vs. Medium and Short trials (90.1% vs. 93.0% and 94.2%; p = 0.02 and p = 0.05), with no differences between Short and Medium. Pre-frontal cortex oxygenation was lower (-5.1%) during all IHE interventions vs. control (p < 0.02), independently of cycle length. Perceived breathlessness was unaffected during IHE, but increased 15-min after exposure vs. baseline (+34%; p = 0.04). Breathlessness was lowest after Short vs. control from 15–60-min (-7%; p = 0.01).

Conclusions: When implementing IHE, greater desaturation is observed during longer compared to shorter hypoxic/normoxic cycles in adults with obesity. However, IHE tends to be better tolerated perceptually with shorter rather than longer cycles.

Key words: Obesity, acute hypoxia, tissue oxygenation, perception.
Introduction

In the UK, ~26% of adults are overweight (body mass index [BMI] = 27–29.9 kg/m$^2$) and ~35% obese (BMI = 30–40 kg/m$^2$) (Baker, 2018). In addition to exacerbated weight-bearing (Wearing et al., 2006), obesity is associated with negative health conditions such as hypertension (Hall et al., 2015) and type II Diabetes (Toplak et al., 2016). To reduce body weight and improve health in obese populations, lifestyle interventions (i.e., dietary manipulation, physical activity) have been implemented, yet with low adherence and success (Forman & Butryn, 2015). Therefore, there is an urgent need for new strategies to reduce obesity prevalence and improve the health and wellbeing of this population. One innovative strategy evidencing therapeutic benefits in this demographic is passive hypoxic exposure (Urdampilleta et al., 2012; Verges et al., 2015; Hobbins et al., 2017).

Normobaric hypoxia is defined as a reduced/insufficient oxygen supply (lower inspired oxygen fraction or FiO$_2$) to tissues leading to decreased arterial oxygen saturation (SpO$_2$) (Heinonen et al., 2006). Workman and Basset (2012) found increased energy expenditure in overweight males immediately following one continuous resting 3-h session at a target SpO$_2$ of 85% versus normoxia. However, continuous hypoxic exposure (several hours) versus normoxia may exacerbate sympathetic nervous system activity, raising the risk of elevated blood pressure, tachycardia and rate pressure product in ‘at-risk’ individuals (White et al., 1985; Wulsin et al., 2017).

Intermittent hypoxic exposure (IHE) includes cycles of hypoxia and normoxia lasting from a few minutes to hours (Urdampilleta et al., 2012). Decreased cerebral oxygenation and blood
pressure, during and immediately after one ~1 h IHE session, were found in healthy and overweight individuals versus a normoxic baseline (Chacaroun et al., 2017; Costalat et al., 2017). Although positive physiological responses have been reported, the aforementioned studies utilised individually-tailored hypoxic intervals (~5-min) to reach a target SpO$_2$ (~75%), rather than replicable standardized hypoxic/normoxic cycles. Currently no consensus regarding best practice IHE (i.e., optimal cycle length and frequency) for maximising physiological responses primarily exists for implementation in adults with obesity.

Between 3–15 cycles per session of moderate hypoxia (FiO$_2$ = 9.0–16.0%) is suggested to reduce blood pressure and increase blood glucose tolerance (daily sessions over four weeks) (Navarrete-Opazo & Mitchell, 2014). Evidence coming from animal studies indicates that longer, less frequent cycles will induce higher physiological (i.e., tissue de-oxygenation) stress versus shorter, more frequent cycles (Almendros et al., 2014). However, this is currently unknown in regards to obese individuals completing IHE for beneficial psycho-physiological responses.

Perception is a necessary component for investigating behaviour changes towards health (Schutzer & Graves, 2004). Stavrou et al. (2018) reported impaired mood state (i.e., greater depression and tension) during a 21-day hypoxic bed rest (FiO$_2$ = 15.0%) versus normoxia. To date, there is a lack of data including perceptual measures during and following (~60-mins) IHE. A low level of enjoyment is commonly cited as a reason for low/non-adherence to lifestyle interventions (King et al., 1988). Therefore, it is relevant to assess perception during IHE and potential influences of different cycles. Although exercise-based, studies have found lower perceived exertion when SpO$_2$ is >94% during hypoxic exercise versus <94% (Romer et al.,
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(2006; Khaosanit et al., 2018). For IHE, perhaps more frequent re-oxygenation phases during shorter versus longer hypoxic cycles will permit more positive perception as SpO₂ will likely increase during normoxic cycles (Urdampilleta et al., 2012).

The aim of this study was to therefore compare the acute effects of 60-min IHE sessions including short, medium and long hypoxic/normoxic exposure cycles (matched total hypoxic and normoxic exposure time) versus a control on the magnitude and time course of psychophysiological responses. We hypothesised that the physiological stimulus (i.e., tissue deoxygenation) would be greater, yet with less favourable perceptual responses (i.e., perceived breathlessness), during longer versus shorter cycles, with medium being the ‘optimal’ trade-off.

Materials and methods

Participants

Eight adults with obesity (5 females, 3 males; age: 37.0 ± 11.1 yrs; height: 169.8 ± 0.1 cm; weight: 93.9 ± 8.7 kg; BMI: 33.0 ± 2.2 kg/m²; systolic and diastolic blood pressure: 117.4 ± 18.3 and 70.6 ± 11.4 mmHg, respectively) were recruited. Participants enrolled onto the study following eligibility assessment that consisted of having a BMI of 30–40 kg/m², being normotensive (90–120 and 60–80 mmHg systolic and diastolic blood pressure, respectively), no known cardiovascular, metabolic or physiological illness/disease, sedentary (<1 h of moderate-intensity exercise/week), and no exposure to altitude (≥2500 m) for >48 h within six months. 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, London South Bank University (SAS1705).
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Experimental design

Participants attended the lab on five separate visits. First, they were familiarised with the study, including procedures/measures involved, and eligibility was determined. Eligible participants returned on four occasions at a similar time of day for the main experimental trials, each separated by seven days to enable a washout period (Kelly & Basset, 2018). Each experimental trial included a 60-min intervention, three of which included 30-min hypoxia ($\text{FiO}_2 = 12.0\%$) and 30-min normoxia, and one in continuous normoxia (control) completed in a randomised order. The cyclical interventions were a) $15 \times 2/2$ mins (SHORT), b) $10 \times 3/3$ mins (MEDIUM) and c) $5 \times 6/6$ mins (LONG) in hypoxia/normoxia. All trials included a 60-min post-intervention period (normoxia). Participants arrived at the lab following an 8-h fast but were permitted to consume water. Participants were asked to replicate their dietary intake for remaining trials after the first (24-h prior), and continue their habitual routine during their time spent enrolled on the study.

Intervention

Figure 1 illustrates the intervention. Participants rested in a seated position for 10 min to enable haemodynamic stabilization (Thijsen et al., 2011). Baseline assessment of blood pressure and perception were made. Participants were fitted with an ‘intervention’ facemask which provided exposure to hypoxic/normoxic gas for 60-min. Perception was assessed at $0$ (T0), $10$ (T1), $22$ (T2), $34$ (T3), $46$ (T4) and $58$ (T5) mins (corresponding to $0\%$, $20\%$, $40\%$, $60\%$, $80\%$ and $100\%$ of the 60-min intervention) in normoxia. Arterial and brain oxygenation was recorded throughout the intervention. The ‘intervention’ facemask was removed after 60 min and blood pressure and perception were re-assessed at 15, 30 and 60 min post-intervention. Entertainment (films/television programmes with similar neutral content across trials) was provided during the intervention.
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**Fig. 1 near here**

Hypoxic simulation

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 simulate hypoxia. The FiO₂ provided was 12.0% (simulated altitude of ~4500 m), deemed safe in the population studied (Navarrete-Opazo & Mitchell, 2014). An additional hypoxic generator was set at a 20.9% FiO₂ for normoxic cycles, both outside of participant viewing, for blinding. A Hans Rudolph two-way valve positioned along the corrugated tubing permitted switching between gases. Participants’ remained seated at all times during the intervention and instructed to maintain a normal breathing pattern by the investigator (“breath as normally as you would without wearing a facemask”) prior to each trial.

Physiological measures

A pulse oximeter (Nellcor N-200E Pulse Oximeter, Medtronic, USA) attached to the participants’ index finger continuously estimated SpO₂ (%) during the 60-min intervention.

Near-infrared spectroscopy bi-polar optode sensors (NIRO-2000NX, Hamamatsu, Japan) attached via double-sided adhesive tape and housed (3 cm apart, 775 Nm wavelength) within rubber-cased hoods assessed oxygenation via tissue saturation index (TSI; %) of the left prefrontal cortex. Probes were placed over the left prefrontal cortex to illuminate cortical area between standard Fp1 and F3 locations according to the international EEG 10-20 system (Chacaroun et al., 2017). TSI was calculated via emission and absorption of near-infrared light to tissue, in accordance with the Beer-Lambert law. The data signal was arbitrarily set to zero via internal factory settings. SpO₂ and TSI data were continuously
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sampled during the 60-min intervention at 10 Hz (Chacaroun et al., 2017) and recorded into

Spike2 (v8, CED, England).

Blood pressure was assessed by attachment of a pressure cuff secured with Velcro around the
participants biceps. The cuff was inflated to 170 mmHg and deflated alongside pulse rate
(Omron M4, Omron, Japan). Systolic and diastolic values were recorded following assessment
at baseline and 15, 30 and 60 min after the intervention. A single measurement was completed
unless there was uncertainty in the values (due to machinery error) by the investigator (Beervers
et al., 2001).

Perceptual measures

Perceived mood state, breathlessness and motivation to exercise were assessed at baseline, T0–
T5, and 15, 30 and 60 min after the intervention. Participants were asked ‘how are you feeling
right now?’ ‘how breathless do you feel right now?’ and ‘how motivated do you feel to
complete exercise right now?’ by the investigator. Participants were instructed to verbally
specify a number on an 11-point scale anchored ‘very bad’ (-5) up to ‘very good’ (+5) for mood
state (Hardy & Rejeski, 1989), a 12-point scale anchored ‘nothing at all’ (0) up to ‘very, very
severe’ (10) for breathlessness (Ward & Whipp, 1989), and to adjust a 20 cm visual analog
scale anchored ‘extremely low’ (1) up to ‘extremely high’ (20) for motivation (Crewther et al.,
2016). Positive and negative affects were assessed via a 20-item 5-point Likert scale at baseline
and 15, 30 and 60 min after the intervention. Participants were instructed to answer how they
feel towards 20 emotions including ‘interested’, ‘distressed’ and ‘excited’, ranging from ‘very
slightly or not at all’ (1) to ‘extremely’ (5). Items were totaled for positive and negative
responses (Watson & Clark, 1988), and the ratio between the two (Diehl et al., 2011). Scale
order presentation was held constant across time points and participants.

Data analysis
Data were processed offline into Excel (Microsoft Office, 2016). SpO\textsubscript{2} data were averaged for time in hypoxia (30-min) and normoxia (30-min) for each IHE condition, and 60-min of normoxia for control. TSI data samples (2-min) were exported in hypoxia (before T1–T5) to compare an equal hypoxic duration between conditions at matched timepoints. Perceptual data were obtained in normoxic conditions to allow meaningful comparisons between IHE cycle variations and the control trial, all under normoxic conditions. TSI data were smoothed using a 5-point moving average, and truncated via removal of the first and last 30-s of each 2-min (T0–T5) data collection period (1 min). TSI data were normalized by calculating percentage change from T0 (in normoxia) in each respective condition for statistical analysis, due to possible sensor placement differences. Perceptual data collected during the 60-min intervention and post-intervention periods were expressed as percentage change from T0 and baseline, respectively.

Statistical analysis

Data distribution was assessed via a Shapiro-Wilk normality test. Normally distributed data was analysed with a parametric two-way analysis of variance, aligned with a Sidak confidence interval adjustment, to investigate the main effects of condition (SHORT, MEDIUM, LONG vs. control), time (during intervention: T0, T1, T2, T3, T4 vs. T5; after intervention: baseline, 15, 30 vs. 60 mins post-intervention) and the condition × time interaction for TSI, blood pressure and perceptual responses. SpO\textsubscript{2} data were compared for main effect of condition (SHORT, MEDIUM, LONG vs. control) and environment (hypoxia vs. normoxia). Sphericity was assessed via a Mauchly test, if violated, a Greenhouse Geisser correction was applied. Partial eta-squared was calculated as an estimation of effect size (ES). Values of 0.01, 0.06 and above 0.14 were considered as small, medium and large, respectively (Cohen, 2013). If data were non-normally distributed, a related samples non-parametric Friedmans test was used. If any significant effects were found, further post-hoc analysis was carried out via pairwise
comparisons to assess where the significance lay. Statistical testing was carried out in SPSS (v21, IBM, Cambridge). Data are presented as mean ± SD, and considered statistically significant if $p \leq 0.05$ and a trend for significance if $p \leq 0.07$. Perceptual data are presented as raw values if statistical significance was not reached for context.

**Results**

**Responses during IHE**

**Physiological measures**

SpO$_2$ was lower during SHORT, MEDIUM and LONG hypoxic vs. normoxic cycles (-6%; $p < 0.001$, $F = 32.351$, ES = 0.822; Fig. 2). Pairwise comparisons revealed SpO$_2$ during hypoxic LONG cycles were lower vs. MEDIUM (-3%; $p = 0.023$) and SHORT (-4%; $p = 0.054$; Fig. 2).

**Fig. 2 near here**

TSI decreased during SHORT (68.9 ± 3.6%), MEDIUM (69.1 ± 4.4%) and LONG (68.7 ± 5.3%) vs. control (72.6 ± 4.9%; $p = 0.009$, $F = 8.237$, ES = 0.543; Fig. 3). Compared to T0, TSI from T1–T5 were lower (-3%; $p = 0.011$; $F = 6.107$; ES = 0.543; Fig. 3). There was no interaction effect on TSI ($p = 0.080$; $F = 2.997$; ES = 0.300).

**Fig. 3 near here**

**Perceptual measures**

No condition, time or interaction effects were observed for perceived mood, breathlessness and motivation to exercise during the 60-min intervention ($p \geq 0.05$; Table 1).
Responses after IHE

Physiological measures

No condition, time or interaction effects existed for blood pressure ($p > 0.05$; Table 2).

Perceptual measures

There was a significant effect of condition ($p = 0.003, F = 6.617, ES = 0.486$) and time ($p = 0.001, F = 17.779, ES = 0.717$) but no interaction ($p = 0.146, F = 1.946, ES = 0.218$; Fig. 5a) on perceived breathlessness. Pairwise comparisons revealed perceived breathlessness greater 15 min after the intervention vs. baseline (+34%; $p = 0.040$; Fig. 6a). Breathlessness during SHORT was lower vs. control 15–60 mins after the intervention ($p = 0.001$; Fig. 6a). Further, SHORT tended to be lower than LONG 15–60 mins after the intervention ($p = 0.06$). Positive affect decreased from 15–60 min post-intervention vs. baseline ($p < 0.05$), but was unaffected by condition ($p > 0.05$; Fig. 6b). Perceived mood state, motivation to exercise, negative affect and the ratio between positive and negative affect were unaffected by condition and did not change over time ($p > 0.05$; Table 1).

Discussion

To our knowledge, this is the first study to compare the acute psycho-physiological responses to SHORT (15 × 2/2 mins), MEDIUM (10 × 3/3 mins) and LONG (5 × 6/6 mins) cyclical variations of IHE. During one 60-min (30 min hypoxia/30 min normoxia) IHE session, arterial and brain oxygenation decreases versus control, independently of cycle length. Compared to
baseline, perceived breathlessness increased 15 min after IHE completion. This increase tended to be smaller following SHORT than LONG. When implementing IHE, greater desaturation is observed during longer compared to shorter hypoxic/normoxic cycles in adults with obesity. However, IHE tends to be better tolerated perceptually with shorter rather than longer cycles.

Responses during IHE

Physiological measures

We found that IHE decreased SpO\textsuperscript{2} (during hypoxic cycles) versus control. Further, LONG (-7\%) led to larger decreases than MEDIUM (-3\%) and SHORT (-1\%) versus normoxic cycles of each respective condition. Although the hypoxic duration was matched across IHE (30 min), the extent of SpO\textsuperscript{2} decrease is aligned with hypoxic/normoxic cycle length. This may be due to acute hypoxic exposure inducing a progressive decline in SpO\textsuperscript{2} (Botek et al., 2015) that is more evident in longer versus shorter cycles. SpO\textsuperscript{2} has been found to decrease continuously during exposure to hypoxia at rest (FiO\textsubscript{2} = 9.6\%) for up to 10 min versus a normoxic baseline in healthy individuals (-26\% 0–10-min) (Krejčí et al., 2018). However, SpO\textsuperscript{2} values during hypoxic cycles of IHE presented in the current study (SHORT = 94.2\%; MEDIUM = 93.1\%; LONG = 90.1\%) may be considered clinically insignificant. Hence, SpO\textsuperscript{2} below 90\% has been defined as a state of hypoxemia (Basnet et al., 2006). To reach greater levels of desaturation (hypoxemic state) alone, it is likely that IHE protocols consisting of longer rather than shorter hypoxic/normoxic cycles would be recommended. Overall, cyclical variations of IHE impacts on the subsequent decreases in SpO\textsuperscript{2}, with longer cycles inducing lower values.

TSI of the pre-frontal cortex decreased during all IHE cycles in reference to control. Here, we speculated that longer cycles of IHE would lead to larger decreases in pre-frontal cortex oxygenation versus shorter cycles (Verges et al., 2012), but this was not the case. Rupp et al.
(2016) reported decreases in pre-frontal cortex oxygenation (-3%) and SpO$_2$ (-9%) during 2-min IHE cycles (FiO$_2$ = 11.0%) for 45 min versus a normoxic baseline in healthy individuals, similar to the current study (TSI: -3%; SpO$_2$: -5%). Chacaroun et al. (2017) also reported decreases in cerebral oxygenation (-6%) during IHE (7 × 5-min hypoxia/3-min normoxia) at a target SpO$_2$ of 70–80%. Overall, it seems that larger SpO$_2$ decreases lead to measurable differences in pre-frontal cortex de-oxygenation. A greater hypoxic dose than that used in the current study (FiO$_2$ = 12.0%) may have led to larger SpO$_2$ decreases, and as such, pre-frontal cortex oxygenation, during both SHORT and LONG. Notably, TSI decreases occurred and were maintained from T1–T5 versus T0. It was previously stated that more than 30-min continuous hypoxic exposure is required to obtain quantifiable decreases in TSI of the pre-frontal cortex (Chacaroun et al., 2017). Under the present circumstances, IHE comprised of varying hypoxic cycles totaling 30-min induced similar de-oxygenation levels in the pre-frontal cortex of adults with obesity, independent of cycle length, which was maintained for 60-min.

**Perceptual measures**

During IHE, no changes in perceived mood state, breathlessness and motivation to exercise between IHE cycles were reported or versus control. During a 21-day bed rest in hypoxia (FiO$_2$ = 15.0%), healthy individuals felt more depressed, tense and confused at days 14 and 21 versus baseline (normoxia) (Stavrou et al., 2018). Although IHE and bed rest are passive modalities, the negative affects during bed rest in combination with hypoxia are unlikely to occur during IHE due to reduced exposure duration and inclusion of normoxic cycles. It was previously reported that mood is negatively impacted during rest in continuous hypoxia (8 h; FiO$_2$ = 13.0%) versus baseline (normoxia) (de Aquino Lemos et al., 2012). Therefore, we anticipated that SHORT would likely lead to better overall perception. However, no perceptual differences were observed between conditions during IHE. This may be due to differences in exposure type (intermittent vs. continuous), duration (30-min vs. 8-h), the hypoxic dose between studies (FiO$_2$
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= 12.0% vs. 13.0%), or little hypoxemia. We conclude here that perceptual responses during IHE are maintained with all tested cycle variations.

Responses after IHE

Physiological measures

Albeit with severe continuous hypoxic exposure (FiO₂ < 8.0%), elevations in blood pressure in humans and animals occur (White et al., 1985). Herein, we assessed blood pressure and found no differences between baseline and post-intervention following IHE of a moderate hypoxic level (FiO₂ = 12.0%). Previous studies have found normalized blood pressure in hypertensive patients following regular IHE (~1–5-min hypoxic/normoxic cycles, FiO₂ = 10–14%, daily for ~60-min, 10–14 days) (Serebrovskaya et al., 2008). As there were no blood pressure assessments during IHE in the current study, we cannot support this evidence. No negative sympathetic nervous system activity effects are realized regardless of IHE cycle length and is thus considered a safe therapy.

Perceptual measures

One unique finding includes greater perception of breathlessness 15-min post-intervention versus baseline, which tended to be exacerbated following LONG versus SHORT. We believe that this response occurred due to dyspnoeagena, i.e., an evoked respiratory exertion without increased physiological ventilation (Ward & Whipp, 1989). In adults with obesity, breathlessness is a symptom often felt during rest (Gibson, 2001), which may explain the increases in perceived breathlessness following control and IHE. Pulmonary ventilation measured before and after IHE did not support the participants’ increased perception of breathing – supporting evidence of dyspnoeagena. Importantly, increases in perceived breathlessness tended to be smaller following SHORT, and greater following LONG. In
summary, shorter IHE cycles may be preferential over longer because of a marginal lowering in the magnitude of post-intervention increases in perceived breathlessness after IHE.

Compared to baseline, positive affect was reduced 15–60-min post-intervention in all conditions (including control). Stavrou et al. (2018) found reduced positive affect following a 21-day bed rest in hypoxic and normoxic conditions. Perceived mood state, motivation to exercise and negative affect were maintained throughout IHE in the current study. As such, a reduced positive affect may not be due to hypoxia per se but the lack of activity over time (>3-h). Although positive affect was reduced following the 60-min intervention, it is unlikely that this was due to the effect of IHE, or in particular, cyclical variation.

Limitations and perspectives

The current study has several limitations. Firstly, our sample size was small (n = 8) implying that our findings should be interpreted with caution. Conclusions from this data are made only from stage I obesity, which may differ to stage II and III, and between genders such as larger psychophysiological stress (Stengel et al., 2013). Secondly, we used one hypoxic dose (FiO₂ = 12.0%) throughout IHE. Further studies should verify whether a more severe FiO₂ (lower than that used in the current study) during longer hypoxic/normoxic cycles, which will likely maximise the desaturation achieved during IHE, does not lead to negative effects on perceptual responses. IHE combined with exercise may potentiate further positive responses, at least short-term, than IHE alone due to added physical activity. As such, chronic studies implementing 2-min IHE (and exercise) cycles on a regular basis (3–4 times per week, over 4–6 weeks) (Hobbins et al., 2017) which are likely to improve aspects of health, are needed since the current study is acute-focused. The findings of this study sheds some light on disregarded perceptual responses.
Conclusion

When implementing IHE, greater desaturation is observed during longer compared to shorter hypoxic/normoxic cycles in adults with obesity. However, IHE tends to be better tolerated perceptually with shorter rather than longer cycles.
Acknowledgements

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Figure legends

**Fig. 1:** Overview of the 60-min intervention completed during each experimental trial. Participants were passively exposed to hypoxia (coloured bars) interspersed with exposure to normoxia (white bars). The time spent in hypoxia and normoxia per intervention was 30 mins, achieved via different cyclical variations: 2:2 (Short; black bars), 3:3 (Medium; dark grey bars) and 6:6 (Long; light grey bars) mins. An additional control trial involving continuous exposure to normoxia was also completed. Measurements were taken in normoxic conditions at 0 (T0), 10 (T1), 22 (T2), 34 (T3), 46 (T4) and 58 (T5) mins, as denoted by the dashed arrows.

**Fig. 2:** Arterial oxygen saturation (SpO$_2$) during the intervention. Values are presented as mean ± SD during hypoxic and normoxic periods (average of 30 min) for Short, Medium and Long, and average of 60 mins for control. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins. * denotes a statistically significant difference ($p < 0.01$) vs. control; ♦ denotes a statistically significant difference ($p = 0.03$) vs. normoxia for a given condition; § denotes a statistically significant difference ($p = 0.05$) vs. Long.

**Fig. 3:** Tissue saturation index (TSI) data of the pre-frontal cortex measured during the intervention at 8 (T1), 20 (T2), 32 (T3), 44 (T4) and 56 (T5) mins in hypoxia during Short, Medium and Long cycles and in normoxia during control. T1 – T5 values are calculated as a percentage difference from T0 and are presented as mean ± SD. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins condition. * denotes a statistically significant difference ($p < 0.01$) vs. control; # denotes a statistically significant difference ($p < 0.01$) vs. T0.
**Fig. 4:** Perceived breathlessness (a) and positive affect (b) data measured during the post-intervention period after 15, 30 and 60 mins. Values are calculated as a percentage difference from baseline and are presented as mean ± SD. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins condition. * denotes a statistically significant ($p < 0.01$) effect of condition; # denotes a statistically significant ($p < 0.01$) effect of time; † denotes a statistically significant ($p < 0.01$) difference vs. baseline; Ø denotes a statistically significant ($p < 0.02$) difference vs. 15 mins; ¥ denotes a statistically significant ($p < 0.01$) difference vs. control; ¢ denotes a statistical trend ($p < 0.06$) difference vs. Long.
Fig. 1: Overview of the 60-min intervention completed during each experimental trial. Participants were passively exposed to hypoxia (coloured bars) interspersed with exposure to normoxia (white bars). The time spent in hypoxia and normoxia per intervention was 30 mins, achieved via different cyclical variations: 2:2 (Short; black bars), 3:3 (Medium; dark grey bars) and 6:6 (Long; light grey bars) mins. An additional control trial involving continuous exposure to normoxia was also completed. Measurements were taken in normoxic conditions at 0 (T0), 10 (T1), 22 (T2), 34 (T3), 46 (T4) and 58 (T5) mins, as denoted by the dashed arrows.

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Fig. 2: Arterial oxygen saturation (SpO2) during the intervention. Values are presented as mean ± SD during hypoxic and normoxic periods (average of 30 min) for Short, Medium and Long, and average of 60 mins for control. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins. * denotes a statistically significant difference (p ≤ 0.01) vs. control; ‡ denotes a statistically significant difference (p = 0.03) vs. normoxia for a given condition; § denotes a statistically significant difference (p = 0.05) vs. Long.

338x190mm (54 x 54 DPI)
Fig. 3: Tissue saturation index (TSI) data of the pre-frontal cortex measured during the intervention at 8 (T1), 20 (T2), 32 (T3), 44 (T4) and 56 (T5) mins in hypoxia during Short, Medium and Long cycles and in normoxia during control. T1 – T5 values are calculated as a percentage difference from T0 and are presented as mean ± SD. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins condition. * denotes a statistically significant difference (p < 0.01) vs. control; # denotes a statistically significant difference (p < 0.01) vs. T0.
Fig. 4: Perceived breathlessness (a) and positive affect (b) data measured during the post-intervention period after 15, 30 and 60 mins. Values are calculated as a percentage difference from baseline and are presented as mean ± SD. Short = 2:2 mins; Medium = 3:3 mins; Long = 6:6 mins condition. * denotes a statistically significant (p < 0.01) effect of condition; † denotes a statistically significant (p < 0.01) effect of time; ‡ denotes a statistically significant (p < 0.02) difference vs. 15 mins; £ denotes a statistically significant (p < 0.01) difference vs. control; ¥ denotes a statistically significant (p < 0.01) difference vs. control; ¢ denotes a statistical trend (p < 0.06) difference vs. Long.

301x179mm (72 x 72 DPI)
Table 1. Perceptual measures recorded at 0 (T0), 10 (T1), 22 (T2), 34 (T3), 46 (T4) and 58 (T5) mins during Short, Medium and Long IHE interventions as well as the control trial.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Condition</th>
<th>Timepoint</th>
<th>ANOVA p value (effect size)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T0</td>
<td>T1</td>
</tr>
<tr>
<td>Mood state</td>
<td>Short</td>
<td>4.13 ± 0.83</td>
<td>3.88 ± 1.13</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>3.63 ± 1.30</td>
<td>3.38 ± 1.69</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>4.00 ± 1.20</td>
<td>3.63 ± 1.51</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.15 ± 1.56</td>
<td>3.75 ± 1.04</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>Short</td>
<td>0.63 ± 1.03</td>
<td>1.19 ± 1.49</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.75 ± 1.16</td>
<td>1.06 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>0.63 ± 0.64</td>
<td>1.08 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.69 ± 0.88</td>
<td>1.00 ± 1.04</td>
</tr>
<tr>
<td>Motivation to</td>
<td>Short</td>
<td>12.63 ± 2.20</td>
<td>13.00 ± 2.75</td>
</tr>
<tr>
<td>exercise</td>
<td>Medium</td>
<td>12.50 ± 2.98</td>
<td>13.00 ± 2.93</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>11.75 ± 5.12</td>
<td>10.88 ± 5.08</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12.13 ± 3.91</td>
<td>12.30 ± 3.29</td>
</tr>
</tbody>
</table>

Values are presented as group mean ± SD. Short = 2.2 mins; Medium = 3.3 mins; Long = 6.6 mins.
Table 2. Physiological and perceptual measures recorded at baseline and 15, 30 and 60 mins post-intervention during Short, Medium and Long IHE interventions as well as the control trial.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Condition</th>
<th>Baseline</th>
<th>+15 mins</th>
<th>+30 mins</th>
<th>+60 mins</th>
<th>ANOVA p value (effect size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>Short</td>
<td>113.38 ± 10.97</td>
<td>108.63 ± 13.33</td>
<td>117.75 ± 12.21</td>
<td>113.75 ± 17.09</td>
<td><strong>0.075 (0.27) 0.661 (0.07) 0.392 (0.13)</strong></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>112.00 ± 15.36</td>
<td>116.57 ± 21.93</td>
<td>112.13 ± 13.58</td>
<td>110.59 ± 14.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>116.14 ± 12.73</td>
<td>116.38 ± 16.03</td>
<td>122.25 ± 15.71</td>
<td>121.43 ± 12.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>112.43 ± 17.55</td>
<td>115.86 ± 15.60</td>
<td>114.14 ± 13.03</td>
<td>114.00 ± 14.47</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>Short</td>
<td>76.63 ± 5.88</td>
<td>74.00 ± 7.19</td>
<td>73.88 ± 8.13</td>
<td>76.75 ± 8.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>72.38 ± 10.27</td>
<td>73.43 ± 12.39</td>
<td>73.75 ± 7.78</td>
<td>77.50 ± 9.44</td>
<td><strong>0.288 (0.16) 0.331 (0.14) 0.584 (0.09)</strong></td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>76.71 ± 6.37</td>
<td>76.25 ± 8.26</td>
<td>79.25 ± 8.82</td>
<td>79.09 ± 7.96</td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>72.71 ± 9.30</td>
<td>75.63 ± 9.50</td>
<td>75.86 ± 10.24</td>
<td>76.43 ± 7.41</td>
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</tr>
<tr>
<td>Mood state</td>
<td>Short</td>
<td>4.63 ± 0.74</td>
<td>3.63 ± 1.69</td>
<td>3.75 ± 1.67</td>
<td>4.25 ± 1.16</td>
<td><strong>0.536 (0.06) 0.710 (0.03) 0.672 (0.05)</strong></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>3.63 ± 1.51</td>
<td>3.75 ± 1.75</td>
<td>3.88 ± 1.46</td>
<td>3.75 ± 1.75</td>
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</tr>
<tr>
<td></td>
<td>Long</td>
<td>4.25 ± 1.16</td>
<td>3.63 ± 1.85</td>
<td>3.50 ± 1.31</td>
<td>3.88 ± 1.73</td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.25 ± 1.28</td>
<td>3.88 ± 1.81</td>
<td>3.75 ± 1.49</td>
<td>3.63 ± 1.41</td>
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<tr>
<td>Motivation to exercise</td>
<td>Short</td>
<td>13.38 ± 3.29</td>
<td>12.88 ± 3.56</td>
<td>12.50 ± 2.98</td>
<td>13.25 ± 2.82</td>
<td><strong>0.473 (0.11) 0.746 (0.05) 0.375 (0.13)</strong></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>12.63 ± 3.38</td>
<td>13.38 ± 3.50</td>
<td>13.25 ± 3.28</td>
<td>12.88 ± 3.18</td>
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<tr>
<td></td>
<td>Long</td>
<td>11.88 ± 5.99</td>
<td>10.38 ± 4.66</td>
<td>10.25 ± 5.20</td>
<td>11.75 ± 4.89</td>
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<tr>
<td></td>
<td>Control</td>
<td>12.38 ± 4.14</td>
<td>13.38 ± 2.97</td>
<td>13.38 ± 2.83</td>
<td>13.13 ± 1.96</td>
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<tr>
<td>Negative affect</td>
<td>Short</td>
<td>10.13 ± 0.35</td>
<td>10.75 ± 1.39</td>
<td>10.75 ± 1.16</td>
<td>10.50 ± 0.76</td>
<td><strong>0.411 (0.12) 0.277 (0.16) 0.444 (0.11)</strong></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>10.25 ± 0.71</td>
<td>11.13 ± 2.10</td>
<td>11.13 ± 1.89</td>
<td>10.75 ± 1.39</td>
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</tr>
<tr>
<td></td>
<td>Long</td>
<td>10.88 ± 1.13</td>
<td>11.00 ± 1.31</td>
<td>10.75 ± 1.16</td>
<td>10.50 ± 0.76</td>
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<tr>
<td></td>
<td>Control</td>
<td>10.50 ± 0.76</td>
<td>11.38 ± 1.77</td>
<td>11.00 ± 1.41</td>
<td>10.63 ± 1.19</td>
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</tr>
<tr>
<td>Positive and negative affect ratio</td>
<td>Short</td>
<td>2.98 ± 0.93</td>
<td>2.64 ± 1.19</td>
<td>2.59 ± 1.36</td>
<td>2.59 ± 1.31</td>
<td><strong>0.346 (0.14) 0.134 (0.28) 0.793 (0.04)</strong></td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>2.69 ± 1.15</td>
<td>2.36 ± 1.41</td>
<td>2.46 ± 1.42</td>
<td>2.44 ± 1.36</td>
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<tr>
<td></td>
<td>Long</td>
<td>2.74 ± 1.24</td>
<td>2.65 ± 1.34</td>
<td>2.38 ± 1.19</td>
<td>2.36 ± 1.32</td>
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<tr>
<td></td>
<td>Control</td>
<td>2.78 ± 0.96</td>
<td>2.43 ± 1.11</td>
<td>2.39 ± 1.14</td>
<td>2.43 ± 1.32</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as group means ± SD. Short = 2.2 mins; Medium = 3.3 mins; Long = 6.6 mins.